High Prevalence of blaNDM-1 Carbapenemase-Encoding Gene and 16S rRNA armA Methyltransferase Gene among Acinetobacter baumannii Clinical Isolates in Egypt

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The main objective of this study was to decipher the molecular mechanism of resistance to carbapenems and aminoglycosides in a large series of 150 Acinetobacter baumannii clinical isolates collected from July 2012 to September 2013 in Egypt. We report for the first time the emergence of blaNDM-1 and the cooccurrence of 16S rRNA methylase armA with blaNDM-1 and blaOXA-23 in Egyptian hospitals. Multilocus sequence typing identified 27 distinct sequence types, 11 of which were novel.

Carbapenem and aminoglycoside resistance in Acinetobacter baumannii is considered an emerging, serious public health problem (1–3). The aim of the present study was to decipher the molecular support of resistance to carbapenems and aminoglycosides among 150 nonrepetitive A. baumannii clinical isolates collected from different clinical samples from inpatients at Mabaret-El-Alasafra hospital (Alexandria, Egypt), El-Demerdash hospital (Cairo, Egypt), and the National Cancer Institute (Cairo, Egypt) from July 2012 to September 2013. Identification was performed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (4) and confirmed via PCR amplification and sequencing of the intrinsic blaOXA-51-like gene (5). Antimicrobial susceptibility was determined using a modified Kirby-Bauer disk diffusion method. MICs were determined using Vitek 2 and confirmed for imipenem and amikacin by using Etest strips. Carbapenemase activity was detected phenotypically using the modified Hodge test (MHT) (6) and the modified Carba NP test (7) and confirmed by MALDI-TOF Ultraflex I mass spectrometry (8), while metallo-β-lactamase activity was detected using the imipenem-EDTA combined-disk test (IECT) (9). All primers and probes used in this study for PCR amplification and sequencing are given in Table S1 in the supplemental material. Genetic mapping of carbapenemases (blaNDM-1 and blaOXA-23-like), aminoglycoside-modifying enzyme (AME)-encoding resistance genes, and armA was performed for 20 selected isolates. Genotyping was done by multilocus sequence typing (MLST) (10). All isolates revealed a high degree of multidrug resistance (MDR) as shown in Fig. S1 in the supplemental material. Interestingly, 30/150 (20%) isolates were falsely identified as amikacin susceptible by Vitek 2, while they were identified as resistant by Etest (Fig. S1) as already reported (11). Overall, 131 out of 150 isolates (87.3%) were resistant to imipenem, with MICs of >16 μg/mL. Among them, 125 isolates (95.4%) were MHT positive, and 59/150 isolates (39.3%) were IECT positive. In Egypt, there have been few reports describing the emergence of the blaOXA-23 gene among A. baumannii isolates (12–14) and one report of an NDM-2 carbapenemase isolate from a patient transferred to Germany from an Egyptian hospital (15). Herein, we found that 115/150 (76.7%) isolates were blaOXA-23-like positive and 150/150 (100%) were blaOXA51-like positive. The blaNDM-1 gene was not previously identified as playing a major role in carbapenem resistance among A. baumannii clinical isolates in Egypt (12). Here, we report for the first time a high prevalence rate of blaNDM-1 among A. baumannii clinical isolates in Egypt, with 59/150 (39.3%) isolates being blaNDM-1 positive. The cooccurrence of blaNDM-1 with blaOXA-23-like genes was found in 53/150 (35.3%) of the isolates. All the MHT-negative isolates (25/150) were found to be negative for the tested carbapenemase-encoding genes. It is well known that blaOXA-23 and blaOXA-51 are regulated in part by ISAba1, which may be located upstream of these genes acting as a promoter and resulting in the overexpression of these genes and in a high level of carbapenem resistance (16, 17). In our study ISAbA1 was found upstream of the blaOXA-23-like and blaOXA-51-like genes in 91.3% (137/150) and 4.7% (7/150) of our isolates, respectively. Conversely, ISAbA1 was found downstream of blaOXA-23- and blaOXA-51-like genes in 4.7% (7/150) and 27.3% (41/150) of our isolates, respectively. To our knowledge, there are no articles reporting the presence of AMEs or armA in Egypt. Here, we report high percentages of aminoglycoside-encoding resistance genes as follows: aac(6)δ (69.3%, 104/150), aac(3) (21.3%, 32/150), aphA6 (80%, 120/150), adaA1 (38.7%, 58/150), adaA2 (8.7%, 13/150), adaA3 (3.3%, 5/150), adaA15 (1.3%, 2/150), adaA21 (0.7%, 1/150), adaA22 (0.7%, 1/150), ant2 (8.7%, 13/150), and armA (94%, 141/150). The cooccurrence of armA, blaOXA-23, and blaNDM-1 has been described in India (18). Here, we report for the first time...
<table>
<thead>
<tr>
<th>Phenotypic resistance</th>
<th>Genotypic resistance</th>
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<td>Class A</td>
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<tr>
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<td>aac(6)-Ib, aac(3)-IIa</td>
<td>Class B</td>
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<td>aac(3)-Ia, aac(6)-Ia, aac(6)-Ib</td>
<td>Class C</td>
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**Table 1. Prevalence of phenotypic and genotypic resistance among 150 MDR A. baumannii isolates from different Egyptian hospitals.**
the cooccurrence of armA with blaOXA-23 and blaNDM-1 among A. baumannii isolates in Egypt. Indeed, armA with blaOXA-23 was found in 114/150 (76%) isolates, armA with blaNDM-1 was found in 59/150 (39.3%) isolates, and armA with blaOXA-23 and blaNDM-1 was found in 52/150 (34.7%) of our isolates. Also, the cooccurrence of blaNDM-1 with aphA6 was found in 59/150 (39.3%) isolates (Table 1). Following plasmid extraction, 10/20 selected isolates were found to harbor 1 to 5 plasmids (25 plasmids analyzed) with sizes ranging from 3 to >10 kb. We found that blaNDM-1, blaOXA-23, and armA were collocated on plasmids in 8/25 (32%) extracted isolates. This is in accordance with previous reports showing that blaOXA-23, blaNDM-1, and armA genes in A. baumannii are mostly located on plasmids (16, 19, 20). Similarly, AME-encoding genes [ant(2), aad, aac(6), aph, and aac(3)] were found to be plasmidic. MLST performed on imipenem-resistant isolates allowed us to identify 27 different sequence types (STs) (Fig. 1), 11 of which were novel and were deposited in the MLST database of the Pasteur Institute (ST602, ST603, ST604, ST605, ST613, ST614, ST615, ST616, ST617, ST618, and ST619). It was found that the ST2 clonal group predominated (41.2%, 54/131) among other ST clonal groups. This clone was found circulating in the 3 hospitals and was associated with the production of blaOXA-23 (92.6%, 50/54), blaNDM-1 (44.4%, 24/54), and armA (100%, 54/54). Moreover, we found an outbreak of isolates from the ST2 clonal group circulating in Mabaret-El-Alasafra hospital. These results are similar to those reported in Italy, where carbapenem resistance was mostly driven by A. baumannii isolates belonging to the ST2 clonal group (21, 22). Our results revealed that different lineages are circulating among Egyptian hospitals. Finally, our study revealed the emergence and spread of blaNDM-1 and blaOXA-23 in addition to the cooccurrence of 16S rRNA methylase armA with blaNDM-1 and blaOXA-23 in Egypt among A. baumannii clinical isolates. This represents a serious public health problem capable of limiting future therapeutic options. Active surveillance is warranted in Egypt to implement strict control measures to prevent further spread of resistance.

(Preliminary results from this study were presented at the 24th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Barcelona, Spain, 10 to 13 May 2014. [23].)

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We declare no conflicts of interest.

REFERENCES


Emergence of NDM-1-Producing A. baumannii in Egypt


