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HOST AND BACTERIAL FACTORS COOPERATIVELY DISRUPT HEALING OF INTESTINAL ANASTOMOSES

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Purpose:
Anastomotic leakage can be a devastating complication for pediatric surgical patients, particularly in those colonized with resistant organisms. Recent studies suggest that the intestinal microbiome contributes to failure of healing after anastomosis and specifically implicates factors elaborated by *Enterococcus faecalis*. We tested the hypothesis that the gelE/sprE operon, known to regulate collagenase production in this microorganism, plays a key role in *E. faecalis*-mediated anastomotic leakage.

Methods:
All experiments were approved by the Institutional Animal Care and Use Committee. Following prophylactic injection of intramuscular cefoxitin (50 mg/kg), 9-week-old, 250-300g male Wistar rats (n=37) were subjected to segmental distal colectomy and primary anastomosis. Rats were then randomized to receive enemas with isogenic strains of *E. faecalis* lacking gelE/sprE (ΔgelE/ΔsprE) or expressing gelE/sprE (VT07). Animals were sacrificed on postoperative day 6 and evaluated for gross evidence of anastomotic leak. Anastomotic tissues were examined for bacterial species identification, collagenase production, and MMP9, a host matrix metalloprotease known to play a key role in anastomotic complications.

Results:
Anastomotic leakage was significantly greater in rats inoculated with the collagenase-producing VT07 strain of *E. faecalis* (12/17, 70%) compared to its isogenic null mutant ΔgelEΔsprE (1/20, 5%) ($p<0.01$). More severe leaks appeared to be associated with high adherence of *E. faecalis* to the mucosa, penetration into the serosa, and high collagenase activity. Zymography demonstrated increased MMP9 activity in VT07 compared to the null mutant.

Conclusions:
Our data suggests that collagenase-producing *Enterococcus faecalis* is associated with anastomotic leak in a rat model of intestinal anastomosis. Mechanisms that involve the quorum sensing-regulated gelE/sprE operon may promote collagenase production and are associated with host MMP9. Further understanding of the contribution of antibiotic-resistant organisms to anastomotic failure may improve the care of chronically hospitalized children.

Notes: