Genomics helps to decipher the resistance mechanisms present in a *Pseudomonas chlororaphis* strain recovered in an HIV patient

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To the Editor

The genus *Pseudomonas* is one of the most diverse and ubiquitous bacterial genera, encompassing more than 140 isolated species [1,2]. The species *Pseudomonas chlororaphis* is primarily used as an agricultural biocontrol because of its unique ability to inhibit the growth of soil-borne pathogens and produce phenazine-1-carboxamide—an antifungal metabolite [3–6]. A literature review of the species revealed the presence of 28 genome assemblies of *P. chlororaphis*, among which, seven were complete genomes.

To the best of our knowledge, there has only been one reported case in a human of pathogenic *P. chlororaphis* [7]. Here we aim to describe the occurrence of two *P. chlororaphis* isolates recovered from an individual with human immunodeficiency virus infection and to characterize the isolate at a molecular level. Our findings, along with the previous reports, demonstrate the ability of this species to serve as a reservoir for resistance determinants and serve as a human pathogen.

Two *P. chlororaphis* isolates (PC190 and PC477) were recovered from the respiratory tract and anal mucous sample of a 63-year-old man. The patient presented with aspirate pneumonia and is AIDS positive, receiving highly active antiretroviral therapy. In February of 2013, the patient was on an R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) regimen—a treatment for aggressive non-Hodgkin lymphoma, diffuse large B-cell lymphoma. After 5 months of treatment, the patient was in remission with intermittent leukopenia.

In October of 2013, he was admitted with a urinary infection due to *Pseudomonas aeruginosa*. He received broad-spectrum antibiotic treatment with imipenem and ciprofloxacin. One month later, the patient presented with a chief complaint of fever (38.5°C) and a cough. A pulmonary infiltrate from the apical segment of the right lower lobe was taken. No bacterial development was observed and he received an empirical treatment with imipenem, colistin and vancomycin. During this period, he also presented with an episode of hypotension, distal coldness, filiform pulse and profuse sweating. A computed tomography scan of the chest revealed the progression of a bilateral pulmonary infiltrate. This prompted the clinicians to take a tracheobronchial aspirate biopsy. Following standard procedure, this was screened for carbapenem-resistant *Enterobacteriaceae*. The tracheobronchial aspirate culture showed the development of *Klebsiella pneumoniae* and *P. chlororaphis*, and the anal swab showed a *P. chlororaphis* infection. As the patient improved with the administration of corticosteroids, it was interpreted as suprarenal insufficiency.

The strains were identified at species level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany), *gyrB* amplification, and sequencing. MALDI-TOF MS identified the strain as *P. chlororaphis* with a score of >2. These results were confirmed with the *gyrB*, *rpoB* and 16S rDNA sequence analysis. The analysis revealed a 99% identity to *P. chlororaphis* Lzh-T5 (AN CP025309), 99% identity with *P. chlororaphis* PA23 (AN CP008696) and a 100% identity with *P. chlororaphis* PA23, respectively, for each gene.

Antimicrobial susceptibility testing was performed using VITEK 2 (bioMérieux, Marcy l'Etoile, France) and the results were interpreted in accordance with the CLSI 2017 standard (Table 1). These strains exhibited resistance to different β-lactam antibiotics, including carbapenems. degenerate oligonucleotide

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TABLE 1. Minimum inhibitory concentrations of antimicrobial agents in PC190 and PC477 isolates pf *Pseudomonas chlororaphis*

Isolate	MIC (mg/L)													
	AMP	AMS	CEP	CTX	CAZ	FEP	TAZ	IMP	MEM	AMK	GEN	CIP	COL	TMS
PC190	≥32 (R)	≥32 (R)	≥64 (R)	≥64 (R)	16 (I)	2 (I)	32 (I)	≥16 (R)	≥16 (R)	≤2 (S)	≤1 (S)	≤0.25 (S)	≤0.5 (S)	80 (R)
PC477	≥32 (R)	≥32 (R)	≥64 (R)	≥64 (R)	16 (I)	2 (I)	32 (I)	≥16 (R)	≥16 (R)	≤2 (S)	≤1 (S)	≤0.25 (S)	≤0.5 (S)	80 (R)

Abbreviations: AMP, ampicillin; AMS, ampicillin-sulbactam; CEP, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; TAZ, piperacillin-tazobactam; IMP, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; COL, colistin; TMS, trimethoprim/sulfamethoxazole.

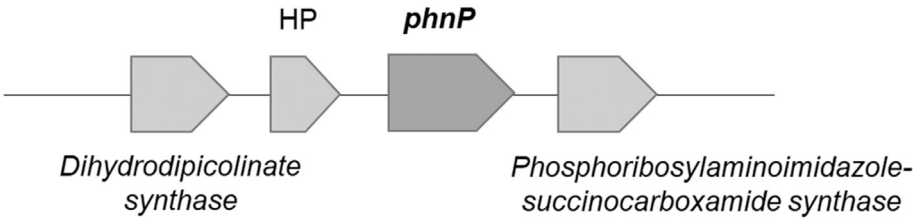

FIG. 1. Genetic environment of *phnP* found in *Pseudomonas chlororaphis* PC190 genome. The genes are shown by grey arrow boxes indicating the transcriptional orientation. HP, hypothetical protein.

primers (DO-PCR)/OD confirmed the genetic relationship between the isolates (data not shown).

Metallo-β-lactamase (MBL) activity was confirmed by synergism between carbapenems and EDTA using a double-disc assay with EDTA/sodium mercaptoacetic acid (SMA) discs (1900 g/750 g per disc, respectively; Laboratorios Britania, Buenos Aires, Argentina) placed 15 mm (centre to centre) from a carbapenem disc (imipenem and meropenem). An 'egg-shaped effect' was observed in the zone of inhibition of a carbapenem-containing disc with the zinc chelating agent (EDTA) disc; these results potentially exposed the presence of MBLs.

To further explore the genetic contents, one isolate (PC190) was selected for whole genome sequencing. The draft genome sequence was obtained with Illumina MiSeq-I and Nextera XT DNA library. *De novo* assembly was performed with SPADES assembler 3.1.0 version [8]. RAST was used to predict the open reading frames and the predictions were confirmed using BLAST (version 2.0) [9]. Further genomic analysis was carried out using ARG-ANNOT [10], ISFinder [11] and PHAST [12].

The draft genome of PC190 comprises 6 791 658 bp. The RAST server predicted 6052 protein-coding genes with a corresponding G+C content of 63.0%. The genome analysis of PC190 exposed the presence of a β-lactamase gene, *ampC*, and two copies of an MBL, *phnP*. The latter gene was flanked by a gene coding for phosphoribosylaminoimidazole-succinocarboxamide synthase downstream and a hypothetical protein upstream, and this was followed by a gene coding for dihydrodipicolinate synthase (Fig. 1). Several efflux pumps such as CmeABC, MexCD-OprJ and MexEF-OprN, were also found. Among resistance genes in PC190, a fosfomycin-resistance protein flanked by *LysE*, coding for a lysine transporter, was found downstream. A hypothetical protein upstream and streptothricin-resistance protein flanked upstream by a transcriptional regulator (LysR) downstream and upstream by a lysine decarboxylase protein. No insertion sequences were found. Using PHAST software, two intact and two incomplete phages were found.

Recent reports of emerging pathogens that have acquired exogenous DNA from other bacterial species, and/or serve as reservoir of resistance genes are more common than was previously thought. The variety of resistance genes found in the PC190 genome can explain the multidrug-resistance phenotype. We also demonstrate the importance of the uncommon multidrug-resistance pathogens isolated from clinical specimens.

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