AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

LETTER TO THE EDITOR

Detection of *poxtA2*, a Presumptive *poxtA* Ancestor, in a Plasmid from a Linezolid-Resistant *Enterococcus gallinarum* Isolate

Ilaria Baccani,[a] Alberto Antonelli,[a,b] Vincenzo Di Pilato,[b,c] Marco Coppi,[a,b] Tiziana Di Maggio,[d] Michele Spinicci,[a,e] Ana Liz Villagran,[f] Carmen Revollo,[g] Alessandro Bartoloni,[a,e] Gian Maria Rossolini[a,b]

[a]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[b]Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy
[c]Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy
[d]Department of Medical Biotechnologies, University of Siena, Siena, Italy
[e]Infectious and Tropical Diseases Unit, Careggi University Hospital, Florence, Italy
[f]Hospital Básico Villa Montes, Villa Montes, Plurinational State of Bolivia
[g]Instituto Nacional de Laboratorios de Salud "Dr. Nestor Morales Villazón" (INLASA), La Paz, Plurinational State of Bolivia

KEYWORDS *poxtA*, linezolid resistance, *Enterococcus*, IS*1216*

The *poxtA* gene encodes a protein belonging to the F lineage of the ATP-binding cassette superfamily, which can protect the bacterial ribosome from some antiribosomal antibiotics, including oxazolidinones (1). After the first identification in a methicillin-resistant *Staphylococcus aureus* of clinical origin (1), several reports of *poxtA*-positive isolates of *Enterococcus* spp. of animal and human origin from different countries have documented the broad and intersectoral dissemination of this transferable resistance gene, mainly among enterococci (2–8).

During a survey on antibiotic-resistant bacteria carried out in 2018 among the rural population living in the Bolivian Chaco region, an *Enterococcus gallinarum* (Eg-IV02) resistant to linezolid (MIC of 8 μg/ml by reference broth microdilution [9]) was isolated from a fecal swab collected from a healthy child after plating the swab on CNA-cv Sh medium (bioMérieux, Marcy l'Etoile, France) supplemented with 16 μg/ml of florfenicol.

Multiplex real-time PCR was positive for the *poxtA* gene but not for the *optrA*, *cfr*, or *cfr*(B) genes, which are the other known transferable linezolid resistance determinants encountered in Gram-positive cocci.

Whole-genome sequencing (WGS) analysis of *E. gallinarum* Eg-IV02, performed using both Illumina MiSeq (Illumina, San Diego, CA) and MinION (Oxford Nanopore Technologies, Oxford, UK) platforms to yield a *de novo* hybrid assembly generated by Unicycler v0.4.6 (10) revealed the presence of a *poxtA*-like gene, named *poxtA2*, carried on a 13,746-bp plasmid, named pIB-BOL (GenBank accession number MZ171245) (Fig. 1a). The plasmid carried a *repB* gene closely related (98% identity) to that of an 11-kb plasmid from a *cfr*-harboring *Enterococcus faecalis* of animal origin from China (GenBank accession number CP028840.1) and also a *fexA* phenicol resistance determinant, located upstream of *poxtA2*. Both genes were flanked by IS*1216*-like insertion sequences, likely involved with their mobilization (Fig. 1a).

Unlike *poxtA*, *poxtA2* was not truncated by an IS*1216* insertion at the 3′ end (Fig. 1b). Consequently, PoxtA2 differed from PoxtA by a few amino acids at the C terminus, which showed some detectable homology with the closest relative of the same protein family, namely, OptrA (Fig. 1c). Altogether, these findings suggest that *poxtA2* likely represents the ancestor of *poxtA*, mobilized by a recombination event that had not truncated the gene at the 3′-end. In fact, the genetic context of *poxtA2* was different from that of *poxtA* (Fig. 1b), supporting the notion that the two genes had been mobilized by independent recombination events.

Citation Baccani I, Antonelli A, Di Pilato V, Coppi M, Di Maggio T, Spinicci M, Villagran AL, Revollo C, Bartoloni A, Rossolini GM. 2021. Detection of *poxtA2*, a presumptive *poxtA* ancestor, in a plasmid from a linezolid-resistant *Enterococcus gallinarum* isolate. Antimicrob Agents Chemother 65:e00695-21. https://doi.org/10.1128/AAC.00695-21.

Address correspondence to Gian Maria Rossolini, gianmaria.rossolini@unifi.it.

Accepted manuscript posted online 7 June 2021
Published 16 July 2021

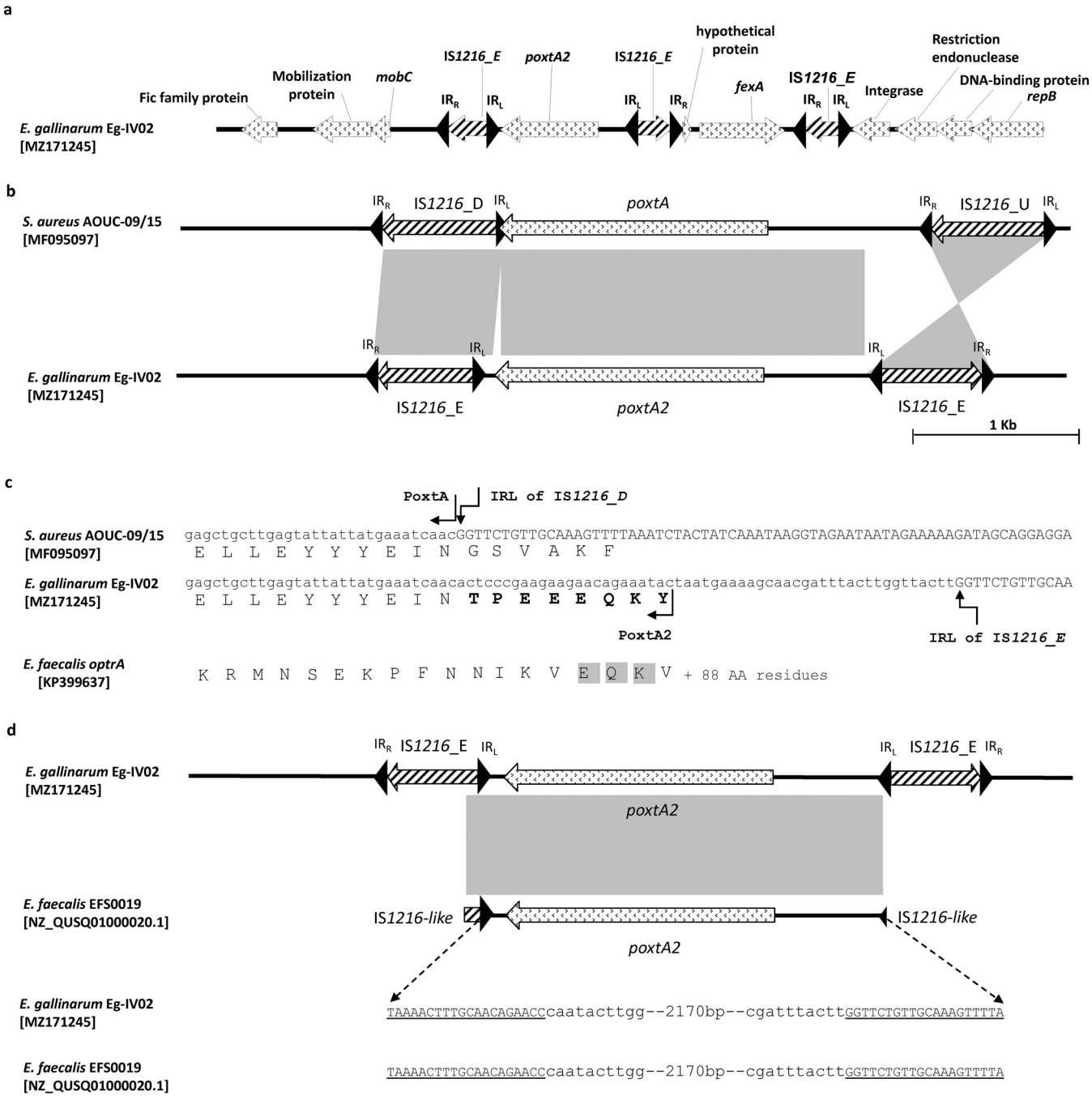

FIG 1 (a) Map of pIB-BOL plasmid from *E. gallinarum* Eg-IV02. (b) Comparison of the genetic context of *poxtA* in the chromosome of *S. aureus* AOUC-09/15 (1) and in plasmid pIB-BOL; regions with >99% nucleotide identity are connected by gray zones, transposase-encoding genes are indicated by striped arrows, and IRs are indicated by black triangles. (c) Nucleotide sequences showing the insertion points of IS*1216*_D at the 3′-end of *poxtA* and of IS*1216*_E downstream *poxtA2*. The sequence of IS*1216* elements is capitalized. Translation of the *poxtA* and *poxtA2* coding sequences is also shown, and the different residues at the C terminus of PoxtA2 are boldfaced. Alignment of the C terminus of PoxtA and PoxtA2 with the homologous region of OptrA is also shown. (d) Comparison of the genetic contexts of *poxtA2* and of the identical gene recently reported from an *E. faecalis* EFS0019 of animal origin from Korea (GenBank accession number NZ_QUSQ01000020.1) (12). The gray shaded zones indicate 100% nucleotide sequence homology. The sequences of the junctions are also shown (uppercase letters for IS*1216* elements), revealing the identity between the two genetic contexts.

Plasmid pIB-BOL was transferred to *E. faecalis* JH2-2 by electrotransformation (11). Transformants were selected on tryptic soy agar containing 8 μg/ml florfenicol. Acquisition of pIB-BOL by *E. faecalis* JH2-2 was associated with a significant increase of linezolid, florfenicol, and chloramphenicol MICs and with a lower increase of doxycycline MIC, while susceptibility to tedizolid, tetracycline, and tigecycline was apparently

not affected (see Table S1 in the supplemental material). Since *poxtA2* and *fexA* were the only resistance determinants carried by the plasmid (Fig. 1a), these results confirmed that *poxtA2* was functional in conferring protection from linezolid, while the effect on phenicols could be ascribed to *fexA*.

A search of the NCBI sequence databases (carried out on 25 May 2021) revealed only another *poxtA*-like gene identical to *poxtA2* from an *E. faecalis* isolate of animal origin from Korea (GenBank accession number NZ_QUSQ01000020.1) (12). Even that gene was flanked by two IS*1216*-like elements and exhibited a genetic context identical to that of *poxtA2* from pIB-BOL (Fig. 1d), suggesting a common origin from a unique mobilization event. Interestingly, the same database search also revealed two *poxtA*-like genes encoding proteins identical to PoxtA but for a single amino acid change (G33H or R256H, respectively) from different *Enterococcus faecium* isolates in China (NCBI:protein accession numbers WP_212481470.1 and WP_159373727.1, respectively).

Overall, these findings identified a *poxtA*-related gene, which (i) appears to be the presumptive ancestor of *poxtA* and (ii) has been mobilized to enterococci circulating in human and animal settings. The intersectoral spreading of these resistance determinants, mediating transferable linezolid resistance, is concerning and mandates for surveillance. In this perspective, it should be noted that the prevalence of *poxtA2* might be underestimated in case of screening using primers targeting external regions of the *poxtA* gene.

Data availability. Sequence data have been deposited in GenBank under the accession number MZ171245.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENT

This study was supported by internal funding.

REFERENCES

1. Antonelli A, D'Andrea MM, Brenciani A, Galeotti CL, Morroni G, Pollini S, Varaldo PE, Rossolini GM. 2018. Characterization of *poxtA*, a novel phenicol–oxazolidinone–tetracycline resistance gene from an MRSA of clinical origin. J Antimicrob Chemother 73:1763–1769. https://doi.org/10.1093/jac/dky088.
2. Fioriti S, Morroni G, Coccitto SN, Brenciani A, Antonelli A, Di PV, Baccani I, Pollini S, Cucco L, Morelli A, Paniccià M, Magistrali CF, Rossolini GM, Giovanetti E. 2020. Detection of oxazolidinone resistance genes and characterization of genetic environments in enterococci of swine origin, Italy. Microorganisms 8:2021. https://doi.org/10.3390/microorganisms8122021.
3. Na SH, Moon DC, Kim MH, Kang HY, Kim SJ, Choi JH, Mechesso A-F, Yoon S-S, Lim S-K. 2020. Detection of the phenicol–oxazolidinone resistance gene *poxtA* in *Enterococcus faecium* and *Enterococcus faecalis* from food-producing animals during 2008–2018 in Korea. Microorganisms 8:1839. https://doi.org/10.3390/microorganisms8111839.
4. Li D, Cheng Y, Schwarz S, Yang M, Du X-D. 2019. Identification of a *poxtA*- and *cfr*-carrying multiresistant *Enterococcus hirae* strain. J Antimicrob Chemother 75:482–484.
5. Papagiannitsis CC, Tsilipounidaki K, Malli E, Petinaki E. 2019. Detection in Greece of a clinical *Enterococcus faecium* isolate carrying the novel oxazolidinone resistance gene *poxtA*. J Antimicrob Chemother 74:2461–2462. https://doi.org/10.1093/jac/dkz155.
6. Freitas AR, Tedim AP, Duarte B, Elghaieb H, Abbassi MS, Hassen A, Read A, Alves V, Novais C, Peixe L. 2020. Linezolid-resistant (Tn6246::*fexB-poxtA*) *Enterococcus faecium* strains colonizing humans and bovines on different continents: similarity without epidemiological link. J Antimicrob Chemother 75:2416–2423. https://doi.org/10.1093/jac/dkaa227.
7. Egan SA, Shore AC, O'Connell B, Brennan GI, Coleman DC. 2020. Linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* from hospitalized patients in Ireland: high prevalence of the MDR genes *optrA* and *poxtA* in isolates with diverse genetic backgrounds. J Antimicrob Chemother 75:1704–1711. https://doi.org/10.1093/jac/dkaa075.
8. Moure Z, Lara N, Marín M, Sola-Campoy PJ, Bautista V, Gómez-Bertomeu F, Gómez-Dominguez C, Pérez-Vázquez M, Aracil B, Campos J, Cercenado E, Oteo-Iglesias J, Spanish Linezolid-Resistant Enterococci Collaborating Group. 2020. Interregional spread in Spain of linezolid-resistant *Enterococcus* spp. isolates carrying the *optrA* and *poxtA* genes. Int J Antimicrob Agents 55:105977. https://doi.org/10.1016/j.ijantimicag.2020.105977.
9. International Organization for Standardization. 2019. ISO 20776–1: Clinical laboratory testing and *in vitro* diagnostic test systems. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices-Part 1: Reference method for the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. International Organization for Standardization, Geneva, Switzerland.
10. Coppi M, Di Pilato V, Monaco F, Giani T, Conaldi PG, Rossolini GM. 2020. Ceftazidime-avibactam resistance associated with increased bla_{KPC-3} gene copy number mediated by pKpQIL plasmid derivatives in sequence type 258 *Klebsiella pneumoniae*. Antimicrob Agents Chemother 64:e01816-19. https://doi.org/10.1128/AAC.01816-19.
11. Brenciani A, Morroni G, Pollini S, Tiberi E, Mingoia M, Varaldo PE, Rossolini GM, Giovanetti E. 2016. Characterization of novel conjugative multiresistance plasmids carrying *cfr* from linezolid-resistant *Staphylococcus epidermidis* clinical isolates from Italy. J Antimicrob Chemother 71:307–313. https://doi.org/10.1093/jac/dkv341.
12. Jung YH, Cha MH, Woo GJ, Chi YM. 2021. Characterization of oxazolidinone and phenicol resistance genes in non-clinical enterococcal isolates from Korea. J Glob Antimicrob Resist 24:363–369. https://doi.org/10.1016/j.jgar.2021.01.009.