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Identification of particular clonal complexes and mobile elements associated with the dissemination of VIM-2-producing *Pseudomonas aeruginosa* and OXA-23-producing *Acinetobacter baumannii* in Romania

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INTRODUCTION

•*Pseudomonas aeruginosa* and *Acinetobacter baumannii* are recognized as serious opportunistic nosocomial pathogens, especially in intensive care units, being frequently associated with therapeutic failures due to their multidrug resistance (MDR) phenotypes (1).

•The VIM-, IMP- and NDM-types are the most worldwide distributed metallo-β-lactamases (MBL) in *P. aeruginosa,* being VIM-2 particularly endemic in certain European countries (2, 3, 4, 5), frequently conveyed in highly disseminated class 1 integrons and mercurial transposons.

•The OXA-23 enzyme is the most worldwide spread carbapenemase in *A. baumannii* and the bla_{OXA-23} gene has been linked to Tn*2006,* Tn*2007,* or Tn*2008* transposon variants, plasmid or chromosomally located (6).

•Romania is one of the European countries with the highest rates of MDR (including carbapenem resistant) *P. aeruginosa* and *A. baumannii* clinical isolates but detailed characterization of these strains is scarce and/or confined to isolates from certain regions of the country.

OBJECTIVES

•To characterize the population structure and molecular epidemiology of carbapenemase-producing *P. aeruginosa* and *A. baumannii* clinical isolates recovered in two main hospitals from Bucharest (capital, South Romania).

MATERIALS AND METHODS

- Eighteen carbapenemase-producing *P. aeruginosa* (n=11) and *A. baumannii* (n=7) isolates recovered from patients admitted longer than 1.5 weeks to two central hospitals (A, B) from Bucharest (January – November 2012) were selected for further characterization. Most of the patients carrying the strains investigated during this study were submitted to a major surgery (94%) and came from hospitals located in other districts of Romania (60%).
- Identification
  - ❖ commercial systems (VITEK2 and Microscan);
  - ❖ bla_{OXA-51} detection and *rpoB* sequencing (for *A. baumannii* isolates).
- Carbapenemases detection
  - ❖ Phenotypic tests (Kirby-Bauer, E-test);
  - ❖ Biochemical tests [Blue-Carba, spectrophotometric assays (7)];
  - ❖ Genotypic tests (PCR and sequencing);
- Population structure – PFGE (8), MLST (http://pubmlst.org/paeruginosa/, http://pubmlst.org/abaumannii/);
- Plasmid analysis and location of carbapenemase genes (S1-PFGE, I-CeuI-PFGE, hybridization) (9);
- Transferability of *bla* (subset of *A. baumannii* isolates): transformation using *A. baylyi* ADP1 as recipient and imipenem as selective agent (0,5/1 mg/L)
- MBL and carbapenem-hydrolysing class D β-lactamases (CHDL) genetic support (integrons, transposons): PCR mapping and sequencing.

RESULTS AND DISCUSSION

Carbapenemase-producing *P. aeruginosa* isolates

•All *P. aeruginosa* isolates were VIM-2 producers, although exhibiting different resistance phenotypes (Table 1).

•bla_{VIM-2} was associated in most cases with a class 1 integron (*aacA7-*bla_{VIM-2}) or an atypical integron configuration. The *aacA7-*bla_{VIM-2} integron has been previously detected in VIM-2-producing isolates in the North-East region of Romania during 2007-2011, suggesting countrywide spread. Additionally, a class 1 integron containing *aadB* [encoding adenylytransferases *ant(2 ")-1a*, which confers streptomycin resistance] was detected in most isolates.

•Most isolates (n=8/11) belonged to ST233 (PFGE-type A), although other sporadic clones were also detected among clinical isolates from the same hospital (n=2 ST364, PFGE-type B) and in the coproculture isolate (n=1 ST1074, PFGE-type C) (Table 1).

Table 1. VIM-2-producing *P. aeruginosa* clinical isolates from South Romania.

PFGE-type (N)	MLST	Hospital / Unit (Date)	Acquired Carbapenemase and location of carbapenemases genes	Class 1 integrons	Antibiotic resistance profile
A (8)	ST233	B / ICUV (26.01.2012 - 13.02.2012)	VIM-2 ^{CR}	*aacA7-VIM-2 + aadB*	FEP, CAZ, IMP, ETP, CIP, CTX, NET, STR, AMK, KAN, GEN, TOB
B (2)	ST364	B / ICUV (18.01.2012)	VIM-2 ^{CR}	*aacA7-VIM-2*	ATM, FEP, CAZ, ETP, CIP, CTX, NET, STR, KAN, TOB
C (1)	ST1074	B / ICUV (15.03.2012)	VIM-2 ^{CR}	-	IMP, MEM, ETP, CTX, STR, KAN

N= total of isolates; CR= chromosomal; ICUV = Vascular Intensive Care Unit

Antibiotic susceptibility profiles to different β-lactam (AMC=amoxicillin-clavulanic acid, ATM=aztreonam, FEP=cefepime, FOX=cefotaxime, CAZ=ceftazidime, CTX=ceftriaxone, IMP=imipenem, MEM=meropenem, ETP=ertapenem) and non-β-lactam (AMK=amikacin, CIP=ciprofloxacin ,GEN=gentamycin, KAN=kanamycin, NET=netilmycin, STR=streptomycin, TET=tetracycline, TOB=tobramycin, SXT=trimethoprim-sulfamethoxazole).

Carbapenemase-producing *A. baumannii* isolates

•All *A. baumannii* isolates carried the acquired carbapenemase bla_{OXA-23}, showing variable antibiotic resistance profiles (Table 2). Our results are consistent with previous reports from other regions of the country (Timisoara, Arad and Resita) and suggest that OXA-23-producing *A. baumannii* are widespread all over the country.

•The bla_{OXA-23} gene was found embedded in a Tn*2008* transposon and identified variably in either a ~40kb chromosomal band or in a 60Kb-aci6 (pACICU2-like) plasmid in the same or in different clones (Table 2). Attempts to transfer this plasmid by electro-transformation were unsuccessful (data not shown).

•43% of *A. baumannii* isolates carried a truncated Tn*aphA6* (consisting of an *aphA6* gene which confers resistance to amikacin, kanamycin and neomycin flanked by directly oriented copies of IS*Aba125*) (10), (Figure 1).

•Three clones were identified in different hospitals: ST437 (PFGE-type A, hospital B), ST764 (PFGE-type C, single-locus variant of ST437, hospital A) and ST765 (PFGE-type B, double-locus variant of ST437, Hospital A) (Table 2). All of them belong to the widespread clonal complex CC92.

•Three distinct class 1 integron structures were identified among *A. baumannii* isolates: i) *aacA4-orfF-*bla_{OXA-20} (n=3, coding for an acetyltransferase and a restricted spectrum class D β-lactamase), ii) *aacC1-orfP-orfQ-aadA1* (n=1, conferring streptomycin and amikacin resistance) and iii) *aacC1-orfA-aadA1* (n=1 conferring amikacin and streptomycin resistance).

Table 2. OXA-23-producing *A. baumannii* clinical isolates from South Romania.

PFGE-type (N)	MLST	Hospital / Unit (Date)	Acquired carbapenemase and location of carbapenemases genes	Replicase content	Class 1 integron	Gene cassette content	Antibiotic resistance profile
A (4)	ST437	B/ICUV 18,19.11.2012	OXA-23^{PL} (pACICU2-60Kb) (n=1) OXA-23^{CR} (n=3)	Aci6; p3S18; rep135040	2.7Kb 2.2Kb	*aacC1-orfP-orfQ-aadA1* *aacA4-orfF-OXA20*	MEM, IMP, ETP, CXM, TOB, FEP, AMC, CTX, KAN, AMK, SXT, NET, CIP, FOX
B (2)	ST765	A/Hematology 16.01.-15.03.2012	OXA-23^{PL} (pACICU2-60Kb)	Aci6, p3S18, rep135040	1.6 Kb	*aacC1-orfA-aadA1*	MEM, IMP, ETP, CXM, CAZ, AMC, TET, CTX, KAN, SXT, CIP, AMK
C (1)	ST764	A/Hematology 29.11.2012	OXA-23-^{CR}	Aci6, AciX , p3S18, rep135040	1.6 Kb	*aacA4-orfF-OXA-20*	MEM, IMP, ETP, CXM, TOB, FEP, AMC, CTX, KAN, AMK, SXT, NET, CIP

N – Number of isolates. Hematology= hospital A, ICUvascular=hospital B; location of carbapenemases genes CR= chromosomal, PL= plasmidic;

Antibiotic susceptibility profiles to different β-lactam (AMC=amoxicillin-clavulanic acid, ATM=aztreonam, FEP=cefepime, FOX=cefotaxime, CAZ=ceftazidime, CTX=ceftriaxone, IMP=imipenem, MEM=meropenem, ETP=ertapenem) and non-β-lactam (AMK=amikacin, CIP=ciprofloxacin ,GEN=gentamycin, KAN=kanamycin, NET=netilmycin, STR=streptomycin, TET=tetracycline, TOB=tobramycin, SXT=trimethoprim-sulfamethoxazole).

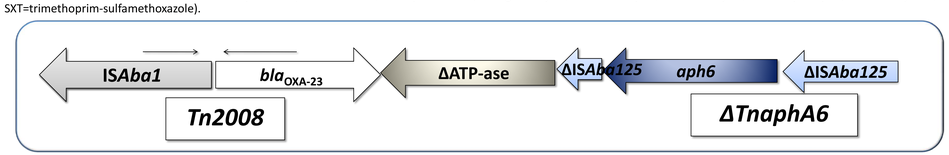

Figure 1. Genetic environment of bla_{OXA-23} detected in some *A. baumannii* isolates (ST765 and ST437).

CONCLUSIONS

- These results demonstrate the presence of acquired carbapenemases among *P. aeruginosa* and *A. baumannii* isolates in Bucharest and their association with epidemic clones (ST233 *P. aeruginosa* and ST437/CC92 *A. baumannii*) and particular genetic structures, which is firstly reported in Romania.
- It is of interest to highlight that most of the patients came from hospitals located in other districts of Romania (60%), suggesting widespread distribution in the country.
- The identification of similar bla_{OXA-23}-carrying elements (encompassing Tn*2008* and surrounding structures) both on pACICU2 plasmid and the chromosome of different clones highlights a higher mobility than that previously reported for these elements.

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