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OXA-24 Carbapenemase Gene Flanked by XerC/XerD-Like Recombination Sites in Different Plasmids from Different *Acinetobacter* Species Isolated during a Nosocomial Outbreak▽

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A clinical strain of *Acinetobacter calcoaceticus* resistant to carbapenems was isolated from a blood culture sample from an inpatient in a hospital in Madrid (Spain) during a large outbreak of infection (affecting more than 300 inpatients), caused by a multidrug-resistant *Acinetobacter baumannii* clone. The carbapenem resistance in both the *A. calcoaceticus* and *A. baumannii* clones was due to a bla_{OXA-24} gene harbored in different plasmids. The plasmids were fully sequenced, revealing the presence of site-specific recombination binding sites putatively involved in mobilization of the bla_{OXA-24} gene. Comparison of plasmids contained in the two strains revealed possible horizontal transmission of resistance genes between the *Acinetobacter* species.

Since 1986, members of the genus *Acinetobacter* have been identified by Southern hybridization. Genospecies 1 (*Acinetobacter calcoaceticus*), 2 (*Acinetobacter baumannii*), 3, and 13TU are genetically closely related and are commonly known as the *A. calcoaceticus-A. baumannii* complex. With the exception of genospecies 1, the other members of this complex have been reported to be involved in nosocomial infections and are known to have the ability to spread within hospitals (1, 12). *Acinetobacter calcoaceticus* has traditionally been considered an environmental species and has never been associated with serious clinical infections.

Among β-lactamases, the most prevalent carbapenemases in *Acinetobacter* spp. are the class D β-lactamases, which are divided into 4 different groups: OXA-23, OXA-24, and OXA-58 with all their variants (17) and the OXA-51 family, which has been described as being intrinsic to *A. baumannii* (6). Although these β-lactamases have mainly been isolated from *A. baumannii*, recent studies have demonstrated the presence of OXA-58 in *Acinetobacter* genomic species 3, as well as in *Acinetobacter* phenon 6/ct13TU (10, 11) and in *Acinetobacter* genomospecies 13TU (8). None of these class D β-lactamases have been described in *A. calcoaceticus* (7).

During an outbreak of infection caused by a multidrug-resistant (including carbapenems) *A. baumannii* clone (named AbH12O-A2), which occurred in the 12 de Octubre Hospital (Madrid, Spain) and affected more than 300 patients, an *A. calcoaceticus* strain (named Acal H12O-07) was isolated from blood cultures from a 58-year-old man. The patient was admitted to the hospital with a cranial encephalic trauma. After admission and during the course of his stay, the patient presented several nosocomial infections. The patient was empirically treated with different cycles of antibiotics, starting with ceftriaxone plus levofloxacin, then piperacillin-tazobactam, meropenem plus vancomycin, and finally linezolid plus imipenem. After 4 months of hospitalization and in light of persistent fever, two sets of blood cultures were drawn and yielded isolation of a Gram-negative bacillus. The microorganism was identified with the automated WIDER system as a member of the *A. baumannii-A. calcoaceticus* complex. The antibiotic susceptibility profile obtained by microdilution revealed the following MICs (μg/ml): piperacillin-tazobactam, >64/4; ceftazidime, >16; cefepime, >16; imipenem, >8; meropenem, >8; tobramycin, ≤2; amikacin, >16; gentamicin, ≤2; ciprofloxacin, 1; trimethoprim-sufamethoxazole, ≤2/38. After clinical evaluation, the case was considered a primary bacteremia and the patient was administered a course of antimicrobial treatment with ciprofloxacin and gentamicin for 2 weeks, with a favorable clinical response.

The 16S rRNA of the isolate was also sequenced and yielded identification of an *A. calcoaceticus* strain. A putative β-lactamase enzyme with carbapenemase activity was detected by the Hodge test (9). A PCR amplification with primers designed from class D carbapenemase genes and metallo-β-lactamase genes (bla_{IMP}, bla_{VIM}, and bla_{SIM}) from *Acinetobacter* (18) yielded isolation of a bla_{OXA-24} gene. A plasmid, named pMMCU1, was isolated from the clinical *A. calcoaceticus* strain. This plasmid was used to transform an *A. baylyi* ADP1 isolate. Imipenem and meropenem MICs for the *A. baylyi* ADP1 strain increased from 0.094 and 0.25, respectively, to >32 μg/ml in both cases, when the strain was transformed with plasmid pMMCU1. Moreover, a band corresponding to the bla_{OXA-24} gene was also obtained by PCR, with the plasmid isolated from the transformed *A. baylyi* ADP1 strain as template. The gene product revealed 100% identity with the previously described bla_{OXA-24} gene (2). The full plasmid was sequenced and was found to be 8,771

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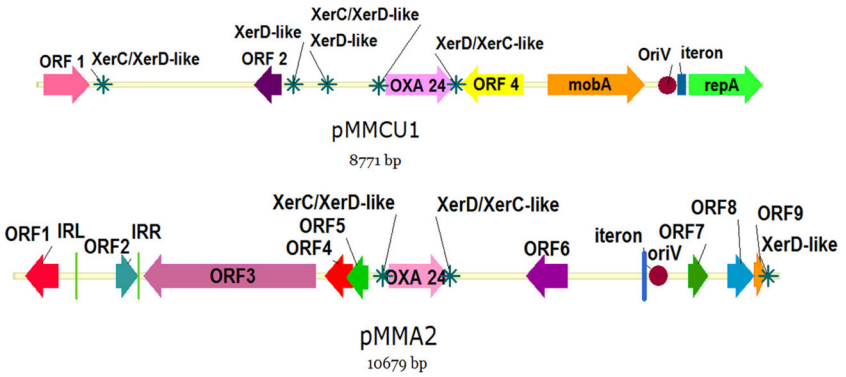

FIG. 1. (Top) Diagram of the 8,771-bp pMMCU1 plasmid obtained from the *A. calcoaceticus* clinical strain (Acal H12O-07). (Bottom) Diagram of the 10,679-bp pMMA2 plasmid obtained from the *A. baumannii* strain (AbH12O-A2) that caused the large outbreak of infection. The asterisks indicate the Xer-like recombination sites.

bp in size (GenBank accession code GQ342610). Moreover, the *A. baumannii* clone that caused the large outbreak was found to carry the pMMA2 plasmid (GenBank accession code GQ377752), which was also isolated, analyzed, and found to be 10,679 bp in size.

Comparative analysis among the sequences of the two plasmids revealed different scaffolds and coding regions, as shown in Fig. 1 and Tables 1 and 2. The pMMCU1 plasmid showed the highest homology with plasmid pABO2 (GenBank accession code AY228470) and carried the mobilization region derived from the previously described pMAC plasmid (GenBank accession code AY541809). The pMMA2 plasmid displayed the highest homology with the previously described p2ABAYE plasmid (GenBank accession code CU459138.1).

The bla_{OXA-24} gene was detected in both the pMMCU1 and pMMA2 plasmids and was found to be flanked by 11-bp conserved inverted repeats separated by a 6-bp variable region (GenBank accession codes GQ342610 and GQ377752). The 5′ sequence flanking the bla_{OXA-24} gene in the pMMCU1 plasmid was ATTTCGCATAACGCCCATTATGTTAAAT, and the 3′ sequence was AATTAACATAATACGCCTTATGCGAAAT. Similarly, in the case of the pMMA2 plasmid, the sequence located at the 5′ position was ACTTCGGATAACGCCCATT ATGTTAAAT, and that located at the 3′ position was TTAA CATAATACACCTTATACGAAATGC. The Xer-like binding site sequences described in the pMMCU1 and pMMA2 plasmids showed 79.5, 76, 76, 91, 74, 81.5, and 90.5% and 76.5, 73, 73, 88.5, 80, 76.5, and 88.5% identity, respectively, with their counterparts found in plasmids pABVA01, p2ABAYE, pAB0057, pAB02, pAB2, pAV1, and pABIR, respectively, and in both cases they showed the highest identity, 100% and 88.5%, respectively, with the XerC/XerD-like binding sites located in the chromosomal region of the bla_{OXA-24} gene previously described in the RYC52763/97 strain (2, 5).

The bla_{OXA-24} gene was already observed as part of one of the discrete DNA modules flanked by XerC/XerD-like sites within *Acinetobacter* plasmids. These Xer-like binding sites have been suggested to be involved in the mobilization of discrete DNA modules within *Acinetobacter* plasmids and chromosomes by site-specific recombination mechanisms (5).

Moreover, other plasmids were also isolated from minor *A. baumannii* clones that appeared during the outbreak (GenBank accession codes GQ904226, GQ476987, and GQ904227). These plasmids also harbored the bla_{OXA-24} gene integrated in different locations flanked by XerC/XerD-like binding sites. These results show that during the outbreak there was no exchange of a common plasmid carrying the bla_{OXA-24} gene among the strains isolated. On the

TABLE 1. Description of the pMMCU1 plasmid isolated from *A. calcoaceticus* (AcalH2O-O7)

Feature[b]	Positions	Properties and/or putative function	GenBank/EMBL accession no. of match	Plasmid or chromosomal homology (reference)
ORF 1	74–631	Hypothetical protein	AY228470	pAB02
XerC/XerD-like	782–809	Recombination sites	FM210331.1	pABVA01
ORF 2	2621–2941	Hypothetical protein	CU468230	ABSDF
XerD-like	3090–3100	Recombination sites	FM210331.1	pABVA01
XerD-like	3503–3513	Recombination sites	FM210331.1	pABVA01
XerC/XerD-like	4116–4143	Recombination sites	AJ239129	RYC52763/97 (2)[a]
bla_{OXA-24}	4217–5044	Carbapenem-hydrolyzing oxacillinase	AJ239129	RYC52763/97 (2)[a]
XerD/XerC-like	5055–5082	Recombination sites	AJ239129	RYC52763/97 (2)[a]
ORF 4	5113–5874	Hypothetical protein	AY541809	pMAC
MobA	6174–7343	Plasmid mobilization protein	AY541809	pMAC
OriV	7524–7724	Origin of DNA replication	AY228470	pAB02
Iteron	7741–7836	Imperfect 4-repeat iterons; control of DNA replication	AY228470	pAB02
RepA	7881–8768	repA_AB; DNA replication protein	AY228470	pAB02

[a] Chromosomal DNA of strain RYC52763/97 (2).
[b] ORF, open reading frame.

TABLE 2. Description of the pMMA2 plasmid isolated from the *A. baumannii* strain that caused a large outbreak of infection (AbH12O-A2)[a]

Feature	Positions	Properties and/or putative function	GenBank/EMBL accession no. of match(es)	Plasmid or chromosomal homology (reference)
ORF 1	141–602	Septicolysin (endotoxin)	CU459138.1	p2ABAYE
IRL	855–872	IS*4* inverted repeat left	YP_001957893.1	NA
ORF 2	1418–1719	Putative IS*4* transposase family	YP_001957893.1	NA
IRR	1722–1739	IS*4* inverted repeat right	YP_001957893.1	NA
ORF 3	1793–4204	Putative TonB-dependent receptor	CU459138.1	p2ABAYE
ORF 4	4332–4715	Putative cytoplasmic protein	CU459138.1	p2ABAYE
ORF 5	4633–4929	Putative inner membrane protein	CU459138.1	p2ABAYE
XerC/XerD-like	5128–5155	Recombination sites	AJ239129, AY228470, NC_010481	RYC52763/97 (2)[b] pABO2 pABIR
bla_{OXA-24}	5229–6056	Carbapenem-hydrolyzing oxacillinase	AJ239129	RYC52763/97 (2)[b]
XerC/XerD-like	6069–6096	Recombination sites	AJ239129, AY228470, NC_010481	RYC52763/97 (2)[b] pABO2 pABIR
ORF 6	7145–7714	DNA replication protein	ZP_04663577	AB900
Iteron	8791–8878	Imperfect 4-repeat iterons; control of DNA replication	CU459138.1	p2ABAYE
OriV	8896–9100	Replication origin	CU459138.1	p2ABAYE
ORF 7	9410–9682	Hypothetical protein	CU459138.1	p2ABAYE
ORF 8	9953–10324	Hypothetical protein	CU459138.1	p2ABAYE
ORF 9	10324–10497	Hypothetical protein	CU459138.1	p2ABAYE
XerD-like	10521–10531	Recombination sites	CU459138.1	p2ABAYE

[a] Abbreviations: ORF, open reading frame; IRL, inverted repeat, left; IRR, inverted repeat, right; NA, not applicable.
[b] Chromosomal DNA of strain RYC52763/97 (2).

contrary, the plasmid structures provide evidence supporting the hypothesis that different plasmids exchange the bla_{OXA-24} gene comprised within a very limited region between the two closest XerC/XerD-like binding sites.

DNA recombination through the Xer system in plasmids requires XerC and XerD (recombinases); XerC/XerD-like binding sites; accessory proteins, such as PepA, ArgR, and ArcA; and an accessory sequence of about 180 bp located near the core site (3, 4, 13, 15). Recombination occurs via formation of a heterotetrameric complex in which each recombinase catalyzes the exchange of one pair of DNA strands in a reaction that proceeds through the Holliday junction intermediate. The accessory proteins bind the accessory sequence and induce formation of a synaptic complex that is required for recombination (3). These recombination events are involved in site-specific integration and excision of lysogenic genomes, transposition of conjugative transposons, termination of chromosome replication, and plasmid stability (3, 14, 16).

Sequence analysis revealed that all the plasmids isolated from the outbreak carried the same bla_{OXA-24} mobilization cassette, which contained the following regions, from 5′ to 3′: a region of 142 bp followed by XerC/XerD-like binding sites, a region of 72 bp, and the bla_{OXA-24} gene finally followed by 10 bp and the downstream XerD/XerC-like binding sites (GenBank accession codes GQ342610, GQ377752, GQ904226, GQ476987, and GQ904227, respectively). We suggest that both of these regions, of 142 and 72 bp, may act as targets for the accessory proteins required for Xer recombination. Moreover, we found that XerC and XerD recombinases are encoded in the *A. baumannii* chromosome of strains AB0057, SDF, AYE, and ACICU (GenBank accession codes for XerC: YP_002320364.1, YP_001706416.1, YP_001712817.1, and YP_001847531.1, respectively; for XerD: ZP_02977538.1, YP_001708368, YP_001715285.1, and YP_001844923.1, respectively). PepA has been also found in the genomes of *A. baumannii* AB0057, SDF, and AYE (GenBank accession codes YP_002317727.1, YP_001708379.1, and YP_001715297.1, respectively).

These findings suggest that Xer recombination may be responsible for mobilization of the bla_{OXA-24} gene, and experiments are in progress to confirm this hypothesis. As the Xer-like binding sites are located in opposite directions, recombination through the Xer system should occur by gene inversion. Therefore, the most likely explanation would seem to be that putative Xer-mediated recombination events led to the dissemination of the bla_{OXA-24} gene among different plasmids and that acquisition of the resistance gene by *A. calcoaceticus* was mediated by the transfer of one of these plasmids. Note that *A. calcoaceticus* is usually regarded as an environmental species and this is the first description of a plasmid-mediated carbapenem-hydrolyzing oxacillinase, OXA-24, isolated from an *A. calcoaceticus* strain causing bacteremia.

Dissemination of resistance genes via Xer recombination in plasmids has previously been suggested (5). The present study emphasizes the threat associated with this mechanism in relation to the dissemination of carbapenemase genes among different *Acinetobacter* species in hospital environments.

Nucleotide sequence accession numbers. The sequences of plasmids pMMCU1 and pMMA2 were deposited in GenBank under nucleotide sequence accession numbers GQ342610 and GQ377752, respectively. Other plasmid sequences from outbreak clones were deposited under accession numbers GQ904226, GQ476987, and GQ904227.

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