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Co-occurrence of bla_{NDM-1} with bla_{OXA-23} or bla_{OXA-58} in clinical multidrug-resistant *Acinetobacter baumannii* isolates in Algeria

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ABSTRACT

The aim of this study was to characterise the mechanisms of carbapenem resistance in *Acinetobacter baumannii* strains isolated in an Algerian hospital. A total of 43 imipenem-resistant *A. baumannii* clinical isolates collected between 2010 and 2013 were identified using API 20NE and were confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Antibiotic susceptibility testing was performed by the disk diffusion and Etest methods. Carbapenemase activity was detected using microbiological tests and PCR. Genetic transfer of the bla_{NDM-1} gene was performed by conjugation using sodium azide-resistant *Escherichia coli* J53 as recipient strain. Clonal relationships were studied by multilocus sequence typing (MLST) using partial sequences of the *csuE* and bla_{OXA-51} genes. All 43 *A. baumannii* isolates were resistant to imipenem with high minimum inhibitory concentrations (MICs) (>32 μg/mL). The strains harboured bla_{OXA-23}, bla_{NDM-1}, bla_{OXA-58} and/or bla_{OXA-24} genes. Co-existence of bla_{NDM-1} and bla_{OXA-23} or bla_{OXA-58} was detected in two isolates and one isolate, respectively. NDM-1 plasmid transfer to *E. coli* J53 was successful only for one of the three strains harbouring both bla_{NDM-1} and bla_{OXA-23} or bla_{OXA-58}. The phylogenetic tree obtained from concatenation of the partial sequences of *csuE* and bla_{OXA-51} showed that there was no genetic relationship between the isolates and the bla_{NDM-1} resistance gene. Here we report for the first time the co-occurrence of bla_{NDM-1} along with bla_{OXA-23} or bla_{OXA-58} in recent clinical isolates of *A. baumannii* from Northeast Algeria. These findings re-emphasise the dissemination and rapid spread of bla_{NDM-1} carbapenemase genes in multidrug-resistant clinical *A. baumannii* isolates in Algeria.

1. Introduction

Acinetobacter baumannii is an important opportunistic and emerging pathogen causing nosocomial infections worldwide [1]. Infections caused by *A. baumannii* are associated with adverse clinical outcomes, including high rates of morbidity and mortality, prolonged hospital stay and substantial healthcare expenses [2]. *Acinetobacter baumannii* exhibits a remarkable ability to rapidly develop antibiotic resistance, leading to multidrug resistance [1]. To date, some strains of *A. baumannii* have become resistant to almost all currently available antibacterial agents [3], including colistin [1]. Carbapenems are the first choice in the treatment of severe *A. baumannii* infections [4].

In Europe, carbapenem resistance rates are lower in France, Germany and Sweden (10–20%, 8% and 4%, respectively) compared with those observed in Turkey (50–80%), Greece (85%), Italy (60%) and Spain (45%) [4]. However, in recent years in Algeria there has been a high emergence of imipenem-resistant *A. baumannii* strains [5–8].

Acinetobacter baumannii may develop resistance to carbapenems through plasmid-mediated acquisition of carbapenem-hydrolysing class A, D and B metallo-β-lactamase enzymes [9]. Many reports on the emergence of New Delhi metallo-β-lactamase 1 (NDM-1) among *A. baumannii* have been described throughout the world [3], including Algeria [7,8,10–12].

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NDM-1 is a metallo β-lactamase conferring resistance to almost all β-lactam antibiotics, including carbapenems [3]. The bla_{NDM-1} gene can have a chromosomal [13] or plasmid [3,14] location, as described for other NDM-producing *A. baumannii* isolates. bla_{NDM-1} can be located in co-existence with bla_{OXA-23} [15–17].

The aim of this study was to determine the molecular support of imipenem resistance in *A. baumannii* clinical strains isolated between January 2010 and May 2013 in Algeria. Here we report the first coexistence of NDM-1 in OXA-58 carbapenemase-producing *A. baumannii* in a hospital in Algeria.

2. Materials and methods

2.1. Bacterial strains and antibiotic susceptibility testing

A total of 43 *A. baumannii* clinical isolates were collected from January 2010 to May 2013 in the University Hospital of Annaba (Annaba, Algeria) from bronchial aspirate, pus, blood, urine and wound samples. Strain identification was performed using an API 20NE system (bioMérieux, Marcy-l'Étoile, France) and was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using the Biotyper database and a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). A correct identification at the species level was defined as a MALDI-TOF score >1.9 as previously described [18].

Antibiotic susceptibility testing was performed on Mueller–Hinton agar (bioMérieux, Craponne, France) by the standard disk diffusion procedure according to the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (http://www.sfm-microbiologie.org). A total of 14 antibiotics were tested including ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, aztreonam, gentamicin, tobramycin, ciprofloxacin, colistin, amikacin and tigecycline. Isolates were considered resistant to imipenem if the diameter of the zone of inhibition was <17 mm. For isolates with an inhibition diameter <17 mm, minimum inhibitory concentrations (MIC_S) for imipenem were determined by Etest (AB BIODISK, Solna, Sweden) and resistant strains were defined as isolates with an imipenem MIC > 8 μg/mL (CA-SFM). MIC_S for tigecycline, colistin and meropenem were evaluated for the three isolates that harboured both bla_{NDM-1} and bla_{OXA-23} or bla_{OXA-23} (A4183, A41 and A696).

2.2. PCR amplification and sequencing

Real-time PCR was performed to screen for the presence of $bla_{OXA-51-like}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$ and bla_{NDM-1} genes, and conventional PCR was performed for bla_{IMP}, bla_{SIM} and bla_{VIM} genes as previously described [2]. Positive PCR products for any carbapenemase-encoding gene tested were sequenced using BigDye® terminator chemistry on an automated ABI 3130 sequencer (PE Applied Biosystems, Foster City, CA). The sequences of the genes obtained were analysed using BlastN and BlastP against the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/blast) for identification.

2.3. Conjugation experiments

To determine the transferability of carbapenem resistance, a conjugation experiment was performed using *Escherichia coli* J53 (azide-resistant) as the recipient strain as previously described [19]. Transconjugants were selected on tryptic soy agar plates (Becton, Dickinson & Co., France) containing 100 μg/mL sodium azide (LABOSI, Paris, France) for counterselection and 0.5 μg/mL imipenem (Tienam® 500; Merck Sharp & Dohme, France) for selection of plasmid-mediated carbapenem resistance. Following incubation, the culture of three strains harbouring bla_{NDM-1}, bla_{OXA-23} and bla_{OXA-58} [two strains harbouring both bla_{NDM-1} and bla_{OXA-23} (A41 and A4183) and one strain harbouring both bla_{NDM-1} and bla_{OXA-58} (A696) (Table 1)] was characterised using MALDI-TOF/MS. PCRs for bla_{NDM-1}, bla_{OXA-23} and bla_{OXA-58} from transconjugant strains were performed using the primers described previously [2]. Susceptibly to the above mentioned antibiotics as well as the MIC of imipenem were determined for the donor isolate (A4183), recipients and transconjugants (Table 2). Disk diffusion susceptibility testing and the MICs of antibiotics for donors, transconjugants and recipients were measured in accordance with CA-SFM guidelines.

2.4. Multilocus sequence typing (MLST)

The phylogenetic tree inferred from the concatenation of partial sequences of *csuE* and bla_{OXA-51} genes for all 43 *A. baumannii* isolates was sequenced and compared with all identified variants as previously described [18].

Molecular typing of the three isolates (A41, A4183 and A696) was determined by full MLST using the seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB*) as described on the MLST website of the Institut Pasteur (http://www.pasteur.fr/mlst).

3. Results

3.1. Bacterial strains and antibiotic susceptibility testing

From January 2010 to May 2013, 43 clinical isolates, identified as *A. baumannii* both by the API20 NE identification system and MALDI-TOF/MS, were recovered from 43 patients (24 adult males, 12 adult females and 7 children) hospitalised in the University Hospital of Annaba and isolated mostly from medical intensive care units (58.1%).

Among the 43 isolates, 22 (51.2%) were recovered from bronchial aspirate, 7 (16.3%) from urine, 7 (16.3%) from blood, 6 (14.0%) from pus and 1 (2.3%) from wound (Table 1).

A minimum spanning dendrogram generated using the Biotyper 3.0 program demonstrated that isolates were significantly separated into three clusters; six of the seven isolates harbouring the bla_{NDM-1} gene were included in the same cluster, however one isolate (A696) carrying bla_{NDM-1} and bla_{OXA-58} genes was in another cluster (Fig. 1).

The results of antibiotic susceptibility testing for the 43 *A. baumannii* isolates revealed a high prevalence of resistance to ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, aztreonam, ceftazidime and ciprofloxacin (100%), however 41.9% of the strains were resistant to amikacin and 51.2% to gentamicin. In addition, all 43 strains were resistant to imipenem (MICs > 32 μg/mL) and cefepime, but no strain was resistant to colistin and tigecycline.

The MICs evaluated for the three isolates harbouring both bla_{NDM-1} and bla_{OXA-23} or bla_{OXA-58} showed that they were not resistant to tigecycline (MIC = 0.5 μg/mL for strains A41 and A4183 and MIC = 0.75 μg/mL for strain A696) or colistin (MIC = 0.94, 0.19 and 0.25 μg/mL for strains A41, A4183 and A696, respectively), however the three strains presented a high level of resistance to meropenem (MIC > 8 μg/mL).

3.2. Molecular detection of carbapenemase-encoding genes

Standard PCR results for carbapenemase-encoding genes showed that all of the isolates were positive for the bla_{OXA-51} gene. Among them, 28 contained bla_{OXA-23}, 7 contained bla_{NDM-1}, 6 contained bla_{OXA-58} and only 1 contained bla_{OXA-24}. It is noteworthy that among these isolates, two harboured both bla_{OXA-23} and bla_{NDM-1}