Genetic characteristics of VanA-type vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in Cuba

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Abstract

VanA-type vancomycin-resistant enterococci isolates from bloodstream infections in Cuba were genetically characterized. *Enterococcus faecalis* isolates were assigned to sequence type (ST) 28, closely related to Eastern Europe, while *Enterococcus faecium* belonged to ST262, ST656 and ST1349, and showed different genetic profiles.

Keywords: Cuba, *Enterococcus faecalis*, *Enterococcus faecium*, ST, VanA

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Enterococci are commensal organisms of the alimentary tract in humans; nonetheless, they have been identified as a cause of opportunistic infections in immunocompromised hosts. Emergence and spread of vancomycin-resistant enterococci (VRE) pose a threat to public health worldwide, mainly in Europe [1]. In Cuba, VRE was first reported in Havana in 2000 and 2001 (one isolate each of *Enterococcus faecalis* (VanB) and *Enterococcus faecium* (VanA) among 99 isolates) [2,3], and low prevalence of VRE (0.4%) had been observed until 2005 [4]. During the period 2010 to 2013, five VanA-type VRE were detected among 596 isolates collected through national surveillance, but systemic surveillance of VRE has since stopped. In the present study, the five VRE isolates (two *E. faecalis* and three *E. faecium* isolates) stored previously were further genetically analysed to obtain key information for control policies in Cuba.

These VRE isolates were derived from blood samples collected from different patients in different provinces in Cuba (Table 1). All the isolates were resistant to vancomycin and teicoplanin, having *vanA* gene with identical sequences, which were deposited to GenBank under accession numbers MG460317 and MG460318 for *E. faecalis* isolate CU709 and *E. faecium* isolate CU710, respectively, and showed high-level resistance to gentamycin associated with *aac(6′)-aph(2″)* (Table 1). Except for *E. faecium* CU710, all the isolates had mutations in quinolone-resistance–determining regions in *gyrA* and/or *parC* showing resistance to levofloxacin. *E. faecalis* isolates had various virulence factors including *esp* and *gelE*, while *E. faecium* harboured a few virulence genes, with *esp* being carried only by the isolate CU207. Except for *E. faecium* isolate CU710, all the isolate had *vanA* plasmid stabilization loci ω-ε-ζ, but not *axe-txe*. Two *E. faecalis* exhibited *rep*-family profile 2-9, which represents commonly found plasmids in this species [5]. In contrast, *rep*-4 of a rare plasmid in *E. faecium* was found in the three *E. faecium* isolates. It was of note that insertion element IS*16*, a nosocomial marker generally associated with clonal complex (CC) 17 isolates, was not found in sequence type (ST) 262 *E. faecium* isolate CU710, while it was positive in ST1349 (non-CC17 lineage) isolate CU850. Moreover, genetic profiles were also different between CU710 and CU850, suggesting that these are genetically unrelated, although ST1379 is a single-locus variant (SLV) of ST262.

Both *E. faecalis* isolates were classified into ST28 (CC87), which was previously reported as one of the common lineages in Poland [6,7]. *E. faecium* isolates belonged to three different genotypes (ST262, ST656 and ST1349). ST656 is a SLV of ST412 belonging to CC17, and ST1349 (SLV of ST262) is a new ST identified in the present study. ST412 had been identified in South America (Brazil, Peru, Colombia, Venezuela) [8,9] and ST262 in China, Russia and Denmark [10–12]. However, relatedness of the Cuban *E. faecium* isolates to those countries was not definite by only ST because the *E. faecium* genome is subject to a high rate of recombination, causing change in ST [13]. In the first report of VRE in Cuba [3], genetic backgrounds of VRE were distinct from vancomycin-susceptible isolates; therefore, occurrence of autochthonous VRE was not evident. Genetic diversity and unique traits of *E. faecium* found in the present study suggest differences in origin or in the molecular

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TABLE 1. Genetic characteristics and drug resistance of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in Cuba

Isolate	CU709	CU789	CU207	CU710	CU850
Enterococcus species	*faecalis*	*faecalis*	*faecium*	*faecium*	*faecium*
Year of isolation	2011	2012	2010	2011	2013
Province	Villa Clara	Holguin	Havana	Havana	Santiago de Cuba
Specimen	Blood	Blood	Blood	Blood	Blood
ST (CC)	ST28 (CC87)	ST28 (CC87)	ST656 (CC17)	ST262 (CC17)	ST1349
Resistance pattern	CMZ, FMX, GEN, ABK, FOX, ERY, CLI, VAN, TEC, Q-D, SXT, LVX	CMZ, FMX, GEN, ABK, FOX, ERY, CLI, VAN, TEC, Q-D, SXT, LVX	AMP, OXA, CFZ, CMZ, FMX, IPM, GEN, FOX, ERY, CLI, VAN, TEC, SXT, LVX	AMP, OXA, CFZ, CMZ, FMX, IPM, GEN, FOX, ERY, CLI, VAN, TEC, SXT	AMP, OXA, CFZ, CMZ, FMX, IPM, GEN, MIN, FOX, ERY, CLI, VAN, TEC, SXT, LVX
Virulence factors[a]	*gelE, esp, cpd, ccf, cob, efaAfs, ace*	*gelE, esp, cpd, ccf, cob, efaAfs, ace*	*esp, ccf, efaAfm, acm*	*efaAfm, acm*	*efaAfm, acm*
Drug resistance gene[b]	*aac(6′)-Ie-aph(2″)-Ia, tet(M), erm(B)*	*aac(6′)-Ie-aph(2″)-Ia, tet(M), erm(B)*	*aac(6′)-Ie-aph(2″)-Ia, aac(6′)-Ii, ant(6)-Ia, aph(3′)-IIIa, tet(M), tet(O), erm(B), msrB*	*aac(6′)-Ie-aph(2″)-Ia, aac(6′)-Ii, ant(6)-Ia, aph(3′)-IIIa, tet(U), erm(B)*	*aac(6′)-Ie-aph(2″)-Ia, aac(6′)-Ii, ant(6)-Ia, aph(3′)-IIIa, tet(M), tet(O), erm(B), msrB*
Mutation in QRDR					
gyrA	S 84 I	S 84 I	S 84 R	No mutation	No mutation
parC	S 85 I	S 85 I	S 82 R	No mutation	S 82 R
IS16	–	–	+	–	+
Toxin–antitoxin system[c]	ε-ω-ζ	ε-ω-ζ	ε-ω-ζ	—	ε-ω-ζ
rep family profile	2–9	2–9	2–4–14	2–4	2–4–14

ABK, arbekacin; AMP, ampicillin; CC, clonal complex; CFZ, cefazolin; CLI, clindamycin; CMZ, cefmetazole; ERY, erythromycin; FMX, flomoxef; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MIN, minocycline; OXA, oxacillin; QRDR, quinolone resistance–determining region; Q-D, quinupristin/dalfopristin; ST, sequence type; SXT, sulfamethoxazole/trimethoprim; TEC, teicoplanin; VAN, vancomycin. All isolates showed high-level resistance to GEN (MIC >1024 μg/mL) but were susceptible to fosfomycin and linezolid.

[a] *cylA*, egg and *asaI* were not detected in *E. faecalis* strains. *gelE, cylA, cpd, agg, cob* and *hyl* were not detected in *E. faecium* isolates.

[b] The following genes were not detected in any isolates: *blaZ, tet(K), tet(L), tet(S), tet(T), erm(A), erm(C), msrA, msrC, aac(6′)-Ib-cr, aac(6′)-Im, ant(3″)-Ia, ant4′-1a, ant(9)-Ia, ant(9)-Ib, aph(2″)-Ib, aph(2″)-Ic, aph(2″)-Id, qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxAB, vatA, vatB, vatC, vatD, vatE, mefAE, lnuA, lnuB.*

[c] *vanA*-plasmid stabilizations loci ε-ω-ζ and *axe-txe* were examined; *axe-txe* was not detected in any isolates.

evolutionary process. To understand the recent trend of VRE and their origins, it is imperative to resume surveillance and molecular epidemiologic studies on VRE in Cuba.

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Conflict of interest

None declared.

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