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First report of Pseudomonas aeruginosa strains co-harboring bla_{NDM}-bla_{VIM} and bla_{VIM}-bla_{IMP} metallo–β–lactamase genes in Morocco.

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Letter to the Editor

First report of *Pseudomonas aeruginosa* strains co-harboring *bla*$_{NDM}$-bla$_{VIM}$ and bla$_{VIM}$-bla$_{IMP}$ metallo- β- lactamase genes in Morocco.

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To the Editor

Dear Editor,

Pseudomonas aeruginosa (PA) has emerged as a significant clinical opportunistic pathogen due to the number and severity of infections caused, especially nosocomial infections. This germ is associated with high levels of morbidity and mortality, especially in immunodeficient patients. It poses serious therapeutic difficulties around the world due to its multidrug resistance and even the emergence of "pan-resistant" strains (Aubin et al., 2021; Pang et al., 2019). Carbapenem-resistant PA has been classified by the World Health Organization (WHO, 2017) as one of a critical group of pathogens (ESKAPE), for which new antimicrobial development is urgently needed in order to counteract the silent and imminent pandemic of antimicrobial resistance (WHO, 2017). The acquired genes for metallo-β-lactamases (MβLs), enzymes that can hydrolyze almost all β-lactam antibiotics and carbapenems, deserve special attention because the interspecies transfer of mobile genetic elements is common, and they, unfortunately, accelerate the dissemination of the carbapenem-resistant PA (Elsherif et al., 2016).

We conducted a study to determine the prevalence of MBL-producing PA among community- and clinical strains isolated from patients in Casablanca in Morocco. This investigation is a part of a research project on the dissemination and genetic characterization of antimicrobial-resistant PA in an environmental and clinical setting to determine the genetic relationship between clinical and environmental PA isolates. A total of 134 isolates of PA were obtained in Casablanca, Morocco, over the year 2021 from different specimens including urine (48), sputum (47), pus (22) and 17 from other samples, which 88 were isolated from community origin and 46 from hospital origin. Among them, three PA strains; PA SC33, PA SC84 and

PA SC90 were carbapenemase producers with a particular interest. PA SC33, PA SC84 and PA

SC90 strains were recovered from the nasal sample and the sputum of two women aged 49 and 70 respectively, as well from the urine of a 62 year old man.

The species identification was performed by the MALDI Biotyper IVD 2.2. Susceptibility testing was realized by the standard disk diffusion on Mueller-Hinton agar (Biorad, France) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) using Biorad disks of ticarcillin (75µg), ticarcillin-acid-clavulanic (75/10µg), piperacillin (30µg), piperacillin-tazobactam (30/6µg), imipenem (10µg), meropenem (10µg), aztreonam (30µg), cefepime (30µg), ceftazidim (10µg), ceftazidim-avibactam (10/4µg), amikacin (30µg), gentamicin (10µg), tobramycin (10µg), ciprofloxacin (5µg) and ceftolozan-tazobactam (30/10µg). The minimal inhibitory concentrations (MICs) were determined by sensititre microdilution method (Sensititre™ Vizion, Manual Viewer) towards these antibiotics in addition to colistin and cefiderocol. Antimicrobial susceptibility results were interpreted according to EUCAST clinical breakpoints 2021. PA ATCC 27853 was used as a wild-type susceptible control.

The carbapenemase activity among carbapenem-resistant PA was detected using the Carba NP® rapid diagnostic technique (Rapidec®, BIOMERIEUX) and Carba NG®test (NG.BIOTECH®, LABORATORIES) was used to confirm the metallo-β-lactamase production and their types (Dortet et al., 2012).

These carbapenem-resistant PA were screened for the following β-lactamases genes : broad/extended-spectrum β-lactamases (*bla*$_{\text{OXA-9-like}}$, *bla*$_{\text{OXA-2-like}}$, *bla*$_{\text{OXA-1/30-like}}$, *bla*$_{\text{OXA-10-like}}$, and *bla*$_{\text{OXA-50-like}}$), extended-spectrum β-lactamases (*bla*$_{\text{GES}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{VEB}}$, *bla*$_{\text{BEL}}$, *bla*$_{\text{OXA-18-like}}$, *bla*$_{\text{OXA-20-like}}$) and carbapenemases encoding genes (*bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{OXA-48-like}}$, bla$_{\text{OXA-198-like}}$) by end-point PCR according to the ISO15189 procedure validated at the Belgian National reference center for antimicrobial resitance in Gram-.

The antimicrobial susceptibility testing results revealed that among the studied isolates, PA SC33, PA SC84, and PA SC90 strains are resistant to all the tested antibiotics except colistin (MIC= 2; 1 and 1 mg/L, respectively) and cefiderocol (MIC= 2; and 1 mg/L for SC33 and SC90, respectively). Nevertheless, PA SC84 was also resistant to this antibiotic (8 mg/l). This has not yet been reported in Morocco. Cefiderocol received U.S. Food and Drug Administration approval in October 2019 for treating urinary tract infections and in September 2020 to include hospital-acquired pneumonia and ventilator-associated bacterial pneumonia. It is one of the best novel available therapies with a broad range of activity for

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treating serious infections caused by carbapenem-resistant Gram-negative bacteria (McCreary et al., 2021).

The Carba NP® test confirms the carbapenemase activity of the 3 isolates. Carba NG test and PCR both confirm the presence of *bla*$_{NDM}$ and *bla*$_{VIM}$ in PA SC33 and PA SC84 and of *bla*$_{VIM}$ and *bla*$_{IMP}$ in PA SC90.

Table 1: Characteristics of CRPa strains co-harboring two MβL genes.

Strains Code	CMI (mg/l)																Resistance genes detected
	AK	AZM	FEP	CAZ	CIP	COL	GM	IMP	MEM	PTZ	TIC	TCC	TOB	CZA	CLT	CEF	
PA SC33	128	16	64	64	8	2	64	32	32	128	256	128	64	32	32	2	bla_{NDM}-bla_{VIM}
PA SC84	128	16	64	64	8	1	64	32	32	128	256	128	64	32	32	8	bla_{NDM}-bla_{VIM}
PA SC90	64	64	64	64	8	1	64	32	32	128	256	128	64	32	32	1	bla_{VIM}-bla_{IMP}

AK, amikacin; ATM, aztreonam; ; CAZ, ceftazidime; CEF, cefiderocol ; CIP, ciprofloxacine ; CLT, ceftolozan-tazobactam; COL, colistin; CZA, ceftazidim-avibactam; FEP, cefepime; GEN, gentamicin; MEM, meropenem; PIP, piperacillin; PTZ, piperacillin-tazobactam; TIC, ticarcillin, TCC, ticarcillin-acid-clavulanic; TM, tobramycin.

To the best of our knowledge, this is the first report of clinical PA carbapenem-resistant strains co-harboring both *bla*$_{VIM}$-*bla*$_{NDM}$ and *bla*$_{VIM}$-*bla*$_{IMP}$ genes in Morocco. In addition, we report the emergence of PA resistance to cefiderocol in Morocco. More extensive genomic studies are needed to determine the sequence types of the isolates and to identify the origin of the cefiderocol resistance in PA SC84.

Conflicts of Interest

The authors declare no conflict of interest in the collection, analyses, or interpretation of data; in the writing of the manuscript, nor in the decision to publish the results.

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Ethical approval : This study was approved by the Ethical Committee of Faculty of Medicine and Pharmacy Rabat, Morocco (Date: 23.12.2021, No: 85/21).

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