ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2011, p. 5946–5948 Vol. 55, No. 12
0066-4804/11/$12.00 doi:10.1128/AAC.05124-11

Genetic Features of CTX-M-15-Producing *Acinetobacter baumannii* from Haiti[▽]

Anaïs Potron,[1] L. Silvia Munoz-Price,[2] Patrice Nordmann,[1]* Timothy Cleary,[3] and Laurent Poirel[1]

Service de Bactériologie-Virologie, INSERM U914 Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, Le Kremlin-Bicêtre Cedex, France,[1] and Department of Infectious Diseases[2] and Department of Pathology,[3] University of Miami, Jackson Memorial Hospital, Miami, Florida 33136-1096

Received 18 June 2011/Returned for modification 31 July 2011/Accepted 11 September 2011

***Acinetobacter baumannii* isolates T23, W35, and H1 were isolated from three patients who had been injured in the Haiti earthquake in January 2010. Those isolates, corresponding to two distinct clones, were identified as extended-spectrum β-lactamase (ESBL) producers and found to be $bla_{CTX-M-15}$-positive. That ESBL gene was associated with IS*Ecp1*, involved in its acquisition by a one-ended transposition mechanism. In all isolates, the IS*Ecp1*-$bla_{CTX-M-15}$ compound transposon was apparently chromosomally located.**

A growing number of β-lactamases conferring resistance to broad-spectrum cephalosporins have been identified in *Acinetobacter* spp. Extended-spectrum β-lactamase (ESBL) genes identified in those species are mostly of VEB or PER types but TEM, SHV, GES, and CTX-M derivatives have also been reported (1, 5, 10, 14). In particular, it is noteworthy that bla_{CTX-M} genes that are so widespread in *Enterobacteriaceae* have been identified so rarely in *Acinetobacter* spp., with one CTX-M-2-producing *Acinetobacter baumannii* isolate in Japan (11) and a few CTX-M-15-producing *A. baumannii* isolates in India (19).

Acinetobacter baumannii isolates T23, H1, and W35 were obtained, respectively, from wound specimens ($n = 2$) and a rectal swab specimen ($n = 1$) from three victims of the earthquake that struck southern Haiti in January 2010 (8). Isolates H1 and W35 had been recovered in Haiti at the field hospital, whereas isolate T23 was obtained in Miami, FL, within 24 h of admission at the Jackson Hospital. Identification of those isolates at the species level was performed by using the API32GN system (bioMérieux, Marcy l'Etoile, France) and was confirmed by 16S rRNA gene sequencing as described previously (6). Susceptibility testing was performed at 37°C on Mueller-Hinton-containing plates by disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) and using the Etest technique (bioMérieux, Marcy l'Etoile, France), and results were interpreted in accordance with the guidelines of the CLSI (2). The three *A. baumannii* isolates were resistant to penicillins, broad-spectrum cephalosporins, and aztreonam but remained susceptible to carbapenems (2). Addition of clavulanic acid or tazobactam significantly decreased the MICs of amoxicillin, ticarcillin, and piperacillin, as well as ceftazidime and cefotaxime (Table 1). Synergies between ceftazidime and clavulanic acid suggested the production of an ESBL. In addition, two isolates (T23 and W35) showed resistance to fluoroquinolones, whereas isolate H1 remained susceptible. All isolates showed susceptibility to colistin (Table 1). *A. baumannii* W35 and T23 were susceptible to amikacin, and *A. baumannii* H1 was of intermediate susceptibility to amikacin (Table 1). Whole-cell DNA of *A. baumannii* isolates was extracted as described previously (13). Those DNAs were used as templates under standard PCR conditions (18) with a series of primers designed for the detection of class A β-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{PER-1}, bla_{VEB-1}, bla_{GES-1}, and bla_{CTX-M}) (3, 16). Genes encoding CTX-M-type ESBL were detected in all three *A. baumannii* isolates, and sequencing identified $bla_{CTX-M-15}$.

Genotypic comparison was performed by multilocus sequence typing (MLST), sequence-type multiplex PCR, and pulsed-field gel electrophoresis (PFGE) according to the manufacturer's recommendations (Bio-Rad, Marnes-la-Coquette, France). PFGE analysis, performed as described previously (9), showed that two out of the three isolates (T23 and W35) were clonally related (and designated clone A), although isolate H1 corresponded to a distinct clone (clone B) (data not shown). The occurrence of the same clone in two different patients could result from a nosocomial transmission originally at the field hospital.

Further analysis with sequence-type multiplex PCR as described by Turton et al. (20) showed that clone A belonged to international clone II, whereas clone B did not correspond to any of the well-defined international clones I to III, amplicons being obtained with group I-specific *ompA* and bla_{OXA-51}-like primers but with group II-specific *csuE* primers. The bla_{OXA-51}-like gene identified in isolates T23 and W35 corresponded to $bla_{OXA-180}$, whereas that identified in isolate H1 encoded a newly described point mutant derivative of OXA-64.

MLST analysis performed for each clone as described by Diancourt et al. (4) showed that clones A and B belonged to two new sequence types (STs) named ST117 (12-37-2-2-9-2-14) and ST118 (35-2-11-2-9-1-2), respectively (http://www.pasteur.fr/cgi-bin/genopole/PF8/mlstdbnet.pl?file=acin_isolates.xml). Those two novel STs are distantly related to all of the others known (no more than 4 out of the 7 alleles in common).

Transfer of the $bla_{CTX-M-15}$ gene from the three *A. baumannii* isolates by conjugation or electroporation, as described previously (9), using rifampin-resistant *A. baumannii* BM45.47

* Corresponding author. Mailing address: Service de Bactériologie-Virologie-Hygiène, Hôpital de Bicêtre, 78 rue de Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.aphp.fr.

▽ Published ahead of print on 19 September 2011.

TABLE 1. MICs of β-lactams for *A. baumannii* T23, W35, and H1 and *E. coli* TOP10(pT23), TOP10(pW35), TOP10(pH1), and the TOP10 reference strain[a]

β-Lactam(s)[b]	MIC (μg/ml) for:						
	A. baumannii			*E. coli*			
	T23	W35	H1	TOP10(pT23)	TOP10(pW35)	TOP10(pH1)	TOP10
Amoxicillin	>256	>256	>256	>256	>256	>256	2
Amoxicillin + CLA	16	16	16	8	8	8	2
Ticarcillin	>256	>256	>256	>256	>256	>256	2
Ticarcillin + CLA	32	32	32	32	32	32	2
Piperacillin	>256	>256	>256	>256	>256	>256	1
Piperacillin + TZB	2	2	2	2	2	2	1
Cephalothin	>256	>256	>256	>256	>256	>256	4
Cefuroxime	>256	>256	>256	>256	>256	>256	2
Cefoxitin	64	64	64	2	2	2	2
Cefotaxime	>256	>256	>256	256	256	256	0.06
Cefotaxime + CLA	16	16	16	0.5	0.5	0.25	0.06
Ceftazidime	64	64	64	128	128	128	0.12
Ceftazidime + CLA	2	2	2	0.25	0.25	0.12	0.06
Cefepime	>256	>256	>256	32	32	32	0.06
Aztreonam	>256	>256	>256	>256	>256	>256	0.03
Meropenem	0.5	0.5	0.5	0.03	0.03	0.03	0.03
Imipenem	0.25	0.25	0.25	0.25	0.25	0.25	0.12
Colistin	1	1	1	0.5	0.5	0.5	0.5
Tigecycline	0.5	0.5	0.25	0.25	0.25	0.25	0.25
Amikacin	4	4	16	1	1	1	1
Rifampin	2	2	2	ND[c]	ND	ND	ND

[a] *E. coli* TOP10(pT23), TOP10(pW35), and TOP10(pH1), respectively, correspond to the *E. coli* TOP10 recombinant strains harboring plasmids containing inserts obtained from the *A. baumannii* T23, W35, and H1 isolates.
[b] CLA, clavulanic acid (4 μg/ml); TZB, tazobactam (4 μg/ml).
[c] ND, not determined.

as the recipient failed, suggesting a chromosomal location for the $bla_{\text{CTX-M-15}}$ gene.

In order to further investigate the genetic support and environment of the $bla_{\text{CTX-M-15}}$ gene in those isolates, shotgun cloning using HindIII-restricted genomic DNA and HindIII-restricted pACYC184 plasmid was performed as described previously (12). Recombinant plasmids were selected onto Trypticase soy agar (TSA) plates containing cefotaxime (10 μg/ml) and chloramphenicol (30 μg/ml). The resulting recombinant strains, *Escherichia coli* TOP10(pW35), TOP10(pT23), and TOP10(pH1), displayed an ESBL phenotype with high-level resistance to broad-spectrum cephalosporins and aztreonam, remaining susceptible to cefoxitin and carbapenems (Table 1). Sequencing of the insert of these three recombinant plasmids revealed that the entire IS*Ecp1* element was always present immediately upstream of the $bla_{\text{CTX-M-15}}$ gene. The right boundary of IS*Ecp1* was located 48 bp upstream of the start codon of the $bla_{\text{CTX-M-15}}$ gene in the three isolates, as previously described (7). The 701 bp located immediately downstream of the $bla_{\text{CTX-M-15}}$ gene were identical in all three isolates, the first 345 bp corresponding to a truncated part of Orf477 previously described downstream of the chromosome-borne $bla_{\text{CTX-M-3}}$ gene in the *Kluyvera ascorbata* progenitor (17). The last 356 bp corresponded to a Tn*3* family transposase, which was truncated in *A. baumannii* H1 (Fig. 1). These observations further suggest that structures identified in *A. baumannii* derive from those identified in the progenitor. In *A. baumannii* T23 and W35, the whole sequence of the Tn*3* family transposase was identified and was followed by a truncated part of IS*26*, as already found in the $bla_{\text{CTX-M-3}}$-carrying plasmid pEK204 (22). The 14 bp located at the 3′ extremity of IS*26* corresponded to an inverted repeat 2 (IRR2) sequence that could be used as a right inverted repeat (IRR) by IS*Ecp1*, as demonstrated previously (15). This sequence shared 8 out of the 14 bp with the original IRR1 of IS*Ecp1*. The identification of a 5-bp target site duplication (AATCA) at the 5′ extremity of IS*Ecp1* and at the right-hand extremity of the IS*26* fragment was the signature of a transposition event and led us to identify a new compound transposon named Tn*Ab15*, comprising IS*Ecp1*, $bla_{\text{CTX-M-15}}$, Δ*orf477*, the Tn*3* family transposase, and a truncated IS*26* (Fig. 1). This transposon was inserted into a gene encoding a transposase sharing 100% amino acid identity with that of IS*N1* previously identified in *A. baumannii* AB0057 (accession no. NC_011586.1) (Fig. 1). An *filD* gene encoding a pilus assembly protein, sharing 100% amino acid identity with that identified in *A. baumannii* AYE (21), was identified upstream of the IS*N1* transposase, further supporting chromosomal integration of the IS*Ecp1*-$bla_{\text{CTX-M-15}}$ transposon. In *A. baumannii* H1, transposon Tn*Ab15* had been truncated at its right extremity, and no target site duplication was identified, suggesting some deletions/rearrangements (Fig. 1). However, this acquired genetic structure was inserted into a gene encoding a pyrimidine utilization transporter sharing significant similarity with that identified among *A. baumannii* genomes, again supporting a chromosomal location.

We report here three CTX-M-15-producing *A. baumannii* isolates. In all isolates, the $bla_{\text{CTX-M-15}}$ gene was linked to IS*Ecp1*, which might be responsible for its acquisition. IS*Ecp1* and $bla_{\text{CTX-M-15}}$ were part of a transposon that integrated into the chromosome of *A. baumannii* through a likely transposition process. This corresponds to the second description of CTX-M-15-producing *A. baumannii* isolates after that re-

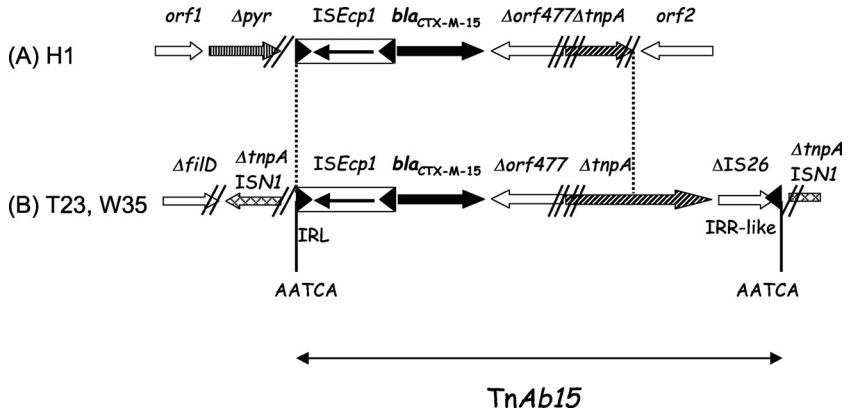

FIG. 1. Schematic map of $bla_{CTX\text{-}M\text{-}15}$-positive structures identified in *A. baumannii* isolates. (A) The structure identified in *A. baumannii* H1; (B) the structures from *A. baumannii* isolates T23 and W35. The genes and their corresponding transcriptional orientations are indicated by horizontal arrows. The AATCA target site duplications identified on both extremities of transposon Tn*Ab15* are indicated. Sequences between vertical dashes are strictly identical. Δ*pyr* corresponds to a truncated gene encoding a putative pyrimidine utilization transporter.

ported in India (19). Despite the high prevalence of $bla_{CTX\text{-}M\text{-}15}$ in *Enterobacteriaceae*, that gene is still rarely identified in *A. baumannii.* This is probably due to the fact that $bla_{CTX\text{-}M\text{-}15}$-carrying plasmids, often of the IncF or IncH1 types, are of narrow host range and cannot replicate in *A. baumannii.* However, since IS*Ecp1* is a very efficient genetic tool in terms of gene mobilization, it is possible that integration of the IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$ element into the chromosome of *A. baumannii* may have occurred.

The emergence and spread of ESBL-producing *A. baumannii* strains are worrisome, as this combination of events will enhance extensive use of carbapenems, thus increasing the risk of emergence of carbapenem-resistant isolates. In addition, it must be emphasized that detection of ESBL production by phenotypic methods is quite difficult in *A. baumannii* because of the frequent accumulation of enzymatic and nonenzymatic resistance mechanisms such as impermeability and efflux. Therefore, it could facilitate a "silent" spread of those particular isolates.

Nucleotide sequence accession number. The nucleotide sequences data reported in this work have been deposited in the GenBank nucleotide database under accession no. JN788266 and JN788267.

This work was partially funded by a grant from the INSERM (U914) and by grants from the European Community (TROCAR, HEALTH-F3-2008-223031 and TEMPOtest-QC, HEALTH-2009-241742). It was also partially funded by an IISP Research Grant from Merck & Co., Inc.

REFERENCES

1. **Bonnin, R. A., et al.** 2011. PER-7, an extended-spectrum β-lactamase with increased activity toward broad-spectrum cephalosporins in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **55:**2424–2427.
2. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing: 21st informational supplement. CLSI M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Cuzon, G., et al.** 2010. Worldwide diversity of *Klebsiella pneumoniae* that produce β-lactamase $bla_{KPC\text{-}2}$ gene. Emerg. Infect. Dis. **16:**1349–1356.
4. **Diancourt, L., V. Passet, A. Nemec, L. Dijkshoorn, and S. Brisse.** 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One **5:**e10034.
5. **Endimiani, A., et al.** 2007. Spread in an Italian hospital of a clonal *Acinetobacter baumannii* strain producing the TEM-92 extended-spectrum β-lactamase. Antimicrob. Agents Chemother. **51:**2211–2214.
6. **Ibrahim, A., P. Gerner-Smidt, and W. Liesack.** 1997. Phylogenetic relationship of the twenty-one DNA groups of the genus *Acinetobacter* as revealed by 16S ribosomal DNA sequence analysis. Int. J. Syst. Bacteriol. **47:**837–841.
7. **Lartigue, M.-F., L. Poirel, and P. Nordmann.** 2004. Diversity of genetic environment of genes. FEMS Microbiol. Lett. **234:**201–207.
8. **Lichtenberger, P., et al.** 2010. Infection control in field hospitals after a natural disaster: lessons learned after the 2010 earthquake in Haiti. Infect. Control Hosp. Epidemiol. **31:**951–957.
9. **Mugnier, P., L. Poirel, M. Pitout, and P. Nordmann.** 2008. Carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* isolates in the United Arab Emirates. Clin. Microbiol. Infect. **14:**879–882.
10. **Naas, T., F. Namdari, H. Réglier-Poupet, C. Poyart, and P. Nordmann.** 2007. Panresistant extended-spectrum β-lactamase SHV-5-producing *Acinetobacter baumannii* from New York City. J. Antimicrob. Chemother. **60:**1174–1176.
11. **Nagano, N., Y. Nagano, C. Cordevant, N. Shibata, and Y. Arakawa.** 2004. Nosocomial transmission of CTX-M-2 β-lactamase-producing *Acinetobacter baumannii* in a neurosurgery ward. J. Clin. Microbiol. **42:**3978–3984.
12. **Nordmann, P., et al.** 1993. Characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **37:**962–969.
13. **Philippon, L. N., T. Naas, A. T. Bouthors, V. Barakett, and P. Nordmann.** 1997. OXA-18, a class D clavulanic-acid inhibited extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **41:**2188–2195.
14. **Poirel, L., R. A. Bonnin, and P. Nordmann.** Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life, in press.
15. **Poirel, L., M.-F. Lartigue, J.-W. Decousser, and P. Nordmann.** 2005. IS*Ecp1B*-mediated transposition of $bla_{CTX\text{-}M}$ in *Escherichia coli*. Antimicrob. Agents Chemother. **49:**447–450.
16. **Poirel, L., I. Le Thomas, T. Naas, A. Karim, and P. Nordmann.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum-β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.
17. **Rodriguez, M. M., et al.** 2004. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. Antimicrob. Agents Chemother. **48:**4895–4897.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19. **Shakil, S., and A. U. Khan.** 2010. Detection of CTX-M-15-producing and carbapenem-resistant *Acinetobacter baumannii* strains from urine from an Indian hospital. J. Chemother. **22:**324–327.
20. **Turton, J. F., S. N. Gabriel, C. Valderrey, M. E. Kaufmann, and T. L. Pitt.** 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii.* Clin. Microbiol. Infect. **13:**807–815.
21. **Vallenet, D., et al.** 2008. Comparative analysis of Acinetobacter: three genomes for three lifestyles. PLoS One **3:**e1805.
22. **Woodford, N., et al.** 2009. Complete nucleotide sequences of plasmids pEK204, pEK499, and pEK516, encoding CTX-M enzymes in three major *Escherichia coli* lineages from the United Kingdom, all belonging to the international O25:H4-ST131 clone. Antimicrob. Agents Chemother. **53:**4472–4482.