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Spread of imipenem-resistant *Acinetobacter baumannii* co-expressing OXA-23 and GES-11 carbapenemases in Lebanon

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SUMMARY

Objectives: The acquisition of carbapenemases by *Acinetobacter baumannii* is reported increasingly worldwide, but data from Lebanon are limited. The aims of this study were to evaluate the prevalence of imipenem-resistant *A. baumannii* in Lebanon, identify resistance determinants, and detect clonal relatedness.

Methods: Imipenem-resistant *A. baumannii* were collected from nine Lebanese hospitals during 2012. Antimicrobial susceptibility, the cloxacillin effect, and ethylenediaminetetraacetic acid (EDTA) synergy were determined. Genes encoding carbapenemases and insertion sequence IS*Aba1* were screened via PCR sequencing. IS*Aba1* position relative to genes encoding Acinetobacter-derived cephalosporinases (ADCs) and OXA-23 was studied by PCR mapping. Clonal linkage was examined by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR).

Results: Out of 724 *A. baumannii* isolated in 2012, 638 (88%) were imipenem-resistant. Of these, 142 were analyzed. Clavulanic acid–imipenem synergy suggested carbapenem-hydrolyzing extended-spectrum β-lactamase. A positive cloxacillin test indicated ADCs, while EDTA detection strips were negative. Genotyping indicated that 90% of isolates co-harbored $bla_{\text{OXA-23}}$ and $bla_{\text{GES-11}}$. The remaining strains had $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{GES-11}}$, or $bla_{\text{OXA-24}}$ with $bla_{\text{GES-11}}$. IS*Aba1* was located upstream of bla_{ADC} and $bla_{\text{OXA-23}}$ in 97% and 100% of isolates, respectively. ERIC-PCR fingerprinting revealed 18 pulsotypes spread via horizontal gene transfer and clonal dissemination.

Conclusion: This survey established baseline evidence of OXA-23 and GES-11-producing *A. baumannii* in Lebanon, indicating the need for further surveillance.

1. Introduction

Acinetobacter baumannii is a Gram-negative, non-fermenting opportunistic pathogen that is frequently associated with nosocomial epidemics in intensive care and burn therapy units, where it causes septicemia, pneumonia, and urinary tract infections with high mortality. The treatment of infections due to *A. baumannii* is a challenge because of resistance to the antimicrobial agents of last resort, β-lactams.[1] Resistance to this class of antibiotics in *A. baumannii* results mainly from the production of β-lactamases, and also from other non-enzymatic pathways, like changes in outer membrane proteins or over-expression of efflux pumps.[1,2] All A, B, C,

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and D Ambler classes of β-lactamase have been described in *A. baumannii*: (1) class A extended-spectrum β-lactamases (ESBLs), (2) class B metallo-β-lactamases, (3) class C Acinetobacter-derived cephalosporinases (ADCs), and (4) carbapenem-hydrolyzing class D β-lactamases (CHDLs).

Resistance to extended-spectrum cephalosporins is mainly attributed to the over-production of chromosomal ADCs or, less frequently, to the acquisition of an ESBL (PER, VEB, GES).[3–6] Resistance to carbapenems is mainly due to transferable class D oxacillinases of subfamilies OXA-23, OXA-24/33/40, OXA-58, and OXA-143, or to over-production of the intrinsic OXA-51-like enzymes.[7] Some carbapenem-resistant *A. baumannii* expressing class B enzymes (IMP, VIM, SIM, SPM, GIM, and NDM) and, more recently, acquired GES and KPC carbapenemases of class A, have been reported.[8–10] Among the GES carbapenemases, GES-11 was the first described conferring reduced susceptibility to carbapenems and initially detected in *A. baumannii*.[8] The presence of insertion sequence IS*Aba1* upstream from $bla_{\text{OXA-23}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-51}}$, and bla_{ADC} provides an efficient promoter for the expression of these genes, leading to higher β-lactam hydrolysis rates.[11–14]

While carbapenem resistance due to the production of CHDLs is the most widespread in *A. baumannii*, Ambler class B enzymes have been associated with carbapenem resistance in *A. baumannii* in several countries, including Italy, Portugal, Japan, and Brazil.[2,15] In addition, *A. baumannii* acquiring GES carbapenemases have been described in France,[8] Belgium,[16] and Turkey.[4] In Lebanon, in 2008, Zarrilli et al. reported a plasmid-borne $bla_{\text{OXA-58}}$ gene in *A. baumannii* strains selected in a Lebanese hospital.[17] Lately, in 2012, NDM-1-harboring *A. baumannii* strains were isolated in another Lebanese hospital from Syrian patients injured during the civil war.[18] However, no countrywide epidemiological survey has addressed the prevalence of carbapenem-resistant *A. baumannii* in Lebanon until now.

The aims of the current study were to evaluate the occurrence of carbapenem-resistant *A. baumannii* in nine Lebanese medical centers, and to describe the types of β-lactamases involved in such resistance, as well as to investigate clonal relatedness of incriminated strains.

2. Materials and methods

2.1. Bacterial strains

From January 1 to December 31, 2012, imipenem-resistant isolates of *A. baumannii* were collected from nine Lebanese hospitals located in diverse geographic areas: Beirut (Hotel Dieu de France, Saint-George Hospital), Mount Lebanon (Bellevue Medical Center), North Lebanon (Mounla Hospital), South Lebanon (Secours Populaire Libanais Hospital, Labib Medical Center), and Bekaa (Bekaa Hospital, Farhat Hospital, Chtaura Hospital). Selection criteria for strains were based upon the recommendations of the Clinical and Laboratory and Standards Institute (CLSI);[19] strains with an inhibition zone diameter of imipenem $\leq$13 mm were included and these were delivered to the central microbiology laboratory at the Faculty of Pharmacy, Saint-Joseph University, Beirut. The isolates were re-identified using the API 20 NE system (BioMérieux, Marcy l'Etoile, France) and confirmed using PCR to detect the intrinsic $bla_{\text{OXA-51-like}}$ gene.[20]

2.2. In vitro susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion on Mueller–Hinton agar using amoxicillin/clavulanic acid (AUG), ceftazidime (CAZ), cefotaxime (CTX), cefepime (CPM), and imipenem (IMI) disks (Mast Diagnostics, Merseyside, UK), in accordance with the recommendations of the CLSI.[19]

2.3. Cloxacillin test and metallo-β-lactamase detection

Antibiotic susceptibility testing was also performed using Mueller–Hinton agar with cloxacillin (200 mg/l) to inhibit intrinsic ADCs. An increase in inhibition zone diameters of cephalosporins in the presence of cloxacillin was a qualitative indicator of ADC hyper-production. Also, the cloxacillin test was used to allow better detection of synergy between clavulanic acid and imipenem, indicating the production of an ESBL with carbapenem-hydrolyzing activity. Metallo-β-lactamases were screened for using the combined imipenem/imipenem+EDTA Etest (Liofilchem, Roseto degli Abruzzi, Italy). A ratio of the minimum inhibitory concentration of imipenem compared to that of imipenem in the presence of EDTA ($\text{MIC}_{\text{imipenem}}/\text{MIC}_{\text{imipenem+EDTA}}$) of $\geq$8 was considered a presumptive diagnosis of metallo-β-lactamase.[21]

2.4. Identification of carbapenem resistance genes

PCR sequencing experiments were used to detect carbapenemase genes $bla_{\text{oxa-23}}$, $bla_{\text{oxa-24}}$, $bla_{\text{oxa-40}}$, $bla_{\text{oxa-58}}$, bla_{KPC}, bla_{GES}, $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, bla_{NDM}, and bla_{VIM}.[22] Isolates were also screened for insertion sequence IS*Aba1*.[14] PCR mapping using IS*Aba1* forward and OXA-23 reverse primers was done on $bla_{\text{oxa-23}}$-positive isolates. Also, IS*Aba1* forward and bla_{ADC} reverse primers were used to locate the position of IS*Aba1* relative to bla_{ADC}.

2.5. ERIC-PCR

The genetic relationship between the isolates was determined using enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), as described by Ferreira et al.[23] The amplification conditions were as follows: an initial denaturation cycle at 95 °C for 7 min, 35 cycles at 95 °C for 1 min, 51 °C for 1 min, and 72 °C for 1 min and 30 s, and a final extension at 72 °C for 15 min. The amplified products were visualized on 1.5% agarose gel stained with 0.5 μg/ml of ethidium bromide. The banding patterns were converted by GelQuest/Sequentix software (Klein Raden, Germany) into a binary matrix, calculated using the Dice coefficient. A dendrogram was constructed via the unweighted pair-group method using arithmetic averages (UPGMAs). Only visible bands in the ERIC-PCR fingerprinting were used to construct the similarity matrix and the dendrogram. Isolates with more than 85% similarity were considered to be clonally related.

3. Results

Out of 723 *A. baumannii* isolated in 2012, 638 (88%) were imipenem-resistant. Of these, 142 non-duplicate imipenem-resistant *A. baumannii* isolates were received by the laboratory for analysis. These isolates were distributed as follows: 63 were from Saint-Georges Hospital, 36 from Labib Medical Center, 22 from Hotel Dieu de France, five from Bellevue Medical Center, five from Bekaa Hospital, five from Chtaura Hospital, four from Farhat Hospital, one from Mounla Hospital, and one from Secours Populaire Libanais Hospital.

3.1. Antimicrobial resistance pattern, cloxacillin effect, and EDTA synergy

All 142 isolates showed high resistance to the tested β-lactams (Table 1). Sixty-eight isolates yielded a positive cloxacillin test, indicating ADC hyper-production. Sixty-one showed synergy between clavulanic acid and imipenem, indicating the production