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Characterization of pABVA01, a Plasmid Encoding the OXA-24 Carbapenemase from Italian Isolates of *Acinetobacter baumannii*▽

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Two epidemiologically unrelated carbapenem-resistant *Acinetobacter baumannii* isolates were investigated as representatives of the first Italian isolates producing the OXA-24 carbapenemase. Both isolates were of European clonal lineage II and carried an identical OXA-24-encoding plasmid, named pABVA01. Comparative analysis revealed that in pABVA01, bla_{OXA-24} was part of a DNA module flanked by conserved inverted repeats homologous to XerC/XerD binding sites, which in other *Acinetobacter* plasmids flank different DNA modules, suggesting mobilization by a novel site-specific recombination mechanism.

Acinetobacter baumannii is an opportunistic nosocomial pathogen of increasing clinical relevance (3, 4, 22). The species is naturally resistant to several antimicrobial agents and exhibits a remarkable propensity to acquire new resistances (4, 14, 22). Carbapenems are elective agents for treatment of *A. baumannii* infections, and the emergence of carbapenem-resistant strains is a matter of increasing clinical concern (22, 24). Acquired class D carbapenemases of the OXA-23, OXA-24 (also named OXA-40), and OXA-58 lineages are playing a major role as determinants of acquired carbapenem resistance in *A. baumannii* (24).

In Italy, production of OXA-58 is the dominant carbapenem resistance mechanism in *A. baumannii*, and several outbreaks caused by OXA-58-producing strains related to European clonal lineage II have been documented (11, 15, 17), while strains producing OXA-23 and OXA-24 have not been reported. Here we report the characterization of the first Italian isolates of carbapenem-resistant *A. baumannii* producing the OXA-24 carbapenemase.

(Part of this study was presented at the 18th European Congress of Clinical Microbiology and Infectious diseases, Barcelona, Spain, 2008. [M. M. D'Andrea, T. Giani, F. Luzzaro, and G. M. Rossolini, oral communication O300].)

Characterization of OXA-24-positive *A. baumannii* isolates. In a survey of carbapenemase genes in carbapenem-resistant *A. baumannii* isolated from several Italian hospitals during the past decade, only two *A. baumannii* isolates, VA-566/00 and N50, tested positive for bla_{OXA-24}-like genes by PCR analysis, while they lacked genes for other acquired class D carbapenemases (OXA-23- and OXA-58-like class D carbapenemases) and metallo-β-lactamases (IMP-, VIM-, or SIM-type metallo-β-lactamases). The primers and PCR conditions used for the detection of carbapenemase genes are described in Table 1. VA-566/00 was isolated from an inpatient at a tertiary care hospital in Varese (in northern Italy) in September 2000. No epidemiological links with areas of OXA-24 endemicity could be traced for this patient by analysis of clinical records. N50 was isolated from an inpatient at a general hospital in Rome (in central Italy) in January 2004. In this case, analysis of clinical records revealed a previous history of hospitalization (2 months earlier) in Spain, which is a country where OXA-24 is endemic (20, 24). Both isolates were from the respiratory tract and had apparently played the role of colonizers. Identification at the species level was performed by automatic identification systems (Phoenix; Becton Dickinson, Sparks, MD; and Vitek-2; bioMérieux, Marcy-l'Etoile, France) and confirmed by PCR detection of bla_{OXA-51}-like alleles (33).

Susceptibility testing carried out by broth microdilution and interpreted according to CLSI guidelines (8, 9) revealed that both isolates were resistant to all β-lactams (including carbapenems), aminoglycosides, and fluoroquinolones. Variable susceptibilities toward colistin and tigecycline were observed (Table 2). PCR performed with the primers described in Table 1 revealed IS*Aba1* upstream of the resident bla_{ADC} gene but not upstream of the resident bla_{OXA-51}-like gene in both isolates, suggesting that bla_{OXA-51}-like gene overexpression was not involved in the carbapenem resistance phenotype.

Multiplex PCR for the definition of *A. baumannii* sequence groups, which has been proven to be a useful method for assigning isolates to major clonal lineages (31, 32), was performed using *ompA*, *csuE*, and bla_{OXA-51}-like target genes as described previously (32). PCR amplicons were purified by using a Wizard PCR purification kit (Promega, Madison, WI) and were sequenced on both strands, using custom primers at an external sequencing facility (Macrogen, Seoul, Korea). Genotyping by macrorestriction analysis of ApaI-digested *A.*

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TABLE 1. Nucleotide primers used in this work for detection of β-lactamase genes and their linkages with insertion sequences in *A. baumannii* isolates

Primer	Sequence (5′–3′)[a]	Target(s)	Expected amplicon size (bp)	Conditions for denaturation (°C/min)[b]	Conditions for annealing (°C/min)[b]	Conditions for extension (°C/min)[b]	Source or reference	Positive control (gene) [source or reference]
IMP-DIA-fwd	GGAATAGAGTGGCTTAATTCTC	bla_{IMP} alleles	361	94/1	50/1	72/1	13	*A. baumannii* AC-54/97 (bla_{IMP-2}) [25]
IMP-DIA-rev	GTGATGCGTCYCCAAYTTCACT							
VIM-DIA-fwd	CAGATTGCCGATGGTGTTTGG	bla_{VIM} alleles	523	94/1	50/1	72/1	26	*P. aeruginosa* VR143/97 (bla_{VIM-1}) [18]
VIM-DIA-rev	AGGTGGGCCATTCAGCCAGA							
SIM1-F	TACAAGGGATTCGGCATCG	bla_{SIM-1}	570	94/1	50/1	72/1	19	*A. baumannii* YMC 03/9/T104 (bla_{SIM-1}) [19]
SIM1-R	TAATGGCCTGTTCCCATGTG							
OXA23-F	GATGTGTCATAGTATTCGTCG	bla_{OXA-23} alleles	748	94/1	50/1	72/1	2	*A. baumannii* Ab13 (bla_{OXA-23}) [10]
OXA23-R	TCACAACAACTAAAAGCACTG							
OXA24-F	TTCCCCTAACATGAATTTGT	bla_{OXA-24} alleles	582	94/1	50/1	72/1	2	*A. baumannii* RYC 52763/97 (bla_{OXA-24}-type) [6]
OXA24-R	GTACTAATCAAAGTTGTGAA							
OXA-58_I_Fw	GCTGAGCATAGTATGAGTCG	bla_{OXA-58} alleles	691	94/1	48/1	72/1	This work	*A. baumannii* VA-900/05 (bla_{OXA-58}) [this work]
OXA-58_I_Rev	AAGCAAATGCCACCACTTGC							
OXA-69A	CTAATAATTGATCTACTCAAG	bla_{OXA-51} alleles	975 or 2,168[c]	94/1	48/1	72/2	16	*A. baumannii* VA-804/03 (IS*Aba1* + bla_{OXA-90}) [this work]
OXA-69B	CCAGTGGATGGATGGATAGAT TATC							
ISAba2_Fw	CCTTATCCTATCAGGGTTCTG	IS*Aba1* and bla_{ADC} alleles	2,300	94/1	50/1	72/2	This work	*A. baumannii* 16x46 (bla_{ADC}-type) [this work]
AmpCaba_Rev	GCATTCAGCACAGCATAAG							

[a] For degenerate primers, the following code was used: Y, C/T.
[b] All reactions included an initial denaturation step of 5 min at 94°C, 30 cycles of amplification, and a final extension step of 20 min at 72°C.
[c] Primers give a larger amplicon in the presence of an IS*Aba1* upstream of the bla_{OXA-51} allele.

baumannii genomes was performed by pulsed-field gel electrophoresis (PFGE) as previously described (28), using a CHEF mapper (Bio-Rad, Hemel Hempstead, United Kingdom). PFGE profiles were interpreted according to the criteria proposed by Tenover et al. (30), with a difference of six bands or less used to define epidemiological relatedness, and were analyzed by BioNumerics software (Applied Maths, Kortrijk, Belgium). *A. baumannii* RUH875 and RUH134 were used as reference strains representative of European clonal lineages I and II, respectively (12, 21). *A. baumannii* isolates VA-900/05, N33, and N40, obtained from the same hospitals in which the OXA-24-producing isolates were detected, were used as representatives of the major carbapenem-resistant OXA-58-producing clone spreading in Italy. N33 and N40 showed close genetic relatedness to the OXA-58-producing prototypic Italian strain ACICU (11, 17).

Sequencing of the *ompA*, *csuE*, and bla_{OXA-51}-like alleles showed that VA-566/00 belonged in sequence group 1 (allelic profile 1-1-1), similar to RUH134 and the OXA-58 producers VA-900/05, N33, and N40 used for comparison, while N50 belonged in a variant of sequence group 1 called group 4, which does not yield an amplicon for the *csuE* allele (31) (Fig. 1A). As expected, RUH875 yielded results typical of sequence group 2, with an allelic profile of 2-2-2. The ApaI macrorestriction profiles of genomic DNA confirmed that the two OXA-24-positive isolates were related to each other (a difference of four bands) and, although to a lower extent, to RUH134 and the OXA-58-producing isolates from the same hospitals (differences of six to seven bands) (Fig. 1B). The topology of the dendrogram highlighted two major clusters at a similarity threshold of at least 82.4%, which differentiated the OXA-24-producing isolates from the OXA-58-producing isolates. Moreover, both clusters were related, having a 74.2% similarity with the prototypic strain RUH134 (Fig. 1B). Altogether, these results demonstrate a genetic correlation between the two OXA-24-producing isolates and that they belong to European clonal lineage II.

Genetic support of bla_{OXA-24}. Plasmid extraction from VA-566/00 and N50, carried out with a Wizard Plus SV Minipreps DNA purification system (Promega), revealed in both cases the

TABLE 2. Antibiotic susceptibility of the bla_{OXA-24}-positive *A. baumannii* isolates investigated in this work and of the RUH134(pABVA01) transformant[a]

Isolate	MIC (mg/liter) of the indicated antibiotic[b]:								
	CIP	GEN	AMK	CAZ	IPM	MEM	SAM	CST	TGC
VA-566/00	128	>256	64	>256	256	256	64	0.5	4
N50	128	>256	256	>256	128	128	32	>8	1
RUH134	1	>256	4	16	0.25	1	64	0.5	1
RUH134(pABVA01)	1	>256	4	16	64	128	64	0.5	1

[a] The susceptibility of RUH134 is shown for comparison.
[b] CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; SAM, ampicillin/sulbactam; CST, colistin; TGC, tigecycline.

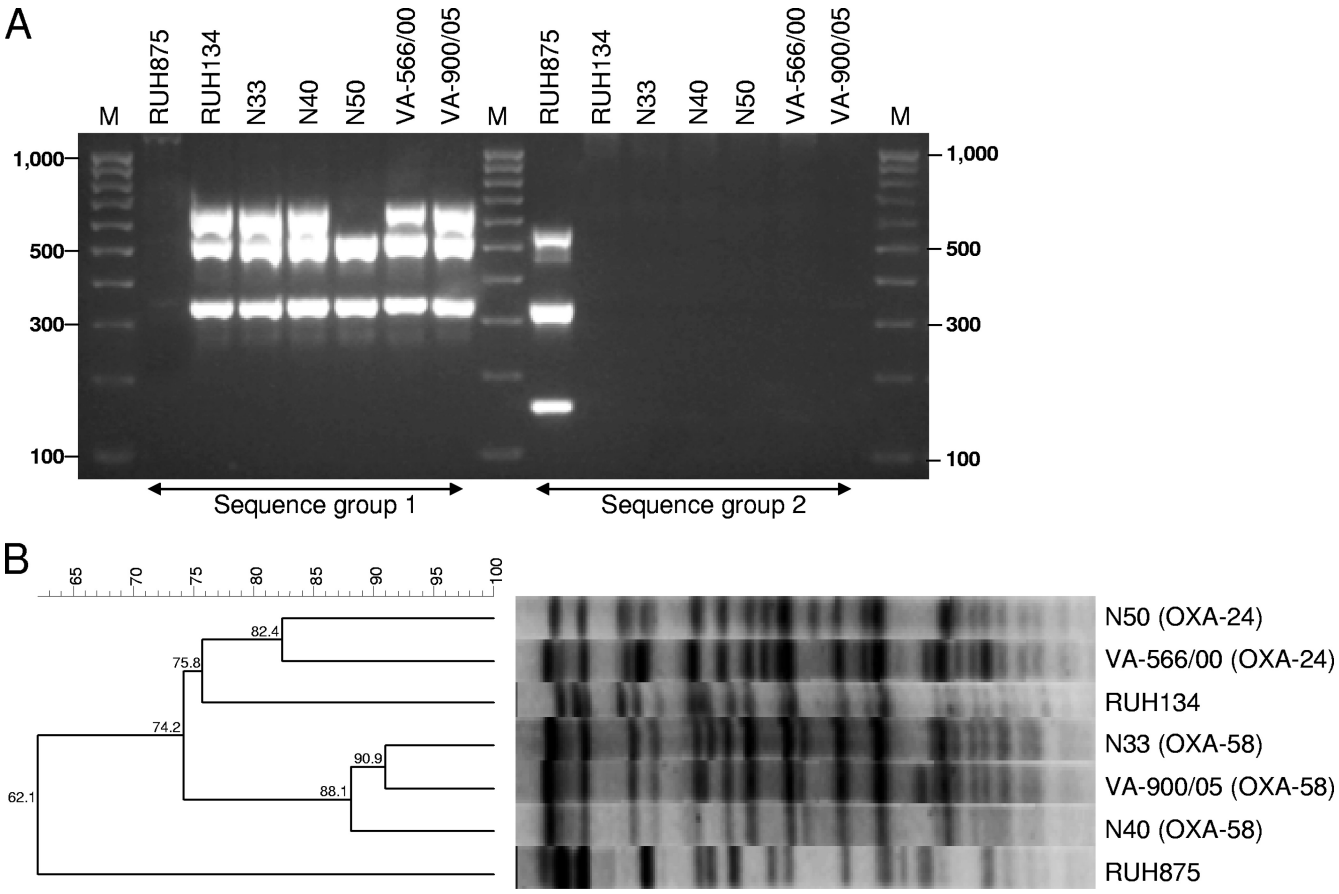


FIG. 1. Results of genotyping of *A. baumannii* isolates investigated in this work and of the reference strains RUH875 and RUH134, representative of European clonal lineages I and II, respectively. (A) Sequence-based typing resulting from multiplex PCR targeting the *ompA*, *csuE*, and bla_{OXA-51}-like genes. Lane M contains 100-bp molecular weight markers. (B) PFGE profiles. The dendrogram was generated with BioNumerics software (Applied Maths), using the unweighted-pair group method with arithmetic averages and the Dice coefficient. The percentage of similarity is shown at each node. Strain descriptions with the corresponding carbapenemases (in parentheses) are shown on the right.

presence of a small plasmid, which in a Southern blotting experiment (27) hybridized to a bla_{OXA-24} probe (data not shown).

The plasmid from VA-566/00, named pABVA01, was transferred to RUH134 by electroporation, using a Gene Pulser apparatus (Bio-Rad). Competent cells were prepared as described for *Escherichia coli* (27). Transformants were selected on LB agar supplemented with imipenem (4 mg/liter). The carriage of bla_{OXA-24} in the transformants was confirmed by PCR. The RUH134(pABVA01) transformants showed high MICs of carbapenem (similar to those for parent strain VA-566/00), while their susceptibilities to other agents, including ceftazidime, were not affected (Table 2), confirming that OXA-24 is not active on this substrate (6). Ceftazidime resistance in the original host was likely related to overexpression of the bla_{ADC} cephalosporinase gene due to upstream insertion of IS*Aba1*.

The nucleotide sequence of pABVA01 was determined on both strands by a primer-walking technique with a purified plasmid preparation. Plasmid pABVA01 is 8,963 bp long and carries eight open reading frames (ORFs) (Table 3, Fig. 2A). PCR mapping, followed by restriction analysis of amplicons and partial sequencing, showed that the plasmid obtained from N50 was indistinguishable from pABVA01 (data not shown). In particular, the nucleotide sequence of the region encompassing bla_{OXA-24} and its flanks (Fig. 2A) was identical.

Structure of plasmid pABVA01 and context of bla_{OXA-24}. Comparative analysis with other sequenced *Acinetobacter* plasmids revealed that the genetic organization of pABVA01 was overall very similar to that of p2ABAYE (35) and pAB0057 (1), two small *Acinetobacter* plasmids previously detected in French and American clinical isolates, respectively. The major differences between pABVA01 and these plasmids were found in the replicon region (the iteron and *repB* region of pABVA01 are more divergent; the 3′ end of the *repA* gene of pABVA01 carries a 7-bp deletion resulting in a premature stop codon) and in the region containing bla_{OXA-24}, which in the other plasmids was replaced by unique regions of different size and composition (Fig. 2B).

A similar arrangement of intervening DNA modules inserted at the same position suggested the occurrence of a conserved recombination site where different DNA modules could be inserted by a site-specific recombination mechanism. Analysis of the sequences at the recombination junctions revealed, on both sides, the presence of conserved inverted repeats (IRs) separated by a 6-bp variable region (Fig. 2C). These structures, which partially overlap the previously de-

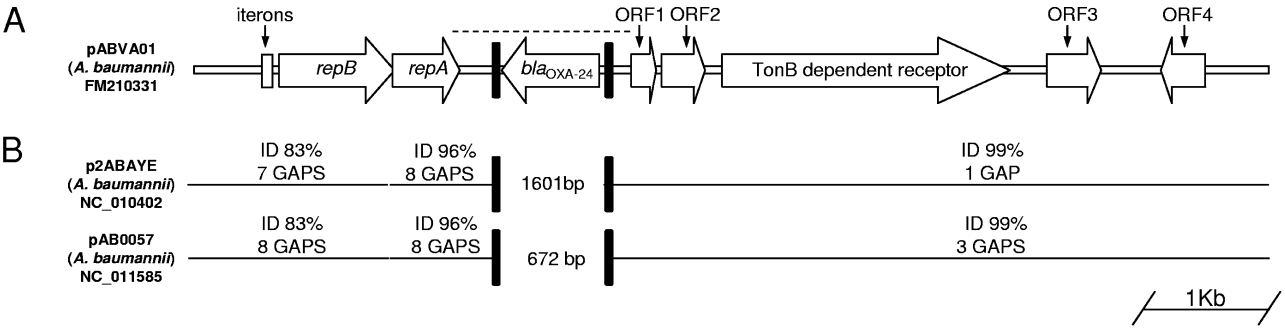
TABLE 3. ORFs and other features of plasmid pABVA01

Nucleotide position[a]	Strand[b]	Feature	Gene product (no. of amino acids)[b]	Properties and/or putative function
319–519	+	*oriV*	NA	Origin of DNA replication
520–607	NA	Repeat region	NA	Imperfect 4-repeat iterons; control of DNA replication
663–1613	+	*repB*	316	Replicase
1606–2181	+	*repA*	191	Replicase
2481–2491	+	Putative XerC binding site	NA	Protein binding site
2498–2508	+	Putative XerD binding site	NA	Protein binding site
2519–3346	−	bla_{OXA-24}	275	Carbapenem-hydrolyzing oxacillinase
3420–3430	−	Putative XerD binding site	NA	Protein binding site
3437–3447	−	Putative XerC binding site	NA	Protein binding site
3617–3832	+	ORF1	71	Putative inner membrane protein
3860–4243	+	ORF2	127	Putative cytoplasmic protein
4371–6782	+	TonB-dependent receptor	803	Putative TonB-dependent receptor
7087–7548	+	ORF3	153	Hypothetical protein
8044–8415	−	ORF4	123	Hypothetical protein

[a] Position no. 1 corresponds to the first nucleotide of p2ABAYE (NC_010402).
[b] NA, not applicable.

scribed Re27-1 and Re27-2 regions associated with bla_{OXA-58} (23, 36), share high homology with *dif*-like binding sites that act as targets of the XerC and XerD recombinases that normally convert plasmid and chromosome dimers to monomers during cell division (29). XerC and XerD proteins and cognate recombination sites are also involved in other site-specific recombination mechanisms such as the integration of phage CTX-Φ at the *dif1* site of the larger chromosome of *Vibrio cholerae* (34) and have also been exploited for artificial gene excision by site-specific recombination in *E. coli* and *Bacillus subtilis* (5). Interestingly, the conserved IRs homologous to the XerC/XerD binding sites were found to flank not only different DNA modules in related *Acinetobacter* plasmids but also the bla_{OXA-24} gene in the partially sequenced plasmid pAB02 and in the chromosome of strain RYC 52763/97 (6) (Fig. 2C). Altogether, these findings suggest that the XerC/XerD-like sites could act as site-specific recombination targets responsible for the mobilization of discrete DNA modules within *Acinetobacter* plasmids and chromosomes and that bla_{OXA-24} could be mobilized by a similar mechanism.

Concluding remarks. To the best of our knowledge, this is the first description of carbapenem-resistant *A. baumannii* iso-



C

Accession no. /reference

	XerC	XerD		XerD	XerC	
pABVA01	ATATACAAATGT--ACTTCGTATAATCGCCATTATGTTAAAT##	911 bp	##	AATTAACATAATAC-ACGTTATACGAAAT	GCTGTATAATTATCCTTA	FM210331
p2ABAYE	.....G...--.....##	1601 bp	##	.C.....-..C.....		NC_010402
pAB0057	.....G...--.....##	672 bp	##	.....-..C.....		NC_011585
pAB02	.....--.....##	911 bp	##	.....-G.C....G.....TG.A.T..TCTTA..		AY228470
pAB2	.....G...--.....AT.....##	3389 bp	##	..A.....-..C.....		NC_009084
pAV1	.....G...--.T.G.....G.....##	2890 bp	##	.....-..C.....GT.....TA..		NC_010309
pABIR	T.T.CA.TT...GC.T.....CGC.....T.##	21802 bp	##	.....-..C.....		NC_010481
bla_{OXA-24} RYC52763/97	T.T.CAT...T.--.T....C....CGC.....##	912 bp	##	.....CG.C....G.....TG.A.T..TCTTA..		Ref. 6

FIG. 2. (A) Linear map of plasmid pABVA01. Plasmid features are detailed in Table 3. The dashed line indicates the sequenced region of the plasmid from isolate N50. (B) Comparison of plasmid pABVA01 with plasmids p2ABAYE and pAB0057. For each homologous segment, the percentage of nucleotide identity and the number of gaps inserted into the alignments are shown. Filled boxes represent the putative recombination sites represented by the IRs homologous to the XerC/XerD binding sites. The sizes of the different DNA modules inserted between the IRs in place of the bla_{OXA-24} module are also shown. (C) Nucleotide sequences flanking different DNA modules (including that containing bla_{OXA-24}) in different *Acinetobacter* plasmids. The IRs homologous to the XerC/XerD binding sites that resulted from comparison with sequences reported by Bui et al. (7) are boxed. The sizes of intervening DNA modules are also indicated. The regions flanking bla_{OXA-24} in the chromosome of strain RYC 52763/97 (6) are also shown. Accession numbers for each sequence and the reference for strain RYC 52763/97 are reported on the right.

lates carrying the bla_{OXA-24} determinant from Italy. The two isolates have a common ancestry and carry the same OXA-24-encoding plasmid, but they are not identical by PFGE profile and sequence type, which suggests a history of independent acquisition of the same resistance plasmid by the two related strains. Analysis of the genetic context of bla_{OXA-24} did not reveal structures typically involved in DNA mobilization (insertion sequences or other genes encoding known recombinases). However, bla_{OXA-24} was carried on a DNA module inserted between conserved inverted repeats homologous to XerC/XerD binding sites, which, in other plasmids, flank DNA modules of different sizes and compositions. This finding suggests the occurrence of a novel site-specific recombination mechanism that could play a role in the plasticity of *Acinetobacter* plasmids and in the mobilization of resistance genes.

Nucleotide sequence accession number. The nucleotide sequence determined in this work has been submitted to the EMBL/DDBJ/GenBank database and assigned accession number FM210331.

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REFERENCES

1. **Adams, M. D., K. Goglin, N. Molyneaux, K. M. Hujer, H. Lavender, J. J. Jamison, I. J. MacDonald, K. M. Martin, T. Russo, A. A. Campagnari, A. M. Hujer, R. A. Bonomo, and S. R. Gill.** 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J. Bacteriol. **190:** 8053–8064.
2. **Afzal-Shah, M., N. Woodford, and D. M. Livermore.** 2001. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D β-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **45:**583–588.
3. **Bergogne-Bérézin, E.** 2001. The increasing role of *Acinetobacter* species as nosocomial pathogens. Curr. Infect. Dis. Rep. **3:**440–444.
4. **Bergogne-Bérézin, E., and K. J. Towner.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148–165.
5. **Bloor, A. E., and R. M. Cranenburgh.** 2006. An efficient method of selectable marker gene excision by Xer recombination for gene replacement in bacterial chromosomes. Appl. Environ. Microbiol. **72:**2520–2525.
6. **Bou, G., A. Oliver, and J. Martinez-Beltran.** 2000. OXA-24, a novel class D β-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob. Agents Chemother. **44:**1556–1561.
7. **Bui, D., J. Ramiscal, S. Trigueros, J. S. Newmark, A. Do, D. J. Sherratt, and M. E. Tolmasky.** 2006. Differences in resolution of *mwr*-containing plasmid dimers mediated by the *Klebsiella pneumoniae* and *Escherichia coli* XerC recombinases: potential implications in dissemination of antibiotic resistance genes. J. Bacteriol. **188:**2812–2820.
8. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing; 17th informational supplement. M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Corvec, S., L. Poirel, T. Naas, H. Drugeon, and P. Nordmann.** 2007. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-23} in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **51:**1530–1533.
11. **D'Arezzo, S., A. Capone, N. Petrosillo, and P. Visca.** 2009. Epidemic multidrug-resistant *Acinetobacter baumannii* related to European clonal types I and II in Rome (Italy). Clin. Microbiol. Infect. **15:**347–357.
12. **Dijkshoorn, L., H. Aucken, P. Gerner-Smidt, P. Janssen, M. E. Kaufmann, J. Garaizar, J. Ursing, and T. L. Pitt.** 1996. Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. J. Clin. Microbiol. **34:**1519–1525.
13. **Docquier, J. D., M. L. Riccio, C. Mugnaioli, F. Luzzaro, A. Endimiani, A. Toniolo, G. Amicosante, and G. M. Rossolini.** 2003. IMP-12, a new plasmid-encoded metallo-β-lactamase from a *Pseudomonas putida* clinical isolate. Antimicrob. Agents Chemother. **47:**1522–1528.
14. **Fournier, P. E., D. Vallenet, V. Barbe, S. Audic, H. Ogata, L. Poirel, H. Richet, C. Robert, S. Mangenot, C. Abergel, P. Nordmann, J. Weissenbach, D. Raoult, and J. M. Claverie.** 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS. Genet. **2:**e7.
15. **Giordano, A., P. Varesi, A. Bertini, L. Villa, A. M. Dionisi, M. Venditti, P. Carfagna, I. Luzzi, C. Mancini, and A. Carattoli.** 2007. Outbreak of *Acinetobacter baumannii* producing the carbapenem-hydrolyzing oxacillinase OXA-58 in Rome, Italy. Microb. Drug Resist. **13:**37–43.
16. **Héritier, C., L. Poirel, P. E. Fournier, J. M. Claverie, D. Raoult, and P. Nordmann.** 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**4174–4179.
17. **Iacono, M., L. Villa, D. Fortini, R. Bordoni, F. Imperi, R. J. Bonnal, T. Sicheritz-Ponten, G. De Bellis, P. Visca, A. Cassone, and A. Carattoli.** 2008. Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. Antimicrob. Agents Chemother. **52:**2616–2625.
18. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla_{VIM}, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1584–1590.
19. **Lee, K., J. H. Yum, D. Yong, H. M. Lee, H. D. Kim, J. D. Docquier, G. M. Rossolini, and Y. Chong.** 2005. Novel acquired metallo-β-lactamase gene, bla_{SIM-1}, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob. Agents Chemother. **49:**4485–4491.
20. **Lopez-Otsoa, F., L. Gallego, K. J. Towner, L. Tysall, N. Woodford, and D. M. Livermore.** 2002. Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in northern Spain. J. Clin. Microbiol. **40:**4741–4743.
21. **Nemec, A., L. Dijkshoorn, and T. J. van der Reijden.** 2004. Long-term predominance of two pan-European clones among multi-resistant *Acinetobacter baumannii* strains in the Czech Republic. J. Med. Microbiol. **53:**147–153.
22. **Perez, F., A. M. Hujer, K. M. Hujer, B. K. Decker, P. N. Rather, and R. A. Bonomo.** 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **51:**3471–3484.
23. **Poirel, L., and P. Nordmann.** 2006. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-58} in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **50:** 1442–1448.
24. **Poirel, L., and P. Nordmann.** 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. **12:**826–836.
25. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla_{IMP} allelic variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.
26. **Riccio, M. L., L. Pallecchi, J. D. Docquier, S. Cresti, M. R. Catania, L. Pagani, C. Lagatolla, G. Cornaglia, R. Fontana, and G. M. Rossolini.** 2005. Clonal relatedness and conserved integron structures in epidemiologically unrelated *Pseudomonas aeruginosa* strains producing the VIM-1 metallo-β-lactamase from different Italian hospitals. Antimicrob. Agents Chemother. **49:**104–110.
27. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
28. **Seifert, H., L. Dolzani, R. Bressan, T. van der Reijden, B. van Strijen, D. Stefanik, H. Heersma, and L. Dijkshoorn.** 2005. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. J. Clin. Microbiol. **43:** 4328–4335.
29. **Summers, D. K., and D. J. Sherratt.** 1988. Resolution of ColE1 dimers requires a DNA sequence implicated in the three-dimensional organization of the *cer* site. EMBO J. **7:**851–858.
30. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
31. **Towner, K. J., K. Levi, and M. Vlassiadi.** 2008. Genetic diversity of carbapenem-resistant isolates of *Acinetobacter baumannii* in Europe. Clin. Microbiol. Infect. **14:**161–167.
32. **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.
33. **Turton, J. F., N. Woodford, J. Glover, S. Yarde, M. E. Kaufmann, and T. L. Pitt.** 2006. Identification of *Acinetobacter baumannii* by detection of the bla_{OXA-51}-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. **44:**2974–2976.
34. **Val, M. E., M. Bouvier, J. Campos, D. Sherratt, F. Cornet, D. Mazel, and**

F. X. Barre. 2005. The single-stranded genome of phage CTX is the form used for integration into the genome of *Vibrio cholerae*. Mol. Cell **19:**559–566.

35. **Vallenet, D., P. Nordmann, V. Barbe, L. Poirel, S. Mangenot, E. Bataille, C. Dossat, S. Gas, A. Kreimeyer, P. Lenoble, S. Oztas, J. Poulain, B. Segurens, C. Robert, C. Abergel, J. M. Claverie, D. Raoult, C. Medigue, J. Weissenbach, and S. Cruveiller.** 2008. Comparative analysis of *Acinetobacters*: three genomes for three lifestyles. PLoS One **3:**e1805.
36. **Zarrilli, R., D. Vitale, A. Di Popolo, M. Bagattini, Z. Daoud, A. U. Khan, C. Afif, and M. Triassi.** 2008. A plasmid-borne bla_{OXA-58} gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. Antimicrob. Agents Chemother. **52:**4115–4120.