

Identification of VIM-2-Producing *Pseudomonas aeruginosa* from Tanzania Is Associated with Sequence Types 244 and 640 and the Location of *bla*_{VIM-2} in a TniC Integron

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Epidemiological data on carbapenemase-producing Gram-negative bacteria on the African continent are limited. Here, we report the identification of VIM-2-producing *Pseudomonas aeruginosa* isolates in Tanzania. Eight out of 90 clinical isolates of *P. aeruginosa* from a tertiary care hospital in Dar es Salaam were shown to harbor *bla*_{VIM-2}. The *bla*_{VIM-2}-positive isolates belonged to two different sequence types (ST), ST244 and ST640, with *bla*_{VIM-2} located in an unusual integron structure lacking the 3' conserved region of *qacΔE1-sul1*.

Pseudomonas aeruginosa is an opportunistic pathogen associated with a number of nosocomial infections. Carbapenems (i.e., meropenem and imipenem) are often the treatment of choice for infections caused by *P. aeruginosa* that is resistant to other antipseudomonal β-lactams (1). However, the emergence and spread of acquired carbapenemases, particularly metallo-β-lactamases (MBLs), among *P. aeruginosa* are threatening the usefulness of carbapenems (2). Resistance to carbapenems in *P. aeruginosa* can also occur due to impermeability, efflux mechanisms, and other β-lactamases, including overexpression of the chromosomal AmpC (3).

Several acquired MBLs have been identified in *P. aeruginosa*, including the VIM, IMP, SPM, GIM, AIM, FIM, and NDM enzymes (2, 4). The genes encoding these MBLs are associated with mobile genetic elements, such as insertion sequence common region (ISCR) elements, transposons, and plasmids, and as gene cassettes in integron structures. The dissemination of the various MBLs among *P. aeruginosa* isolates has been shown to occur in many different genetic backgrounds (5). However, two major clonal complexes (CC), CC235 and CC111, predominate, particularly with respect to the dissemination of VIM enzymes (5).

On the African continent, an increasing number of reports indicates that MBL-producing, and in particular VIM-2-producing, *P. aeruginosa* is widespread in Africa. VIM-2-producing *P. aeruginosa* isolates have been observed in Tunisia (6–8), Kenya (9), the Ivory Coast (10), Algeria (11), and South Africa (12). Further, reports have shown the import of *P. aeruginosa* with VIM-2 from African countries (Ghana, Tunisia, and Egypt) into Europe (13–15).

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The aim of this study was to determine the occurrence and molecular epidemiology of MBL-producing *P. aeruginosa* isolates identified from clinical specimens at a tertiary hospital in Dar es Salaam, Tanzania. This was a cross-sectional study conducted at the Central Pathology Laboratory, Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania. MNH is the largest tertiary

health care facility in Tanzania and serves as a university teaching and referral hospital to the population of Dar es Salaam and the whole country. The study included 90 clinical isolates of *P. aeruginosa* consecutively collected from May 2010 to July 2011. Duplicate isolates were excluded from the study. Only isolates that were tested locally with respect to antimicrobial susceptibility were included. The low total numbers of *P. aeruginosa* isolates in the study period reflect the lack of routine analysis of all isolates with respect to antimicrobial susceptibility. The species identification of *P. aeruginosa* was based on the production of characteristic pigments, biochemical test results (oxidase production), and Vittek 2 (bioMérieux). Antimicrobial susceptibility testing was initially performed on all isolates by disk diffusion (Oxoid). Isolates resistant to imipenem and/or meropenem were further screened for MBL production using the MBL Etest (imipenem ± EDTA) (bioMérieux) and an extended panel of Etests for MIC determination. The results from the antimicrobial susceptibility testing were interpreted according to the clinical breakpoints from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucastr.org/clinical_breakpoints/). The antimicrobial agents tested included piperacillin-tazobactam, ceftazidime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and colistin. The presence of MBL genes was investigated by PCR, as described previously (16). The molecular typing of the MBL-producing isolates was per-

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TABLE 1 Antimicrobial susceptibility and molecular typing characteristics of VIM-2-positive *P. aeruginosa* isolates from Tanzania

Reference no.	Specimen type	Antimicrobial susceptibility (MIC) (mg/liter) ^a										PFGE type ^b	ST ^c
		TZP	CAZ	ATM	IPM	MEM	GEN	AMK	TOB	CST	CIP		
P3-66	Blood	128	64	8	>32	>32	32	32	16	1	0.125	A1	244
P3-70	Pus	128	64	8	>32	>32	32	16	16	1	0.25	A2	244
P3-72	Pus	128	64	8	>32	>32	32	16	16	1	0.125	A2	244
P3-73	Pus	64	64	2	>32	4	64	32	32	2	0.25	B	640
P3-74	Pus	128	64	4	>32	>32	128	64	32	2	0.25	A3	244
P3-75	Pus	128	128	4	>32	>32	64	32	32	2	0.125	A3	244
P3-76	Blood	128	64	8	>32	>32	32	16	16	1	0.125	A1	244
P3-77	Blood	128	64	4	>32	>32	64	32	32	2	0.25	B	640

^a TZP, piperacillin-tazobactam; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; CST, colistin; CIP, ciprofloxacin.

^b PFGE, pulsed-field gel electrophoresis.

^c ST, sequence type.

formed by serotyping, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and sequencing of the integrons, as previously described (13, 15). The genomic localization of *bla*_{VIM-2} was determined by I-CeuI PFGE, followed by Southern blotting and hybridization with nonradioactive labeled *bla*_{VIM-2} and 16S rRNA probes. The study was carried out in accordance with existing standard ethics guidelines. Ethical clearance was obtained from the Senate Research and Publications Committee of Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania.

Of the 90 isolates, 30 (33%), 16 (18%), and 15 (16%) were from the outpatient clinics, burn unit, and surgical ward, respectively. The remaining isolates (33%) were from patients in the pediatric ward (5 [5.6%]), medical ward (12 [13.3%]), intensive care unit (3 [3.3%]), ear, nose, and throat (3 [3.3%]), emergency medicine department (2 [2.2%]), and the psychiatric ward (4 [4.4%]). Eight isolates (8.9%) from pus (*n* = 5) and blood (*n* = 3) specimens were carbapenem resistant (Table 1). All carbapenem-resistant isolates were from children (0 to 10 years). Six of these children were admitted in the burn unit, one was from the pediatric surgery ward, and one child with an infected wound was in the psychiatric ward.

All carbapenem-resistant isolates were phenotypically positive (ratio \geq 8 and phantom zone) for MBL production. PCR followed by sequencing showed that the isolates harbored *bla*_{VIM-2}. The *bla*_{VIM-2}-positive isolates showed broad-spectrum β -lactam resistance, except for against aztreonam (Table 1). All VIM-2 isolates showed high-level resistance to carbapenems (MIC, \geq 32 mg/liter), except one strain that was intermediate susceptible to meropenem (MIC, 4 mg/liter). The reason for the lower MIC in this isolate is likely to be due to lack of non- β -lactamase-mediated resistance mechanisms (e.g., efflux mechanisms and/or reduced permeability), since the isolate had a positive MBL Etest result, as did the other isolates, indicating the expression of *bla*_{VIM-2}. Core-susceptibility or intermediate susceptibility was observed for the aminoglycosides, including gentamicin, tobramycin, and amikacin. All isolates were susceptible to ciprofloxacin and colistin (Table 1). The PCR assays performed for extended-spectrum β -lactamases (GES, PER, and VEB) and 16S rRNA methylases were negative in all *bla*_{VIM-2}-positive isolates (data not shown). The observed high rate (100%) of MBL production among the carbapenem-resistant isolates is surprising, since non- β -lactamase-mediated resistance mechanisms are generally more prevalent (17). The reason for this

high rate is unclear, but carbapenems were introduced in the hospital as late as 2010, and their use is limited due to high costs. Consequently, the selective pressure due to carbapenem usage has been limited, and MBL-producing clones might have emerged before the selection of other non- β -lactamase-mediated carbapenem resistance mechanisms, such as efflux or impermeability, started to emerge. Ciprofloxacin was introduced for use in children after the study period, which might explain the ciprofloxacin susceptibility in the MBL-positive isolates.

The PFGE patterns showed four different pulsotypes (A1, A2, A3, and B), with two isolates belonging to each type (Table 1). Isolates belonging to pulsotype A1 (P3-66 and P3-76) and A2 (P3-70 and P3-72) were isolated from patients admitted to the burn unit within a time period of 23 days, indicating nosocomial spread. Nosocomial spread was also suspected for the isolates of pulsotype B (P3-73 and P3-77), as these were isolated within a 5-day period, also in the burn unit, but \sim 3 months before the isolates of pulsotypes A1 and A2 were identified in the same unit. The isolates belonging to pulsotype A3 (P3-74 and P3-75) were isolated from patients admitted to different wards (psychiatric and pediatric) and \sim 2.5 months apart. MLST showed that ST244 corresponded to pulsotypes A1 to A3 and ST640 to pulsotype B (Table 1). ST244 has been shown to be a global *P. aeruginosa* clone identified in several countries, including Poland (18), Brazil (19), Spain (20–22), South Korea (23), Bulgaria (24), the Czech Republic (25), Greece (26), Russia (27), and Libya (28). ST244 has been associated with VIM-2 (22, 26, 27) and with extended-spectrum β -lactamases, such as PER-1 and VEB-1 (18, 24). eBURST analysis (<http://eburst.mlst.net/>) of the *P. aeruginosa* MLST database (<http://pubmlst.org/paeruginosa/>) showed that ST244 is the founder of CC244, with 18 single-locus variants, indicating that this is a globally dispersed CC of related isolates associated with antimicrobial resistance. ST640, on the other hand, is currently a singleton in the MLST database and has been described in the Czech Republic (25). To our knowledge, this is the first time ST640 has been associated with VIM-2. None of the isolates were typeable by serotyping.

The sequencing of the genetic structure of *bla*_{VIM-2} in four isolates showed that *bla*_{VIM-2} was present as the second gene cassette in an integron, along with *aacA7*, *dhfrB5*, and *aacC-A5*. Interestingly, the integron lacked the 3' conserved region (3'CS) of *qac* Δ *E1-sul1* and harbored the *tniC* at the 3' end, which is characteristic of Tn5090/Tn402 (29). The sequenced part of the integron

was identical to previously described integrons in *P. aeruginosa* from the United States (30, 31), Russia (GenBank accession no. AM749810, AM749811, and DQ522233) (27), Taiwan (32), the Ivory Coast (10), Malaysia (33), Sri Lanka (33), and in a *P. aeruginosa* isolate identified in Norway associated with import from Ghana (13). Also, the integrons were similar, with only a few nucleotide differences, to an integron identified in India (GenBank accession no. HQ005291), as well as an integron from India, where the *aacC-A5* gene cassette was exchanged with *aacC6-II* (29). PCRs and sequencing of the PCR products showed the presence of *orf6*, *tniB*, and *tniA* further downstream of *tniC* at the 3' end of the integron. In all isolates, *bla*_{VIM-2} probes hybridized with 16S rRNA probes on a similarly sized chromosomal fragment (~550 kb) but also separately on an ~40-kb fragment (data not shown). This observation indicates both the chromosomal and plasmid locations of *bla*_{VIM-2}. In addition to the identification of the integron structure in ST244 and ST640, this genetic structure has been identified in several other different genetic backgrounds of *P. aeruginosa*, such as ST233 (13, 31), ST234 (27), ST235 (27, 33), and ST1488 (10), with wide geographical distribution. This suggests that the integron is associated with mobile genetic structures facilitating horizontal transfer and dissemination, as previously suggested (29). Further studies should be performed to investigate the surrounding genetic structures and the transfer of this integron in these different genetic backgrounds.

In conclusion, this study further confirms the global distribution of *bla*_{VIM-2} located in an unusual integron and in new genetic *P. aeruginosa* backgrounds, emphasizing the requirement of global surveillance to fully understand the mechanisms of dissemination.

Nucleotide sequence accession numbers. The nucleotide accession numbers for the sequenced integrons have been registered in GenBank with the accession numbers [KC630980](#) to [KC630983](#).

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