First detection in Europe of the metallo-β-lactamase IMP-15 in clinical strains of *Pseudomonas putida* and *Pseudomonas aeruginosa*

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Abstract

In a prospective study (2009–2011) in healthcare institutions from the Canary Islands (Spain), 6 out of 298 carbapenem non-susceptible *Pseudomonas aeruginosa* isolates produced a metallo-β-lactamase: four IMP-15, two VIM-2 (including one IMP-15-positive isolate) and one VIM-1. Multilocus sequence typing identified the single VIM-1-producing isolate as clone ST111 and two IMP-15-producing isolates as ST606, but, strikingly, bacterial re-identification revealed that the other three isolates (producing IMP-15 and/or VIM-2) were actually *Pseudomonas putida*. Further retrospective analysis revealed a very high prevalence (close to 50%) of carbapenem resistance in this environmental species. Hence, we report the simultaneous emergence in hospitals on the Canary Islands of *P. putida* and *P. aeruginosa* strains producing IMP-15, a metallo-β-lactamase not previously detected in Europe, and suggest an underestimated role of *P. putida* as a nosocomial reservoir of worrying transferable resistance determinants.

Keywords: Class B carbapenemases, environmental reservoirs, metallo-β-lactamases, *Pseudomonas putida*, transferable resistance determinants

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Pseudomonas aeruginosa is one of the most relevant nosocomial pathogens, particularly in the Intensive Care Unit setting [1], as well as the first cause of chronic respiratory infections in patients with underlying diseases such as cystic fibrosis [2]. One of the most striking features of this pathogen is its outstanding capacity for the development of antimicrobial resistance, through the selection of chromosomal mutations and the acquisition of horizontally transferred resistance determinants in genetic elements such as integrons located in transposons and/or plasmids. Particularly noteworthy among these determinants are class B carbapenemases (also called metallo-β-lactamases: MBLs), hydrolysing all β-lactams with the exception of monobactams [3–5]. In this scenario, the prevalence of multidrug-resistant and extensively drug-resistant *P. aeruginosa* strains is globally increasing, significantly compromising our anti-pseudomonal arsenal [6]. Moreover, recent reports have provided evidence of the existence of multidrug-resistant/extensively drug resistant clones disseminated in several hospitals worldwide and for that reason denominated high-risk clones [7]. Among them, ST235, ST111 and ST175 are probably more widespread, linked to multiple transferable and mutational resistance mechanisms [8]. Furthermore, although some environmental *Pseudomonas* species such as *Pseudomonas putida* may not have the clinical relevance of *P. aeruginosa*, their potential role as reservoirs of transferable β-lactamases has recently been suggested [9,10]. In this work, we report the simultaneous emergence of *P. putida* and *P. aeruginosa* strains harbouring an MBL not previously detected in Europe (IMP-15), and highlight the potentially underestimated role of *P. putida* as a nosocomial reservoir of worrying transferable resistance determinants.

A prospective study, from 2009 to 2011, was carried out to determine the prevalence of carbapenem non-susceptible *P. aeruginosa* in healthcare institutions of Gran Canaria, Canary Islands (Spain), including Hospital Universitario de Gran Canaria Dr Negrín, Hospital Universitario Materno Infantil and the primary-care centres. The *P. aeruginosa* isolates showing an intermediate or resistant clinical category (following CLSI breakpoints) to at least one of the tested carbapenems (imipenem and meropenem) were included [11]. The GN card of Vitek2 (bioMérieux, Marcy l'Étoile, France) was used for initial identification and susceptibility testing. From a total of 298 non-susceptible isolates (14.7% of all the *P. aeruginosa* isolates recovered in the study period), six yielded a positive result with the MBL-Etest (bioMérieux). To confirm the presence of MBL determinants and determine the specific genes involved, PCR followed by sequencing of the complete coding regions was performed using previously described primers and protocols [12,13]. Sequencing results revealed the presence of bla_{IMP-15} in four isolates, bla_{VIM-2} in two (including

one of the bla_{IMP-15}-positive isolates) and bla_{VIM-1} in one. Strikingly, the re-identification using the Api-20NE strips (bioMérieux) and 16S rDNA sequencing showed that three of the six isolates (producing bla_{VIM-2} and/or bla_{IMP-15}) were actually *P. putida*.

Clonal relatedness was evaluated by pulsed-field gel electrophoresis (PFGE) following described protocols [13]. Additionally, *P. aeruginosa* isolates were further analysed through multilocus sequence typing (MLST), using described procedures and available databases (http://pubmlst.org/paeruginosa/). The PFGE, MLST and resistance profiles (imipenem, meropenem, ceftazidime, cefepime, aztreonam, piperacillin-tazobactam, amikacin, gentamicin, tobramycin, colistin and ciprofloxacin MICs determined by Etest) documented for the six isolates are shown in Table 1. The single VIM-1-producing *P. aeruginosa* isolate was found to belong to the internationally spread high-risk clone ST111, previously linked to multiple different integron-borne acquired β-lactamases, from narrow to extended spectrum and carbapenemases [14]. Although VIM-2 production has been recently linked to ST111 in several Spanish hospitals [8], this is the first association of this clone with VIM-1 in our territory. On the other hand, the two *P. aeruginosa* isolates producing bla_{IMP-15} showed an identical PFGE pattern, identified as ST606 clone through MLST (Table 1). ST606 has been reported in a few *P. aeruginosa* isolates [15], but has never been related to an acquired β-lactamase, and it is not yet considered one of the high-risk clones [7]. Interestingly, one of the two isolates was recovered from a sputum sample of a patient with cystic fibrosis, accounting for the first documentation of colonization by MBL-producing *P. aeruginosa* among Spanish cystic fibrosis patients (Table 1). The two IMP-15-producing *P. putida* isolates also belonged to a single clone, showing an identical PFGE pattern (despite one of the isolates additionally produced VIM-2), completely different to that of the remaining VIM-2-producing strain (Table 1). Additionally, the three *P. putida* isolates were found to be aztreonam resistant. As shown in Table 1, MICs performed in Müller–Hinton plates containing the efflux pump inhibitor Phe-Arg β-naphthylamide dihydrochloride (final concentration, 20 mg/L) [16] suggested the involvement of efflux in the resistance phenotype.

The re-identification of three of the six isolates as *P. putida* prompted us to retrospectively review the carbapenem-resistance rates in this species during the study period, yielding quite alarming results: up to 22 of 48 isolates (45.8%) were found to be carbapenem resistant. Moreover, carbapenem resistance in *P. putida* significantly increased during the study period reaching 71.4% (10 of 13) in 2011. Unfortunately, a screening for MBL production in the 22 isolates could not be performed retrospectively because the isolates were no longer

TABLE 1. Characteristics of the metallo-β-lactamase (MBL) -producing strains studied

Species	Isolate	PFGE clone	MLST clone	Date of isolation (mm/dd/yy)	Clinical sample (colonization/ infection)	Ward	MBL	MIC (mg/L)										
								IMP	MER	CAZ	FEP	ATM	ATMEPI	PTZ	AMK	GEN	TOB	COL	CIP
PP	364223	PP-A	–	02/09/09	Urine (C)	Internal medicine	VIM-2/IMP-15	>32	>32	>256	>256	64	6	>256	6	0.5	12	0.25	>32
	447490	PP-A	–	09/14/10	Urine (I)	Domiciliary admission	IMP-15	>32	>32	>256	>256	32	4	>256	3	0.5	6	0.38	>32
PA	399327	PP-B	–	10/08/09	Urine (C)	Cardiology	VIM-2	>32	>32	24	64	12	0.75	>256	8	>256	32	0.19	>32
	394851	PA-A	ST606	09/07/09	Sputum (I) (CF)	Pneumology	IMP-15	>32	>32	64	>256	2	ND	>256	4	0.5	1	0.094	2
	508856	PA-A	ST606	11/24/11	Blood (I)	Neurosurgery	IMP-15	>32	>32	64	>256	2	ND	>256	6	0.5	1	0.38	0.75
	496312	PA-B	ST111	08/25/11	Tracheal aspirate (C)	ICU	VIM-1	>32	>32	>256	>256	1	ND	>256	12	64	>256	2	>32

PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; PP, *Pseudomonas putida*; PA, *Pseudomonas aeruginosa*; C, colonization; I, infection; CF, cystic fibrosis; ICU, Intensive care unit; IMP, imipenem; MER, meropenem; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; EPI, ATM MICs in Müller–Hinton plates containing the efflux pump inhibitor (EPI) Phe-Arg β-naphthylamide dihydrochloride (20 mg/L); PTZ, piperacillin-tazobactam; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; COL, colistin; CIP, ciprofloxacin; ND, not determined.

available, but the resistance patterns of many of them suggest a high prevalence of MDR determinants that could be transferred to *P. aeruginosa*, which is consistent with previous evidence [17].

This is the first time that bla_{IMP-15} has been detected in Europe, as well as, to our knowledge, the first report of the simultaneous presence of VIM and IMP enzymes in *P. putida* isolates. IMP-15, showing a 90% identity with IMP-1, was originally detected in a *P. aeruginosa* strain from Thailand (GenBank accession no. AY553333), and later in a strain recovered from a surgical wound of a patient admitted to a US hospital after surgery in a Mexican centre [18]. Other reports have documented an endemic presence in Mexico of *P. aeruginosa* strains harbouring bla_{IMP-15} in several integron structures related to In*95* [19–21]. To analyse whether bla_{IMP-15} of the strains from the Canary Islands were located in the same genetic element, PCR and sequencing of the complete integron was performed following described protocols [13]. However, a different integron structure (designated *In*589, GenBank accession no. KC310496), which contained bla_{IMP-15} and bla_{OXA-4}, was detected, arguing against a direct importation of the Mexican *P. aeruginosa* strains. Finally, regarding the potential plasmid/chromosomal location of bla_{IMP-15} in the studied strains, all attempts to transfer the plasmid DNA (obtained using the Ultraclean Plasmid Prep Kit; MO BIO Laboratories Inc., Carlsbad, CA, USA) to the PAO1 reference strain through electroporation/conjugation yielded negative results. Furthermore, the southern blot hybridization following described protocols [10] and using the North2South Complete Biotin Random Primer labelling and detection kit (Thermo Scientific, Rockford, IL, USA), over the I-CeuI/S1 nuclease-digested genomes, suggested the chromosomal location of bla_{IMP-15} in all the strains, given that the bla_{IMP-15} probe hybridized with bands that also hybridized with the rRNA gene probe (data not shown).

In summary, we report the simultaneous emergence in hospitals from the Canary Islands of *P. putida* and *P. aeruginosa* strains producing IMP-15, an MBL not previously detected in Europe. Moreover, our results suggest a relevant role of *P. putida* as a nosocomial reservoir of worrying transferable resistance determinants, which is probably underestimated because of the lack of active surveillance in environmental *Pseudomonas* species and their misidentification as *P. aeruginosa*.

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Transparency Declaration

The authors declare no conflict of interest.

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