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Pseudomonas aeruginosa infection in cystic fibrosis caused by an epidemic metallo-β-lactamase-producing clone with a heterogeneous carbapenem resistance phenotype

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Abstract

An epidemic IMP-13 metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* clone, causing infections and even large outbreaks in Italian critical care settings, was detected in a young cystic fibrosis patient. In this patient, the chronic infection was sustained by distinct clonal sub-populations of the MBL-producing *P. aeruginosa* clone, either susceptible or resistant to carbapenems. These findings underscore the importance of infection prevention practices in cystic fibrosis settings and pose an important diagnostic and therapeutic challenge.

Keywords: Carbapenems, cystic fibrosis, epidemic clone, metallo-β-lactamase, *Pseudomonas aeruginosa*

Original Submission: 6 October 2010; **Revised Submission:** 20 December 2010; **Accepted:** 26 December 2010
Editor: R. Cantón
Article published online: 17 January 2011

Clin Microbiol Infect 2011; **17:** 1272–1275
10.1111/j.1469-0691.2011.03466.x

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Pseudomonas aeruginosa is a major pathogen for patients with end-stage chronic obstructive pulmonary disease, including patients with cystic fibrosis (CF) [1], in whom it establishes a persistent respiratory infection with recurrent exacerbations [2].

P. aeruginosa is prone to acquire antibiotic resistance and multidrug resistant (MDR) clones represent an important therapeutic challenge [1]. In CF patients, infection by MDR *P. aeruginosa* clones has been associated with a more severe prognosis [3], and cross-contamination with similar clones is a matter of major concern [4].

Acquired metallo-β-lactamases (MBLs) are emerging resistance determinants of increasing clinical importance. MBLs can confer a very broad spectrum of resistance to β-lactams (including the expanded-spectrum cephalosporins and carbapenems), which is not reversible by the available β-lactamase inhibitors. Due to linkage of MBL genes with other resistance genes, MBL-producing strains usually exhibit a complex MDR phenotype [5]. MBL-producing *P. aeruginosa* have been reported worldwide in nosocomial settings, sometimes causing major outbreaks [5]. However, their presence in CF patients has not been reported, except for a single report in Portugal [6].

In this paper we demonstrate the acquisition of an MBL-producing epidemic *P. aeruginosa* clone in a CF patient; the MBL-producing strain caused a chronic infection that was sustained by distinct clonal sub-populations with a different carbapenem resistance phenotype.

A female CF patient, monitored at a paediatric hospital in Rome (Italy), was first colonized by an MDR *P. aeruginosa* at the age of 6 years and subsequently developed a chronic infection. Before infection by *P. aeruginosa*, the patient had received prophylaxis with aerosolized tobramycin and azithromycin (since the ages of 6 months and 5 years, respectively), and had occasionally received expanded-spectrum cephalosporins and ciprofloxacin. In the 6-year follow-up period after *P. aeruginosa* infection, the patient presented with several episodes of acute exacerbations (average 2 per year), with a progressive decline of FEV_1 (61% at the end of the follow-up period), and received intermittent inhaled tobramycin and azithromycin therapy; exacerbation episodes were treated with ciprofloxacin, expanded-spectrum cephalosporins and, only in one case, with carbapenems.

Eighteen isolates of *P. aeruginosa*, taken at different times during the chronic infection, were available for microbiological investigation. Cultures were derived from the original frozen stocks. All isolates exhibited an MDR phenotype, including ceftazidime, tobramycin and ciprofloxacin, while retained susceptibility to piperacillin-tazobactam and colistin. Amikacin susceptibility was variable, with MICs close to the breakpoint. Carbapenem susceptibility was variable, with imipenem and meropenem MICs either in the susceptible range (≤4 mg/L) or well above the resistance breakpoint (>32 mg/L). Carbapenem-susceptible isolates were detected after the initial isolation of a carbapenem-resistant isolate, and they coexisted with carbapenem-resistant isolates over time (Table 1).

Pulsed-field gel electrophoresis (PFGE) genotyping [7] revealed a clonal relationship for all isolates; the infecting clone was named OBG6.

The presence of major carbapenem resistance mechanisms, namely carbapenemase production, reduced OprD expression and upregulation of the MexAB-OprM efflux system, was investigated. Spectrophotometric assay with crude extracts [8] revealed production of EDTA-inhibitable carbapenemase activity in all the OBG6 isolates, without significant differences in enzyme activity between the carbapenem-susceptible and carbapenem-resistant ones (Table 1). Multiplex PCR for detection of bla_{IMP}- and bla_{VIM}-type MBL genes [8] revealed the presence of a bla_{IMP} allele, identified as bla_{IMP-13} by sequencing. MexAB-OprM efflux pump and OprD porin expression levels were analysed by real-time (RT)-PCR; the *gyrB* gene was used as housekeeping reference gene; amplification reactions were performed using the following primers: oprD_Fwd 5′-GAACTCTATGCCACCTACGC-3′ and oprD_Rev 5′-TGTTGAAATCGAAGCCCAGC-3′, MexB2-Fwd 5′-AACGGGATCGACAATCTGCG-3′ and MexB2-Rev 5′-GAAGTTCTTCACCGCCTTGG-3′, gyrB-PSA_Fwd 5′-AAGGTCTGGGAACAGGTCTA-3′ and gyrB-PSA_Rev 5′-AGGATGTCCCAACTGAAGTG-3′. Real-time (RT)-PCR revealed a two- to nine-fold increased expression of the *mexB* gene and a decreased (0.9–0.1-fold) *oprD* gene expression in all isolates (Table 1), without any apparent relation to susceptibility to carbapenems. Sequencing of the *oprD* gene, using primers designed on the gene flanking sequences (oprD-EXT-Fwd 5′-CTATCGCCAAGAAACACTGC-3′, oprD-EXT-Rev 5′-CTACGCCCTTCCTTTATAGG-3′), revealed the presence of an *oprD-TS* allele [9,10] in all isolates, and a single nucleotide deletion (position +594) causing premature translational termination of the protein (OprD-ΔTS) in carbapenem-resistant isolates (Table 1).

As an epidemic IMP-13-producing *P. aeruginosa* clone has recently been reported, even as a cause of nosocomial outbreaks [11,12], the OBG6_1 strain was compared with representatives of the epidemic clone isolated in Italy. Analysis of the PFGE profiles revealed clonal relatedness for all isolates (Fig. 1), showing an identical PFGE profile for OBG6_1 and AV13X-28 strains and a difference of 1–3 bands between OBG6_1 and the other epidemic strains. By multilocus sequence typing, the OBG6 clone belonged in

TABLE 1. Antimicrobial susceptibility and resistance mechanisms of the *Pseudomonas aeruginosa* isolates from a patient with cystic fibrosis

Isolate	Isolation date	MIC mg/L[a]							MBL Activity[c]	Gene expression[d]		*oprD-TS* allele[e]
		CAZ[b]	TZP	IMP	MEM	TOB	AK	CIP		*mexAB*	*oprD-TS*	
OBG6_1	2004 Feb 12	>256	8	>32	>32	>64	32	>32	181	8.47 ± 0.89	0.32 ± 0.05	*oprD-ΔTS*
OBG6_2	2004 Sep 30	>256	8	4	1	>64	32	>32	186	6.93 ± 1.46	0.39 ± 0.08	wt
OBG6_3	2005 Jun 24	>256	4	>32	>32	>64	16	>32	190	ND[f]	ND	*oprD-ΔTS*
OBG6_4	2005 Oct 11	>256	8	4	1	>64	16	>32	192	ND	ND	wt
OBG6_5a	2006 Aug 30	>256	3	4	2	>64	24	>32	199	2.93 ± 0.71	0.13 ± 0.01	wt
OBG6_5b	2006 Aug 30	>256	2	>32	>32	>64	16	>32	189	3.88 ± 0.63	0.10 ± 0.05	*oprD-ΔTS*
OBG6_6a	2007 Jan 03	>256	2	4	1	>64	32	>32	131	5.93 ± 0.37	0.54 ± 0.02	wt
OBG6_6b	2007 Jan 03	>256	3	>32	>32	>64	16	>32	152	9.22 ± 0.61	0.26 ± 0.02	*oprD-ΔTS*
OBG6_7	2007 Feb 20	>256	8	>32	>32	>64	64	>32	130	ND	ND	*oprD-ΔTS*
OBG6_8a	2007 Sep 25	>256	16	>32	>32	>64	32	>32	133	6.94 ± 1.71	0.39 ± 0.01	*oprD-ΔTS*
OBG6_8b	2007 Sep 25	>256	8	>32	>32	>64	16	>32	207	9.26 ± 1.15	0.26 ± 0.03	*oprD-ΔTS*
OBG6_9	2008 Jun 08	>256	1.5	>32	>32	>64	12	>32	158	2.43 ± 0.16	0.35 ± 0.09	*oprD-ΔTS*
OBG6_10	2008 Dec 05	>256	2	>32	>32	>64	24	>32	142	ND	ND	*oprD-ΔTS*
OBG6_11	2009 Jun 03	>256	1	>32	>32	>64	16	>32	173	3.10 ± 0.25	0.33 ± 0.02	*oprD-ΔTS*
OBG6_12	2009 Oct 26	>256	1.5	4	1	>64	12	>32	199	4.64 ± 0.32	0.28 ± 0.05	wt
OBG6_13	2009 Nov 12	>256	1.5	>32	>32	>64	24	>32	133	4.25 ± 1.34	0.65 ± 0.17	*oprD-ΔTS*
OBG6_15a	2009 Nov 27	>256	1.5	3	1	>64	24	>32	204	8.60 ± 0.78	0.89 ± 0.09	wt
OBG6_15b	2009 Nov 27	>256	2	>32	>32	>64	24	>32	150	ND	ND	*oprD-ΔTS*

[a]Antibiotic susceptibility was determined using Etest (AB-Biodisk, Solna, Sweden) and interpreted according to the Clinical Laboratory Standards Institute [14].
[b]CAZ, ceftazidime; TZP, piperacillin-tazobactam; IMP, imipenem; MEM, meropenem; TOB, tobramycin; AK, amikacin; CIP, ciprofloxacin.
[c]Enzymatic activity of metallo-β-lactamase (MBL), obtained using imipenem as substrate (nMol/mg^{-1} min^{-1}).
[d]Gene expression fold-increase/decrease ± standard deviation, analysed using the fluorescent dye *SYBR Green I* with the *Lightcycler* instrument (Roche-Applied Science, Mannheim, Germany); the *P. aeruginosa* susceptible wild-type strain PT5 was used as reference strain; the *nalB* mutant strain PT629, overexpressing MexAB-OprM, was used as positive control strain [15].
[e]Nucleotide sequence of the *oprD* allele; wt, wild-type *oprD-TS* allele; *oprD-ΔTS*, mutated allele.
[f]Not determined.

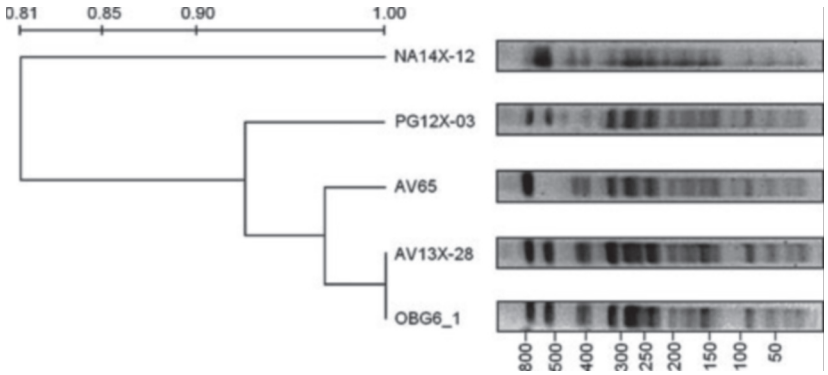

FIG. 1. *Spe*I PFGE profiles of the IMP-13-producing *Pseudomonas aeruginosa* isolate OBG6_1 from a patient with cystic fibrosis, strain AV65, causing a nosocomial outbreak in southern Italy and representing the epidemic IMP-13 *P. aeruginosa* clone (clone C), and strains AV13X-28, NA14X-12 and PG12X-03, representing the clonal variant C_1, C_2 and C_3, respectively, present on the Italian territory [11,12]. Molecular size standards are reported in kilobasepair.

ST621, which was already reported for IMP-13-producing isolates disseminated worldwide, revealing a possibly common ancestor for these *P. aeruginosa* strains [12].

Moreover, analysis of the bla_{IMP-13} locus by PCR-mapping and sequencing, revealed the presence in all OBG6 isolates of the class 1 integron InPSG, harbouring the bla_{IMP-13} and *aacA4* cassettes, which was previously described in an Italian study [11].

This study reports the isolation of an IMP-13-producing *P. aeruginosa* clone, which was found to be epidemic in Italian hospital settings, in a young CF patient; the strain was involved in the first colonization event of the patient and persisted for a prolonged period. To the best of our knowledge, this is the first report of an IMP-type MBL-producing *P. aeruginosa* strain in a CF setting.

Although patient-to-patient transmission of MBL-carrying *P. aeruginosa* strains during chronic respiratory infections in nosocomial settings has already been demonstrated [13], information on transmission of such strains in CF settings is poor. Our findings demonstrate the acquisition of a highly transmissible nosocomial MBL-producing *P. aeruginosa* strain in a CF patient and underline the importance of infection prevention practices in the CF setting. Control practices such as patient segregation, and the implementation of appropriate hygienic measures and of disinfection procedures have been recommended to prevent cross-infection and appear to be of major importance in the case of MDR strain transmission. Moreover, regular culturing of CF pathogens and bacterial typing emerge as mandatory for epidemiological surveillance. Furthermore, the role of chronically colonized patients as a reservoir of such *P. aeruginosa* strains represents a matter of concern and could play a major role in spreading of clinically relevant resistance determinants.

Our study demonstrates the chronic infection by distinct clonal sub-populations of MBL-producing *P. aeruginosa* showing different carbapenem resistance phenotypes; coexistence for a long period of mutants of the OprD porin showing high-level resistance to carbapenems with an apparently susceptible wild-type strain was demonstrated. This finding underlines the need for accurate diagnostic procedures, in order to correctly detect chemoresistant bacterial subpopulations; moreover, it could represent an important therapeutic challenge, due to the possible selection of resistant *P. aeruginosa* variants after repeated antibiotic treatments.

Acknowledgements

We thank Thilo Köhler for kindly providing *P. aeruginosa* strains PT5 and PT629. These results were partially presented at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco (USA), September 2009.

Transparency Declaration

This work was partially supported by grants from the Italian Cystic Fibrosis Research Foundation (Grants FFC#14/2006 and FFC#09/2008). The authors declare no conflicts of interest.

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Understanding the dynamics of imipenem-resistant *Acinetobacter baumannii* lineages within Portugal

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Abstract

A recent collection of 213 imipenem-resistant *Acinetobacter baumannii* (IRAB) clinical isolates was characterized for the presence of acquired carbapenem-hydrolysing class D β-lactamases (CHDLs) and clonality. A population structure analysis of IRAB was also conducted, with five molecular typing methods. Three main clusters, each one associated with a specific CHDL, were observed with multilocus sequence typing. Overall, our results suggest a switch in the dominant clone, with sequence type (ST) 92, carrying bla_{OXA-23} (63.4%), replacing the closely related ST98, carrying $bla_{OXA-24/40}$ (22%). In addition, ST103, an independent lineage, was associated with bla_{OXA-58}-carrying isolates (14.6%).

Keywords: bla_{OXA-51}-like, carbapenemase, multilocus sequence typing, pulsed-field gel electrophoresis type, sequence groups

Original Submission: 24 July 2010; **Revised Submission:** 7 January 2011; **Accepted:** 9 January 2011
Editor: R. Cantón
Article published online: 24 January 2011

Clin Microbiol Infect 2011; **17:** 1275–1279
10.1111/j.1469-0691.2011.03469.x

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During the last decade, an ongoing rise in *Acinetobacter baumannii* carbapenem resistance has been observed worldwide, with recent reports of resistance to all available antimicrobials [1,2]. The production of acquired carbapenem-hydrolysing class D β-lactamases (CHDLs) belonging to the OXA-23, OXA-24/40 and OXA-58 groups is the main reported carbapenem resistance mechanism [1–7]. Despite the description of two particular isolates producing IMP-5 [8] and, more recently, OXA-23 [2], the imipenem resistance observed in Portuguese *A. baumannii* clinical isolates has been mainly associated with the production of OXA-24/40 and linked to the spread of a particular multidrug-resistant clone [3,6]. Several sequence-based typing methods have been proposed for *A. baumannii* population structure analyses [9–12], with multilocus sequence typing (MLST) being increasingly applied as a standard method [4,5,10,11,13]. Nevertheless, studies evaluating the robustness of each particular method are still scarce.

In this work, we have characterized the antimicrobial susceptibility, acquired CHDLs and clonality of 213 previously undescribed imipenem-resistant *A. baumannii* (IRAB) isolates,