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Short communication

First detection of the OXA-40 carbapenemase in *P. aeruginosa* isolates, located on a plasmid also found in *A. baumannii*

Première détection de la carbapénémase OXA-40 dans des souches de *P. aeruginosa*, localisée dans un plasmide aussi trouvé dans *A. baumannii*

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Abstract

The aim of this work was to carry out the molecular investigation of the OXA-40 carbapenemase detected in two isolates of *Pseudomonas aeruginosa* resistant to imipenem. The sequence showed 100% of homology with the gene previously described in *Acinetobacter baumannii*. Hybridization experiments located the gene on a plasmid also found in the OXA-40 control strain of *A. baumannii*.

Résumé

L'objectif de ce travail c'est l'investigation moléculaire de la carbapénémase OXA-40 détectée dans deux souches de *Pseudomonas aeruginosa* résistantes à l'imipénem. La séquence a montré 100 % d'identité avec le gène décrit précédemment dans *Acinetobacter baumanii*. Les expériences d'hybridation ont localisé le gène dans un plasmide aussi trouvé dans la souche contrôle de *A. baumannii* pour le gène bla_{OXA-40}.

Keywords: Plasmid; OXA-40; *Pseudomonas aeruginosa*

Mots clés : Plasmide ; OXA-40 ; *Pseudomonas aeruginosa*

Carbapenems are considered agents of choice to treat infections caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* but increasing identification of multidrug-resistant isolates complicates the treatment.

One growing factor leading to carbapenem resistance is the presence of carbapenemases, being class B metallo-β-lactamases (VIM and IMP type) prevalent in *P. aeruginosa* and class D oxacillinases (OXA type) in *A. baumannii*. The fact that these enzymes could reside on transmissible elements should magnify our concern regarding their spread [1].

In *P. aeruginosa*, several OXA-type enzymes have been described, but very few with carbapenemase activity. Up to now, a naturally occurring oxacillinase OXA-50 has been described, with an homology of 44 and 43% respectively with OXA-23 and OXA-27 carbapenemases from *A. baumannii*.

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OXA-40 carbapenemase, firstly identified in *A. baumannii* strains from a hospital in northern Spain, is endemic in the Iberian Peninsula and it has been recently identified in the United States [2,3].

In this study, we investigated the presence of the OXA-40 carbapenemase in isolates of *P. aeruginosa* resistant to imipenem and located its coding gene on a plasmid also found in *A. baumannii*.

For that purpose, we analyzed two clonally unrelated *P. aeruginosa* isolates (named P15 and P23) PCR-positive for the *oxa40* gene. Isolates were obtained from a hospital in northern Spain and had been previously characterized. [4].

As a part of this study, sequencing of amplified DNA fragment was performed using Dye Terminator Cycle Sequencing Ready Fraction Kit (Perkin Elmer) according to the manufacturer's conditions. Plasmid DNA was extracted with a commercial Midi Plasmid Extraction Kit (Quiagen). Plasmid content was analyzed by electrophoresis on 0.7% agarose gels and plasmid size was determined by comparison with those from the standard strains *Escherichia coli* NCTC 50193 and NCTC 59192 (ranging in size from 163.3 to 2 kb) and *A. baumannii* SM28 (OXA-40). Mapping of plasmids was done by the digestion of 1 μg of plasmid DNA with EcoRI, PstI and HindIII endonucleases, following the manufacturer's conditions (Invitrogen). To locate the *oxa40* gene, gels with plasmid DNA and the corresponding digestions were transferred to nylon membranes by the Southern technique and hybridized with a PCR-generated OXA-40 probe, labeled with dUTP-digoxigenin. Detection of hybrids was done using an antidigoxigenin antibody coupled to alkaline phosphatase following the manufacturer's indications (Roche).

Results obtained showed that the size of the amplification product obtained with the *oxa40* primers from the two *P. aeruginosa* isolates was identical to the amplicon obtained from the *oxa40* producing *A. baumanii* control strain SM28. The complete nucleotide sequence of the two PCR products showed the presence of an ORF identical to the OXA-40 carbapenem-hydrolyzing β-lactamase (Genbank accession AF509241) [2].

To further analyze the location of the *oxa40* gene in the genomes of the two *P. aeruginosa* isolates, total DNA was isolated from *P. aeruginosa* P15 and P23 and also from *A. baumanii* SM28, the control strain for the *oxa40* gene. Total DNA digested with endonucleases, was separated in agarose gels and hybridized with the *oxa40*-derived probe. The results obtained demonstrated the presence of the *oxa40* gene in the two *Pseudomonas* isolates, and furthermore indicated that this gene was probably located in a plasmid rather than in the chromosome of the three strains analyzed.

Plasmid DNA was purified with a commercial kit and separated in agarose gels. Several plasmids were simultaneously present in each strain. With the use of the appropriate plasmid size standards and assuming that the observed bands corresponded to circular covalently closed plasmid DNA, we could establish the presence of four plasmids of 112, 82, 32 and 8 kb approximately (Fig. 1). The three strains analyzed showed the same plasmids with the same electrophoretic mobility.

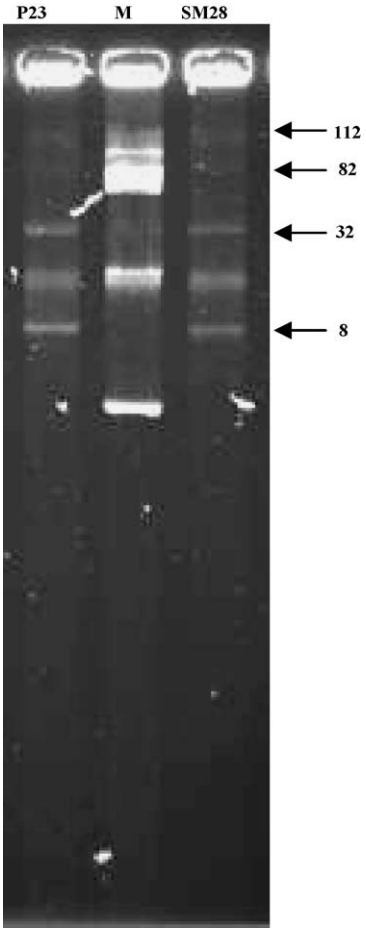

Fig. 1. Plasmid content of *P. aeruginosa* P23 and *A. baumannii* SM28 isolates. M: control strain *E. coli* NCTC 59192.

Purified plasmid DNA was also analyzed by Southern hybridization with the *oxa40* probe. Several hybridization bands could be observed in each strain; however, the detailed analysis of the size and intensity of the hybridization bands in at least six independent experiments led us to the conclusion that probably the 32 kb plasmid contained the *oxa40* gene, and that the accompanying hybridization bands were due to different forms of the same plasmid DNA molecule.

Previous reports had located the *oxa40* gene from *A. baumannii* only in the chromosome [5], but our results showed that the *oxa40* gene could also be plasmid located. This finding is also supported by a recent study carried out in the United States that reported the presence of this gene in both locations [3]. Sequencing experiments of the genetical environment of the *oxa40* gene do not associate it to any transposon or insertion sequence. In addition, the sequence is the same of the one included in the Genbank (accession AY228470) belonging to the parcial sequence of 4162 bp belonging to a plasmid replicon (pAB02) of *A. baumannii*.

All attempts to transfer the 32 kb plasmid from the *oxa40* containing isolates to recipient strains failed. These results were not a surprise, since they are in coincidence with the general failure of this kind of conjugation experiments on *A. baumannii* and *P. aeruginosa* found by other authors [5]. For this reason, we tried to prove indirectly that the plasmids carrying the *oxa40*

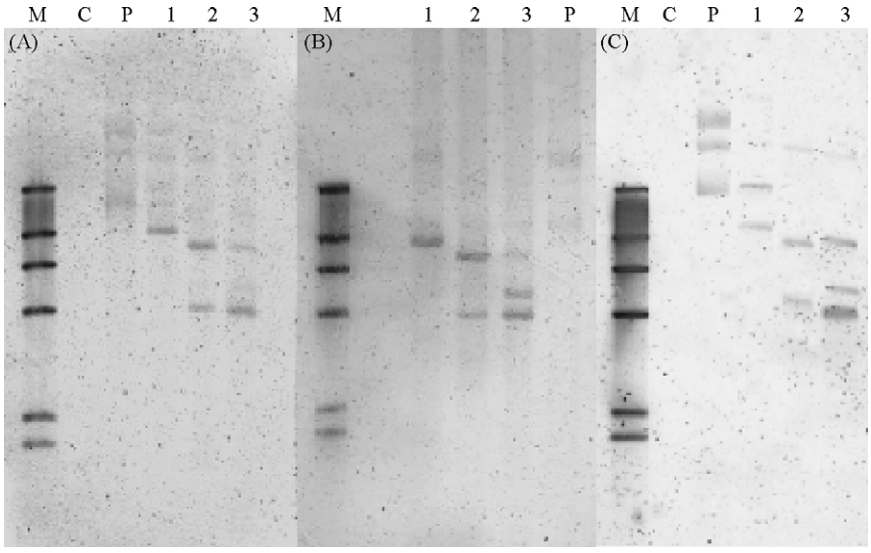

Fig. 2. Plasmid DNA digestion hibridized with the OXA-40 probe. M: DNA molecular-weight marker II DIG-labeled (Roche); C: control strain *E. coli* NCTC 59192; P: plasmid DNA; 1: plasmid DNA digested with PstI; 2: plasmid DNA digested with HindIII and 3: plasmid DNA digested with EcoRI. A: *P. aeruginosa* P23; B: *A. baumannii* SM28; C: *P. aeruginosa* P15.

gene were identical in the two *Pseudomonas* and in the *Acinetobacter* isolates.

For that purpose, we assayed to digest our plasmid preparations with restriction endonucleases and analyzed the products by gel electrophoresis and Southern hybridization with the *oxa40* probe. The band patterns obtained after digestion and the following hybridization were the same in the three isolates analyzed (Fig. 2). This provided a reasonable fingerprint that strongly suggested that the 32 kb plasmid containing the *oxa40* gene was identical in the three examined strains. This hypothesis was also supported by the *oxa40* gene sequence that was exactly the same either in *A. baumanii* or *P. aeruginosa* strains.

To our knowledge, this is the first report of an OXA-40 carbapenemase in *P. aeruginosa* located in a plasmid also found in *A. baumannii*.

Although more experiments are needed to detect the plasmid transfer between both species, this could be of capital importance, since it may represent a mechanism for the spreading of resistance to carbapenems among nosocomial Gram negative bacteria, and could become a serious problem in the hospital environment.

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