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Genomic Characterization of VIM Metallo-β-Lactamase-Producing *Alcaligenes faecalis* from Gaza, Palestine

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ABSTRACT Carbapenemase-producing Gram-negative bacteria (CP-GNB) have increasingly spread worldwide, and different families of carbapenemases have been identified in various bacterial species. Here, we report the identification of five VIM metallo-β-lactamase-producing *Alcaligenes faecalis* isolates associated with a small outbreak in a large hospital in Gaza, Palestine. Next-generation sequencing analysis showed bla_{VIM-2} is harbored by a chromosomal genomic island among three strains, while bla_{VIM-4} is carried by a novel plasmid in two strains.

KEYWORDS *Alcaligenes faecalis*, Palestine, genome, metallo-β-lactamase

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Carbapenemase-producing Gram-negative bacteria (CP-GNB) have spread worldwide, and they have become a significant public health concern. Infections due to CP-GNB are usually associated with high morbidity and mortality rates. Nevertheless, our knowledge of the dissemination of CP-GNB in some isolated regions, such as the Gaza Strip, remains limited. The Gaza Strip is a narrow Palestinian territory (41 km long and 6 to 12 km wide) on the eastern coast of the Mediterranean Sea, with one of the most densely populated areas in the world. Over the past decades, due to the long-term effects of war, economic isolation, and border closures, the socioeconomic situation in Gaza has declined steadily, and the health care infrastructure within Gaza is largely damaged (1). The spread of multidrug-resistant bacteria, such as CP-GNB, into the Gaza Strip will further confound their current medical crisis. Here, we report an outbreak due to VIM metallo-β-lactamase-producing *Alcaligenes faecalis*, an unusual nonfermentative Gram-negative pathogen, in a major hospital in the Gaza Strip. We used next-generation sequencing (NGS) to characterize the VIM-producing *A. faecalis* isolates, the bla_{VIM}-harboring plasmids, and genomic islands.

Between February and April 2012, 5 VIM-producing *A. faecalis* isolates were collected from Al-Shifa Hospital, the largest medical complex and central hospital in the Gaza Strip, during a surveillance study for CP-GNB. The five specimens were collected from 2 outpatients and 3 inpatients from wounds ($n = 4$) and urine ($n = 1$) (Table 1). The first isolate (GZAF1) was collected from a wound sample in a male outpatient in February 2012. In the last week of April, three *A. faecalis* isolates were collected from three inpatients (2 from wound samples from patients in the gynecology ward and one from a male patient in the orthopedic surgery department), while one *A. faecalis* isolate was collected from a urinary sample from a female outpatient (Table 1). Due to the retrospective nature of the study, no additional clinical epidemiological information is available. The species identification was performed using 16S rRNA sequencing as described previously (2). Antimicrobial susceptibility was determined by broth microdilution in cation-adjusted Mueller-Hinton broth (MHB) according to Clinical and Labo-

TABLE 1 Characteristics of bla_{VIM}-harboring *A. faecalis* strains

Isolate	Patient type	Source	Resistance genes	MIC (μg/ml)[a]															
				IPM	MEM	CTX	CAZ	FEP	ATM	TIC/CLA	SXT	GEN	AMK	TOB	CIP	LEV	DOX	TGC	CST	PMB
GZAF1	Outpatient	Wound	bla_{VIM-4}, *aadB*, *sul1*	4	>8	>32	>16	>16	>16	128/2	>4/76	>8	8	>8	≥4	>8	8	1	4	4
GZAF2	Inpatient	Wound	bla_{VIM-4}, *sul1*	4	2	>32	>16	>16	16	>128/2	>4/76	2	≤4	≤1	≥4	4	≤2	0.5	2	2
GZAF3	Outpatient	Urine	*aadB*, bla_{VIM-2}, *sul1*, *aacA4*, *dfrB5*, *dfrA34*	8	2	>32	>16	>16	16	>128/2	>4/76	>8	32	>8	≥4	2	≤2	0.5	2	2
GZAF4	Inpatient	Wound	*aadB*, bla_{VIM-2}, *sul1*, *aacA4*, *dfrB5*, *dfrA34*	8	2	>32	>16	>16	>16	128/2	>4/76	>8	≤4	>8	≥4	>8	4	1	4	2
GZAF5	Inpatient	Wound	*aadB*, bla_{VIM-2}, *sul1*, *aacA4*, *dfrB5*, *dfrA34*	4	≤1	>32	>16	>16	>16	128/2	>4/76	>8	≤4	>8	≥4	>8	4	2	2	2

[a]MICs were determined using broth microdilution. IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; TIC/CLA, ticarcillin-clavulanate; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; DOX, doxycycline; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; CST, colistin; and PMB, polymyxin B.

ratory Standards Institute (CLSI) methods (3, 4) and interpreted using breakpoints for *Pseudomonas aeruginosa*, as breakpoints for *Alcaligenes* spp. have not been established. All five isolates exhibited reduced susceptibility to cefepime, cefotaxime, ceftazidime, and aztreonam and showed intermediate resistance or resistance to imipenem, but were susceptible to meropenem (except for GZAF1) (Table 1). VIM carbapenemases usually have higher hydrolysis activity against imipenem than against meropenem (5, 6). We suspect the discrepant carbapenem susceptibility profile may be attributed to the low level bla_{VIM} expressions and/or alterations in outer membrane permeability, which deserves further study. Moreover, all isolates showed resistance to ciprofloxacin and trimethoprim-sulfamethoxazole, and all except GZAF2 were resistant to gentamicin and tobramycin.

An in-house real-time PCR screening (7) revealed that all five isolates were positive for bla_{VIM}, while further Sanger sequencing showed that GZAF1 and GZAF2 harbor bla_{VIM-4}, but GZAF3, GZAF4, and GZAF5 carry bla_{VIM-2} (Table 1). The five genomes were then subjected to next-generation sequencing using the Illumina NextSeq platform, and the resultant genome assemblies were deposited in GenBank under the accession no. MSZN00000000, MSZO00000000, MSZP00000000, MSZQ00000000, and MSZR00000000. An *in silico* analysis of acquired resistance genes using ResFinder (8) identified 6 antimicrobial resistance genes in all 3 bla_{VIM-2}-harboring strains (GZAF3, GZAF4, and GZAF5), conferring resistance to β-lactams (bla_{VIM-2}), aminoglycoside (*aadB* and *aacA4*), sulfonamide (*sul1*), and trimethoprim (*dfrA34* and *dfrB5*). On the other hand, GZAF1 harbored 3 antimicrobial resistance genes (bla_{VIM-4}, *aadB*, and *sul1*), while GZAF2 carried 2 antimicrobial resistance genes (bla_{VIM-4} and *sul1*).

To explore the phylogenetic relationship among the five *A. faecalis* strains, a core single-nucleotide polymorphism (SNP) (defined as SNPs shared across all genomes) analysis was conducted using kSNP3.0 (9). A core SNP maximum likelihood tree was produced by RAxML 8.2.4 (10) using the GTRGAMMA model and 100 bootstrap replicates. An additional eight genomes from the GenBank whole-genome shotgun (WGS) database were also included in the phylogenetic analysis. The results showed that the 3 bla_{VIM-2}-harboring strains (GZAF3, GZAF4, and GZAF5) clustered together and differed by 1 to 3 core SNPs, suggesting a clonal spread of VIM-2-producing *A. faecalis* (see Fig. S1 in the supplemental material). Similarly, the two bla_{VIM-4}-harboring strains (GZAF1 and GZAF2) were closely related and differ only by a single SNP (Fig. S1). However, the bla_{VIM-2}-harboring strains (GZAF3, GZAF4, and GZAF5) were distinct in comparison to the two bla_{VIM-4}-harboring strains (GZAF1 and GZAF2) by an average of 9,707 SNPs, indicating that bla_{VIM-4}- and bla_{VIM-2}-harboring *A. faecalis* strains belong to two different clones.

To examine the genomic localization of bla_{VIM-4} and bla_{VIM-2} (chromosomal or plasmid borne), we conducted S1 nuclease pulsed-field gel electrophoresis (S1-PFGE), followed by Southern blotting using a digoxigenin (DIG)-labeled (Roche Diagnostics GmbH, Germany) bla_{VIM} probe. The probing results showed that bla_{VIM-4} in isolates GZAF1 and GZAF2 was harbored on a plasmid of ~120 kb (see Fig. S2), but the size of the plasmid in GZAF2 was smaller. In contrast, bla_{VIM-2} was carried on the chromosome

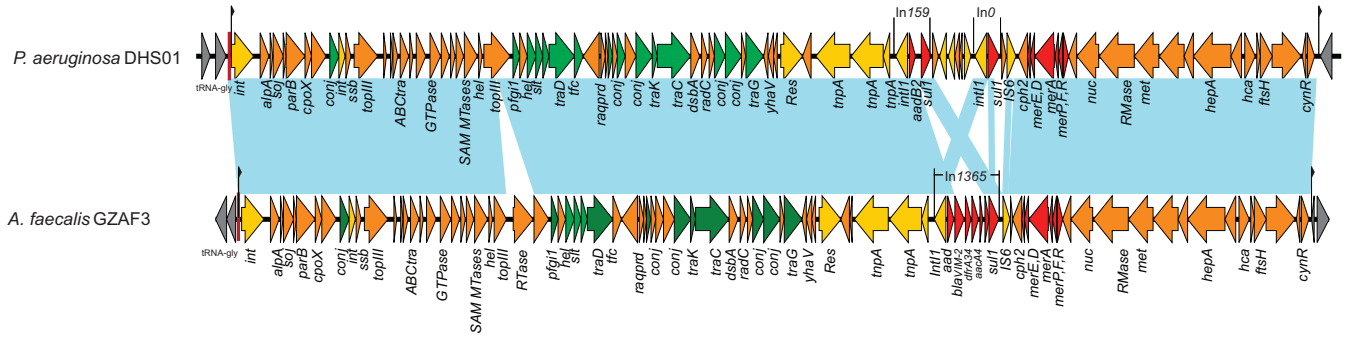

FIG 1 Genomic islands in *P. aeruginosa* DHS01 and *A. faecalis* GZAF3. Light blue shading denotes shared regions of homology with >99% identities. Open reading frames (ORFs) are portrayed by arrows and colored based on predicted gene function. Orange arrows indicate genomic island scaffold regions. The genes associated with gene transfer are indicated by green arrows, antimicrobial and mercury resistance genes are indicated by red arrows, and the accessory genes are indicated by yellow arrows. Small black arrowheads above the genome island indicate the 20-bp direct repeats.

of the three bla_{VIM-2}-harboring strains, GZAF3, GZAF4, and GZAF5 (Fig. S2). Further sequence analysis and PCR gap closure showed that bla_{VIM-2} is located in identical genomic islands on the chromosomes in GZAF3, GZAF4, and GZAF5. The genomic island, named as GZAF3_GI (deposited in GenBank as accession no. KY623658), is 92 kb in length and integrated into a tRNA-Gly site on the *A. faecalis* chromosome, flanked by two 20-bp direct repeats (GATTCCCATCACCCGCTCCA). A BLASTn analysis showed that the overall structure of GZAF3_GI is similar to several genomic islands identified in *P. aeruginosa* strains, including DHS01 (accession no. CP013993), E6130952 (CP020603), RIVM-EMC2982 (CP016955), NCGM257 (AP014651), RI_BP-14 (KX196169), Carb01_63 (CP011317), RI_KMU-P11 (KX196167), and RI_IH-2 (KX196168), with over 94% query coverage and maximal 99% nucleotide identities. In comparison to the other genomic islands in *P. aeruginosa*, the major difference in GZAF3_GI is that it carries an additional group II intron reverse transcriptase (RTase) gene and harbors a novel integron, In*1365*. In*1365* harbors a novel gene cassette array that contains *aadB*-bla_{VIM-2}-*dfrA34-aacA4-dfrB5*. A sequence comparison between the genomic islands in DHS01 and GZAF3_GI is displayed in Fig. 1.

S1-PFGE showed that GZAF1 and GZAF2 each harbor a single plasmid (Fig. S2). Thus, the two natural plasmids (namely pGZAF1_VIM and pGZAF2_VIM) were extracted from the two *A. faecalis* strains and were directly sequenced by Illumina NextSeq. Sequencing reads were assembled *de novo* using SPAdes 3.10.0 (11), resulting in a single head-to-tail contig for both plasmids. The plasmids were closed by PCR using outward-directed primers targeting the two ends of the contigs, followed by Sanger sequencing. The results showed that pGZAF1_VIM (deposited in GenBank as accession no. KY623659) is 124,415 bp in length, with an average GC content of 50.7% (Fig. 2). pGZAF2_VIM is 121,408 bp in length and is nearly identical to pGZAF1_VIM, except for a 3,007-bp deletion encompassing a class I integron, In*7* (harboring *aadB*). A BLASTn search of pGZAF1_VIM and pGZAF2_VIM sequence backbones (excluding the resistance and accessory genes) against GenBank did not identify any matches, suggesting they are novel plasmids. pGZAF1_VIM and pGZAF2_VIM carry a novel replication protein gene, *repA*, with the highest amino acid sequence identity (~46%) and 89% query coverage to the replication protein in *Pseudomonas* sp. HPB0071 (WP_010799484). The bla_{VIM-4} in pGZAF1_VIM and pGZAF2_VIM is located in a novel class I integron, In*1366*, with the gene cassette array of bla_{VIM-4}-*smr2*. To the best of our knowledge, pGZAF1_VIM and pGZAF2_VIM represent the first completely characterized carbapenemase gene-harboring plasmids in *Alcaligenes* spp.

A. faecalis, belonging to the family *Alcaligenaceae*, is a Gram-negative rod-shaped opportunistic pathogen commonly found in soil, water, and other environments (12). Nosocomial infections caused by *A. faecalis* have mostly occurred in immunocompromised hosts and are often caused by contamination of hospital equipment or fluids (12). *A. faecalis* has been rarely found to carry antimicrobial resistance genes,

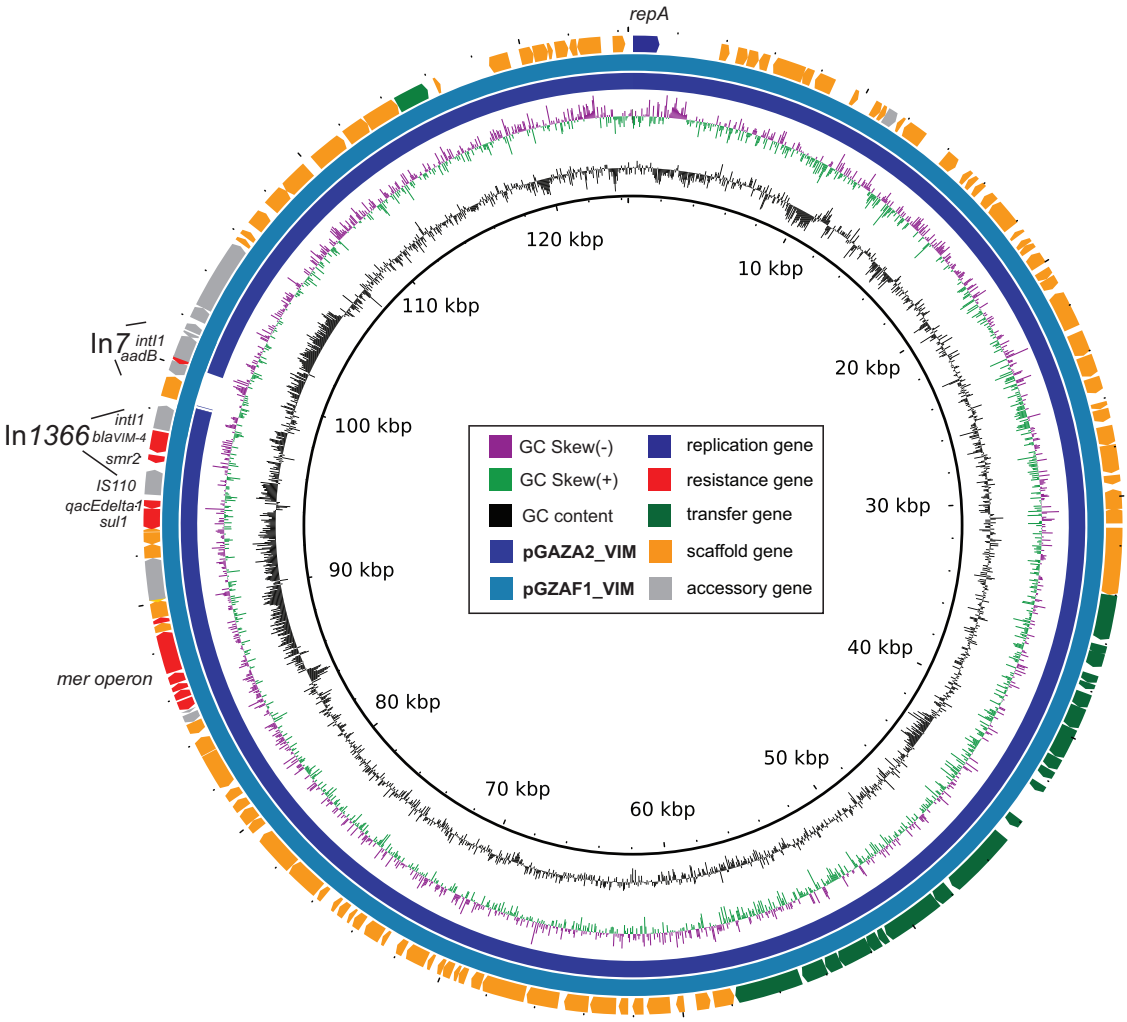

FIG 2 Plasmid structures of pGAZA1_VIM and pGAZA2_VIM.

although sporadic reports of extended-spectrum-β-lactamases (ESBLs) genes bla_{PER-1}- or bla_{TEM-21}-bearing strains have been described (13, 14). More recently, carbapenem resistance was reported for the first time in *A. faecalis* clinical isolates from hospitalized patients in a tertiary care center in India. The carbapenem resistance in these isolates was linked to the presence of the bla_{VIM-6} gene (15). However, no genomic sequences were present in that study; therefore, there is no information regarding the localization of bla_{VIM-6} in these strains nor their phylogenetic makeup, providing no opportunity to compare to the Gaza *A. faecalis* strains.

In summary, we described a small hospital outbreak of VIM-producing *A. faecalis*, caused by two different *A. faecalis* clones, in Gaza, Palestine, a highly populated region with poor public health services and limited medical resources. Two of five patients were outpatients, suggesting that the VIM-producing *A. faecalis* may have spread beyond the hospital setting in Gaza. Notably, we previously reported the pandrug-resistant OXA-48-producing *Proteus mirabilis* and clonal spread of toxic shock syndrome toxin (TSST-1)-producing methicillin-resistant *Staphylococcus aureus* (MRSA) in the same area (16, 17). In addition, ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*, NDM-2-producing *Acinetobacter baumannii*, and VIM-producing *P. aeruginosa* from Gaza have also been documented (18–20). Apparently, besides other challenges, Gaza is facing an antimicrobial resistance crisis as a result of the emergence of these highly multidrug-resistant organisms. Epidemiological surveillance and strict infection control programs should be strongly considered to monitor and prevent the spread of these

multidrug-resistant organisms, such as VIM-producing *A. faecalis*, in the Gaza Strip and other regions.

Accession number(s). The draft genome sequences of the five *A. faecalis* isolates have been deposited within the GenBank whole-genome shotgun (WGS) database under the accession no. MSZN00000000, MSZO00000000, MSZP00000000, MSZQ00000000, and MSZR00000000. The complete nucleotide sequences of GZAF3_GI and pGZAF1_VIM have been deposited as GenBank accession no. KY623658 and KY623659, respectively.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01499-17.

SUPPLEMENTAL FILE 1, PDF file, 1.4 MB.

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