Detection of OXA-231, a new variant of blaOXA-143, in Acinetobacter baumannii from Brazil: a case report

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Sir,

In Brazil, the resistance rates to carbapenems range from 25% to 45% among Acinetobacter baumannii isolates.1 The mechanisms of carbapenem resistance among these isolates are mainly associated with the production of two major carbapenem-hydrolysing class D carbapenemases (CHDLs), OXA-23 and OXA-143.2,3 To date, OXA-143 is the single representative of this recently reported subgroup of CHDLs. In contrast to the OXA-23 subgroup, which is widely disseminated in the Brazilian territory, OXA-143 has only been detected in A. baumannii isolated from hospitals located in São Paulo and Rio de Janeiro states so far.4–5 Analysis of the genetic environment of blaOXA-143 revealed that it was bracketed by two copies of the same replicase gene. This suggested that a homologous recombination process probably took place in the blaOXA-143 acquisition.6 This study reports the occurrence of OXA-231, a novel variant of OXA-143, in an A. baumannii clinical isolate (strain Ac-141) recovered from a tertiary teaching hospital located in southern Brazil.

In late 2007, a woman in her early seventies was admitted to the emergency room of the Hospital Universitário de Londrina, a teaching hospital located in the city of Londrina, Paraná state, Brazil. The patient presented with a history of diabetes and hypertension and had previously been submitted to a transtibial amputation due an infected diabetic foot, at the Hospital Universitário de Londrina, 25 days previously. On the day of admission, the patient presented with fever and dyspnea, and was diagnosed with pneumonia. Empirical therapy with cefepime [2 g, intravenously (iv), twice daily] and vancomycin (1.5 g, iv, once a day) was started. Five days after hospitalization, the patient became febrile and showed signs and symptoms of urinary tract infection. An extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae susceptible only to imipenem and amikacin was isolated from urine. Therapy with imipenem (500 mg, iv, four times a day) was initiated. Six days after imipenem administration, the patient remained febrile and developed signs of sepsis. A carbapenem-resistant A. baumannii isolate (strain Ac-141) was recovered from the urine culture, and antimicrobial therapy was replaced by polymyxin B (500,000 IU, iv, three times a day) that was continued for 10 days. The patient showed clinical improvement and was discharged after 15 days of hospitalization. Further urinary cultures were negative, indicating that microbiological cure was probably achieved.

The Ac-141 strain was initially identified as Acinetobacter calcoaceticus-baumannii complex using a MicroScan WalkAway automated system (Siemens Healthcare Diagnostic). Sequencing analysis of partial regions of the RNA polymerase β subunit gene (rpoB) (zone 1 (350 bp, between positions 2900 and 3250) and zone 2 (450 bp, between positions 3250 and 3700)), as previously published,7 confirmed the identification at species level of the Ac-141 strain as A. baumannii. The obtained partial sequence of the rpoB gene was deposited in GenBank under accession number JX014310. The MICS of ceftazidime, imipenem, meropenem, ampicillin/sulbactam and polymyxin B were confirmed by the agar dilution method, according to the recommendations of the CLSI.8 According to susceptibility tests results, the Ac-141 strain was resistant to ampicillin/sulbactam (MIC >128/64 mg/L), ceftazidime (MIC >128 mg/L), cefepime (MIC >16 mg/L), imipenem (MIC 64 mg/L), meropenem (MIC 64 mg/L), trimethoprim/sulfamethoxazole (MIC >2/38 mg/L), amikacin (MIC >4 mg/L), ciprofloxacin (MIC >4 mg/L), levofloxacin (MIC >4 mg/L) and gentamicin (MIC >8 mg/L), but susceptible to polymyxin B (MIC 1 mg/L). The Ac-141 strain also showed a low tigecycline MIC (1 mg/L).

PCRs targeting genes encoding CHDLs, metallo-β-lactamas and KPC were carried out as previously described.8,9 The PCR results showed that the Ac-141 strain carried the blaOXA-51 and blaOXA-143 genes. A subsequent PCR targeting both blaOXA-51 and the insertion sequence ISAbA1 yielded a positive result. The ISAbA1 upstream of the blaOXA-51 is associated with the over-production of this CHDL and contributes to the increased MICs of carbapenems for A. baumannii, which may result in a high level of carbapenem resistance when other mechanisms are also present. DNA sequencing of the entire sequence of the...
blaOXA-143-like gene, using the primer pair previously published, identified a single mutation at nucleotide 671 that led to a unique amino acid substitution of an aspartic acid by alanine at position 230, according to the DBL numbering system. This new OXA-143 variant was named OXA-231, and its nucleotide sequence has been deposited in GenBank under accession number JQ676953. Interestingly, the Asp230Ala mutation was near the conserved KSG motif (positions 216 to 218) of the active site of the OXA-231 and adjacent to the key residue methionine that is structurally very important for conferring the biochemical properties of OXA-24/40, a closely related CHDL. (88% identity with OXA-143). An in vitro study conducted by Santillana et al. showed that mutation of this residue caused a reduction in the catalytic efficiency of OXA-24/40 against the carbapenems and noticeably increased the specificity for oxacillin. Thus, it is difficult to predict the effect of the substitution Asp230Ala on the structure of OXA-231 and, consequently, on its biochemical properties.

A plasmid extract of the Ac-141 strain was obtained by the method of Kieser, followed by electrophoresis and subsequent Southern blot and hybridization with a blaOXA-231-specific probe using the DIG DNA Labeling and Detection Kit (Roche Diagnostics GmbH, Penzberg, Germany). The results showed that the blaOXA-231 gene was located on a plasmid of ~140 kb. Although it has not been possible to sequence the entire genetic environment of the blaOXA-231 gene yet, the partial flanking sequences obtained up to now (data not show) are similar to those described for the blaOXA-143 gene.

In our hospital, 75% of the A. baumannii isolates are resistant to carbapenems. The production of OXA-23 has been the main mechanism of carbapenem resistance found in these isolates. For instance, from August 2006 to August 2011, blaOXA-23 was detected in 68% of A. baumannii isolates that belonged to multiple clones (80% Gionco, F. E. Carrara-Marroni, E. J. Venancio, E. C. B. Togni and J. S. Pelayo, unpublished data). To our knowledge, this is the first description of an OXA-143 cluster in an A. baumannii strain isolated in our hospital and in southern Brazil, and, coincidently, it is a novel variant of this cluster. Curiously, no additional OXA-143 or OXA-231 variant has been detected among the carbapenem-resistant A. baumannii isolates (n=125) during a 5-year period of study in our hospital.

The detection of OXA-231 in Brazil is a cause of great concern and shows the potential of these new CHDLs to spread to other Brazilian regions. Although only a single case involving OXA-231 was reported, continuous surveillance studies are of paramount importance to prevent its further dissemination. Lastly, we would like to emphasize the clinical and microbiological efficacy of polymyxin B in eradicating the infection caused by the Ac-141 strain. The clinical success was achieved probably because the urinary tract was the source of infection. A worse outcome might have resulted if the respiratory tract was the source of infection. In this manner, novel therapeutic agents are extremely necessary for the treatment of multidrug-resistant Gram-negative bacilli.

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