AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Loss of a Major Enterococcal Polysaccharide Antigen (Epa) by *Enterococcus faecalis* Is Associated with Increased Resistance to Ceftriaxone and Carbapenems

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KEYWORDS *Enterococcus faecalis*, Epa

The enterococcal polysaccharide antigen (Epa) locus of *Enterococcus faecalis* encodes enzymes and transporters involved in *E. faecalis* cell wall polysaccharide metabolism (1–3). We previously showed that deletion of *epaB* (which encodes a putative glycosyl transferase and possibly mediates transfer of rhamnose to cell wall polysaccharides [1]) and disruption of several other *epa* genes resulted in reduced virulence in murine peritonitis (1, 2) and urinary tract infection (4) models. In addition, OG1RFΔ*epaB* and disruption of *epaB* were impaired in colitogenic activity in IL-10$^{-/-}$ mice (5) and showed lysozyme susceptibility (5), attenuation in nonvertebrate models (5), translocation (1), biofilm formation, resistance to polymorphonuclear leukocyte killing, susceptibility to NPV-1 phage (1), and marked alteration in cell morphology, suggesting a role for Epa in cell wall synthesis or function (1).

A recent study on *Enterococcus hirae* LcpA (Psr) (6) postulated that LcpA catalyzes the attachment of "rhamnose-containing polysaccharides" onto the cell's peptidoglycan (6). Reasoning that altering rhamnose-containing polysaccharides of *E. faecalis* could alter its peptidoglycan and thus the activity of beta-lactams, we examined our *E. faecalis epa* mutants, including those lacking cell wall rhamnose, to determine whether the MICs of select beta-lactams against these mutants differ versus OG1RF.

The strains used included wild-type (WT) OG1RF (7, 8), OG1RFΔ*epaB*, reconstituted OG1RFΔ*epaB*::*epaB* (5, 9), and several *epa* disruption mutants (1, 2) [OG1RF*epaA*::*aph(3′)-IIIa*, OG1RF*epaB*::*aph(3′)-IIIa*, OG1RF*epaE*::*aph(3′)-IIIa*, OG1RF*epaL*::*aph(3′)-IIIa*, and OG1RF*epaN*::*aph(3′)-IIIa*] representing different transcripts of the *epa* gene cluster.

MICs were determined with Etest strips of ceftriaxone (CRO), ampicillin (AMP), doripenem (DOR), meropenem (MEM), and daptomycin (DAP; which acts via unrelated mechanisms) (AB Biodisk, Solna, Sweden; Liofilchem, Inc., Waltham, MA) on cation-adjusted Mueller-Hinton II (Oxoid, UK) agar plates. MICs were read according to the manufacturer's guidelines, and MIC determinations were repeated at least twice with reproducible results. Table 1 shows the MICs of test antibiotics against WT OG1RF and *epa* mutants.

The CRO MIC increased from 24 μg/ml (OG1RF and OG1RFΔ*epaB*::*epaB*) to >256 μg/ml with OG1RFΔ*epaB* and the disruption mutants, including OG1RF*epaA*::*aph(3′)-IIIa*, which has the most growth impairment (1). The DOR and MEM MICs were also considerably higher with *epa* mutants than with OG1RF and OG1RFΔ*epaB*::*epaB*.

AMP MIC differences were modest between OG1RF and the *epa* mutants and the growth impaired OG1RF*epaA*::*aph(3′)-IIIa* mutant did not show any change. The DAP MIC was the same for all strains except OG1RF*epaA*::*aph(3′)-IIIa*, likely due to its growth impairment.

Citation Singh KV, Murray BE. 2019. Loss of a major enterococcal polysaccharide antigen (Epa) by *Enterococcus faecalis* is associated with increased resistance to ceftriaxone and carbapenems. Antimicrob Agents Chemother 63:e00481-19. https://doi.org/10.1128/AAC.00481-19.

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Accepted manuscript posted online 11 March 2019
Published 25 April 2019

TABLE 1 MICs against WT and *epa* locus mutants of *E. faecalis* OG1RF

Organism	E-test MIC (μg/ml)[a]				
	CRO	AMP	DOR	MEM	DAP
WT *E. faecalis* OG1RF	24	0.5	6	4	1–2
OG1RF*epaA*::*aph(3′)-IIIa*	>256	0.5	>32	6	0.25–0.38
OG1RF*epaE*::*aph(3′)-IIIa*	>256	1	>32	12	NT
OG1RF*epaL*::*aph(3′)-IIIa*	>256	1	>32	12	NT
OG1RF*epaN*::*aph(3′)-IIIa*	>256	1	>32	24	NT
OG1RF*epaB*::*aph(3′)-IIIa*	>256	1.5	>32	>32	1.5–2
OG1RFΔ*epaB*	>256	2	>32	>32	1–2
OG1RFΔ*epaB*::*epaB*	24	1	4	4	1–2

[a]CRO, ceftriaxone; AMP, ampicillin; DOR, doripenem; MEM, meropenem; DAP, daptomycin; NT, not tested.

Our previous data on polysaccharide (PS) content showed that a major polysaccharide band was missing in *epa* disruption mutants and was restored in the *epaB* complemented strain (1). In addition, our analyses of purified polysaccharide (Epa) from WT OG1RF and the OG1RF*epaB*::*aph(3′)-IIIa* mutant showed that Epa is composed of glucose, rhamnose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose, while there was no rhamnose present in Epa purified from the OG1RF*epaB*::*aph(3′)-IIIa* but, instead, mannose was present (1).

Our results indicate that altered cell wall polysaccharide content, such as lack of rhamnose and/or the presence of mannose in *epa* mutant cell walls, is associated with marked resistance to CRO, DOR, and, MEM. The mechanism for resistance remains to be determined.

ACKNOWLEDGMENT

We thank Karen Jacques-Palaz for technical assistance.

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