Microbiota-derived lantibiotic restores resistance against vancomycin-resistant Enterococcus

Letters

Sohn G. Kim1,2, Simone Becattini1, Thomas U. Moody1,3, Pavel V. Shliaha4, Eric R. Littmann1, Ruth Seok1, Mergim Gjonbalaj1, Vincent Eaton1, Emily Fontana3, Luigi Amoretti8, Roberta Wright1, Silvia Caballero1,2, Zhong-Min X. Wang5, Hea-Jin Jung1, Sejel M. Morjaria2, Ingrid M. Leiner1,3, Weige Qin6, Ruben J. J. F. Ramos6, Justin R. Cross6, Seiko Narushima7, Keny Honda7,8,9, Jonathan U. Peled2,10, Ronald C. Hendrickson4,11, Ying Taur5, Marcel R. M. van den Brink1,2,10 & Eric G. Pamer1,2,3,5a

Intestinal commensal bacteria can inhibit dense colonization of the gut by vancomycin-resistant Enterococcus faecium (VRE), a leading cause of hospital-acquired infections1,2. A four-strained consortium of commensal bacteria that contains Blautia producta BPSCSK can reverse antibiotic-induced susceptibility to VRE infection3. Here we show that BPSCSK reduces growth of VRE by secreting a lantibiotic that is similar to the nisin-A produced by Lactococcus lactis. Although the growth of VRE is inhibited by BPSCSK and L. lactis in vitro, only BPSCSK colonizes the colon and reduces VRE density in vivo. In comparison to nisin-A, the BPSCSK lantibiotic has reduced activity against intestinal commensal bacteria. In patients at high risk of VRE infection, high abundance of the lantibiotic gene is associated with reduced density of E. faecium. In germ-free mice transplanted with patient-derived faeces, resistance to VRE colonization correlates with abundance of the lantibiotic gene. Lantibiotic-producing commensal strains of the gastrointestinal tract reduce colonization by VRE and represent potential probiotic agents to re-establish resistance to VRE.

Preventing transmission of highly antibiotic-resistant pathogens in healthcare settings remains problematic4. A promising approach to reducing antibiotic-resistant infections involves enhancing microbiota-mediated colonization resistance of the host by administering protective commensal bacteria5. Although mechanisms of colonization resistance are being discovered, few bacterial strains that mediate resistance have been identified6. Faecal microbiota transplantation (FMT), although effective for recurrent Clostridium difficile infection7, remains problematic because faecal compositions can be highly variable8. Preclinical studies suggest that commensal bacterial strains that inhabit the lower gastrointestinal tract can be effective at providing resistance9–11. Enterococci colonize the human gastrointestinal tract and have developed resistance to antibiotics, including vancomycin1,2. Antibiotic-mediated depletion of the gut microbiota leads to expansion of VRE in the intestine, predisposing patients to bloodstream infection6,12,13. In mice, FMT can re-establish colonization resistance and reduce intestinal VRE density4,14. We recently described a four-strain-consortium named CBBPSCSK, consisting of Clostridium bolteae, Blautia producta (BPSCSK: SCsK refers to the Blautia strain, which was characterized by S.C. and S.G.K.), Bacteroides sartorii and Parabacteroides distasonis, that restored colonization resistance against VRE in antibiotic-treated mice15.

To determine the mechanism of CBBPSCSK-mediated VRE inhibition, we co-cultured each strain with VRE (Fig. 1a, Extended Data Fig. 1a–d). BPSCSK inhibited VRE growth, as did BPSCSK-conditioned media (Extended Data Fig. 1e–i), and dilution experiments demonstrated that BPSCSK-mediated inhibition is not due to nutrient depletion. In contrast to BPSCSK-conditioned media, culture supernatants of B. producta (Clostridiales VE202-06 (BP control)) and other microbiota-derived Blautia species did not inhibit VRE growth (Extended Data Fig. 1j, Supplementary Tables 1, 2).

Previous studies demonstrated that BPSCSK requires the other CBBPSCSK members to colonize the intestine3. CBBPSCSK, but not a modified consortium in which BPSCSK was replaced with BPcontrol (BP control), reduced VRE colonization (Fig. 1b), even though both consortia colonized mice (Extended Data Fig. 2a, b). CBBPSCSK also reduced VRE colonization in gnotobiotic mice (Extended Data Fig. 2c, d). CBBPSCSK reduced colonization by several VRE strains (Extended Data Fig. 2e–g, Supplementary Table 3) and fluorescence in situ hybridization analysis demonstrated BPSCSK colonization throughout the large intestine (Extended Data Fig. 3).

To determine whether BPSCSK production of an inhibitory factor, VRE was cultured in caecal contents from mice reconstituted with CBBPSCSK or BPcontrol (Extended Data Fig. 4a). Only CBBPSCSK caecal contents inhibited VRE growth. Previous studies demonstrated that the commensal microbiota stimulates secretion of a host-derived antimicrobial peptide16, such as RegIIIγ, which reduces intestinal VRE colonization17. CBBP colonization, however, did not induce RegIIIγ transcripts or RegIIIγ protein in the ileum of antibiotic-treated mice (Extended Data Fig. 4b, c). Host-derived antimicrobial peptides and inflammatory mediators did not differ between mice treated with CBBPSCSK or PBS (Extended Data Fig. 4d–i). CBBPSCSK was effective at reducing VRE density in Rag2−/−Il2rg−/− mice, indicating that T cells, B cells, natural killer cells and innate lymphoid cells do not contribute to CBBPSCSK-mediated VRE inhibition (Extended Data Fig. 4j).

VRE was inhibited by proteins precipitated from BPSCSK, but not BPcontrol-conditioned media (Extended Data Fig. 5a), which suggests that BPSCSK secretes an inhibitor. We performed whole-genome sequencing of BPSCSK and BPcontrol and discovered that only BPSCSK contains an operon for a lantibiotic, a lanthionine-containing antimicrobial peptide (Extended Data Fig. 5b–d, Supplementary Tables 4, 5). Lanthionines are formed by enzymatic dehydration of serine or threonine residues that cyclize with neighbouring cysteine residues18,19. Nisin-A, a lantibiotic expressed by L. lactis20,21, binds lipid II and inhibits the synthesis of peptidoglycan and also forms a membrane pore complex22. Comparison of the lantibiotic operons from BPSCSK and L. lactis (lan and nis, respectively) revealed homologous sequences for all genes except dissimilar signal peptidase sequences (Extended Data Fig. 5c, Supplementary Table 5). Although gene organization and number

1Immunochemistry Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 2Weill Cornell Medical College, New York, NY, USA. 3Lucille Castor Center for Microbes, Inflammation and Cancer, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 4Microchemistry and Proteomics Core Laboratory, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 5Infectious Diseases Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 6Donald B. and Catherine C. Marron Cancer Metabolism Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 7RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. 8JSK-Kees Institute Medical and Chemical Innovation Center, Tokyo, Japan. 9RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. 10Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 11Molecular Pharmacology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 12email: egpamer@uchicago.edu

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BPSCSK expresses a lantibiotic in vivo that inhibits VRE. a, VRE was co-cultured in vitro with each CBBPSCSK isolate (n = 15 biologically independent samples from three independent experiments) and growth was monitored. CFU, colony-forming unit; LOD, limit of detection. b, Antibiotic-treated, VRE-dominated mice (n = 12 mice from three independent experiments) received treatment by oral gavage containing CBBPSCSK. CBBPcontrol or PBS. VRE colonization was monitored by CFU quantification in faecal samples. c, VRE was inoculated in culture broth with commercial nisin-A (100 μM), purified LanA1–LanA4 lantibiotic from BPSCSK (100 μM), or PBS (n = 4 biologically independent samples from two independent experiments). VRE CFUs were enumerated 8 h after inoculation. d, RNA sequencing analysis was performed on caecal content from mice treated with CBBP (n = 3 mice from one independent experiment). RPKM, reads per kilobase of transcript per million mapped reads. VRE (ATCC 700221) was used in experiments in a–c. *P = 0.0286, ****P < 0.0001, two-tailed Mann–Whitney U-test for comparisons with negative control (PBS, VRE culture alone). Data are mean ± s.d. (a, c), median ± range (b) and median values (d).

within the lan and nis operons differ, a lantibiotic operon recently characterized21 in Blautia obeum is similar to that of BPSCSK. Notably, BPSCSK encodes five lantibiotic precursor genes (lanA1–lanA5), in contrast to one encoded by the Nis operon (nisA). The first four precursor sequences (lanA1–lanA4) are identical, whereas the fifth precursor (lanA5) encodes a similar but non-identical sequence (Supplementary Table 4). lanA1–lanA4 and nisA belong to a lantibiotic subset that contains the gallidermin superfamily domain, which conserves two N-terminal lanthionine rings enabling lipid II binding22 and inhibitory activity23.

Nisin-A and other lantibiotics of the gallidermin superfamily carry a net positive charge, which enables electrostatic interactions with the cell membrane and lipid II24. The inhibitory factor of BPSCSK and nisin-A elute similarly during cation exchange chromatography, which suggests that they both carry a positive charge (Extended Data Fig. 5e). In addition, the inhibitory factor of BPSCSK and nisin-A are resistant to heat and proteases, a characteristic of lantibiotics (Extended Data Fig. 5f). Methods to edit the genome of Blautia producta are lacking, so we pursued a gain-of-function approach and heterologously expressed lanA1–lanA4 in Escherichia coli (Extended Data Fig. 6a, b, Supplementary Table 6), purified the lantibiotic to homogeneity, and validated it by mass spectrometry (Extended Data Fig. 6c). VRE was similarly inhibited by the addition of the purified BPSCSK LanA or commercial nisin-A (Fig. 1c).

RNA sequencing of caecal contents from antibiotic-treated mice colonized with CBBPSCSK (Fig. 1d) demonstrated that, relative to the overall transcriptome of BPSCSK, precursor lantibiotic transcripts and associated immunity genes were abundant (greater than the ninety-fifth percentile), whereas genes involved in post-translational modification of the precursor lantibiotic were expressed to a lesser degree. Oral administrations of proteins precipitated from BPSCSK, but not BPcontrol cultures reduced VRE colonization in antibiotic-treated mice challenged with VRE (Extended Data Fig. 7), albeit to a lesser degree than CBBPSCSK administration. This probably reflects reduced concentrations of lantibiotic owing to intestinal absorption, metabolism and intermittent administration. These findings demonstrate that BPSCSK encodes a lantibiotic that is highly expressed and inhibits VRE in vivo.

L. lactis is a lantibiotic-producing probiotic that, theoretically, could be used to reduce VRE colonization. VRE is inhibited after co-culture with BPSCSK or L. lactis (Fig. 2a) and after exposure to precipitated proteins from either species (Extended Data Fig. 8a). By contrast, in vivo VRE colonization was inhibited by CBBPSCSK but not when BPSCSK was replaced by L. lactis (CLBP) (Fig. 2b). Although BPSCSK is prevalent in the microbiota after CBBPSCSK treatment (relative abundance > 25%), L. lactis was not detected after CLBP treatment (Fig. 2c). The failure of L. lactis to colonize the intestine probably explains its inability to reduce VRE density in vivo; L. lactis also does not colonize the porcine intestine or inhibit Listeria monocytogenes or C. difficile in a human distal-colon model25.

To characterize the antibacterial spectrum of the BPSCSK lantibiotic, we cultured 152 commensal strains obtained from human faeces (Supplementary Table 7) with protein precipitated from BPSCSK or BPcontrol cultures or broth spiked with nisin-A diluted to the same minimal inhibitory concentration (MIC) against VRE as BPSCSK. The MIC was determined as the highest dilution that inhibited growth
Fig. 2 BPSCSK colonizes the gastrointestinal tract and broadly inhibits Gram-positive pathogens while preserving some commensal species. a, VRE was co-cultured in vitro with L. lactis or BPSCSK (n = 9 biologically independent samples from three independent experiments) and growth was monitored. b, Antibiotic-treated, VRE-dominated mice (n = 12 mice from three independent experiments) received an oral gavage that contained CBBPSCSK, CLBP, or CBBPcontrol. VRE colonization was monitored by CFU quantification in fecal samples. c, The microbiota composition determined by metagenomic sequencing of 16S rRNA genes from fecal samples collected from mice treated with CBBPSCSK or CLBP. d, Culture broth was conditioned with proteins precipitated from BPSCSK, BPCcontrol or commercial nisin-A and serially diluted. The MIC was determined for 158 strains from a commensal biobank by calculating the highest dilution factor that inhibited growth (n = 2 biologically independent samples from two independent experiments). The resistance index is a ratio between MIC of BPCcontrol-conditioned media to the MIC of BPSCSK or nisin-A-conditioned media. VRE (ATCC 700221) was used for experiments in a–c. ***p < 0.001, ****p < 0.0001, two-tailed Mann–Whitney U-test for comparisons with negative control (a, b) or between experimental conditions (d). Data are median values (a) or mean ± s.d. (b). For the box plots in d, centre line denotes the median, box limits represent the upper and lower quartiles, and errors denote 1.5× the interquartile range.

over 24 h. Protein precipitates from BPSCSK-conditioned or nisin-A-spiked media, but not BPCcontrol-conditioned media, inhibited Gram-positive, but not Gram-negative, bacterial strains. A resistance index that compares the MIC values in conditioned media from BPCcontrol to that from BPSCSK or nisin-A was used to quantify the sensitivity of bacterial strains to both lantibiotics. The Gram-positive population demonstrated greater sensitivity to nisin-A-spiked media than BPSCSK-conditioned media (Fig. 2d). Several VRE strains and other Gram-positive nosocomial pathogens (Extended Data Fig. 8b) demonstrate comparable sensitivity to either conditioned media, but many Gram-positive commensal strains were more resistant to the BPSCSK lantibiotic than nisin-A, including members implicated in resistance to intestinal infections, such as Bifidobacterium longum and Pediococcus acidilactici27,28 (Extended Data Fig. 8c). Thus, the BPSCSK lantibiotic, relative to nisin-A, has a narrower spectrum of activity that targets VRE while preserving commensal bacteria.

Among 32 Blautia isolates cultured from fecal samples from healthy donors, BPSCSK was the only strain that encoded a lantibiotic and inhibited VRE in vitro (Fig. 3a, Supplementary Tables 1, 2). To determine the prevalence of lantibiotic genes in the human intestinal microbiome, we shotgun-sequenced faecal samples collected from 15 healthy donors and identified lantibiotic genes and homologues that contain the gallidermin superfamily domain (Extended Data Fig. 9a) in 7 of 15 samples, with different sequences within and between samples (Fig. 3b, Extended Data Fig. 9b).

We next mined the genomes of commensal biobank isolates for the gallidermin superfamily domain and identified one additional Clostridiales species, Ruminococcus faecis, which encodes a similar lantibiotic and inhibits VRE in vitro, whereas R. faecis strains that did not encode a lantibiotic did not inhibit VRE (Fig. 3c, Extended Data Fig. 9c, Supplementary Table 1). Although only a minority of cultured commensal bacteria encodes lantibiotics, it remains unclear whether this reflects their paucity in the microbiota or their relative resistance to in vitro culture.

Patients undergoing allogeneic haematopoietic cell transplantation frequently have intestinal domination by VRE12,13,29. From a biobank of longitudinally collected faecal samples, we identified 238 samples from
Lantibiotic genes are present in human microbiomes of healthy individuals and gut resident, lantibiotic-producing species inhibit VRE. a. Microbiota-derived Blautia species were whole-genome sequenced, assembled, annotated and mined for lantibiotic precursor sequences. VRE was inoculated in conditioned media from 39 strains sequenced, assembled, annotated and mined for lantibiotic precursor sequences. shotgun sequencing of human faecal samples (n experiments) and monitored for growth. b. Lantibiotic detection from shotgun sequencing of human faecal samples. VRE was plotted against microbiota diversity. The relative abundance of lantibiotic, and the relative abundance of the lantibiotic, was stratified by abundance of the lantibiotic, and the relative abundance of E. faecium was plotted against microbiota diversity. The Spearman correlation coefficient determined by 16S rRNA was plotted against lantibiotic gene abundance (Spearman correlation coefficient = −0.43, P = 2.08 × 10⁻¹⁰) (Fig. 4a). Samples with high lantibiotic abundance (Lan high > 85th percentile) consistently had low abundance of E. faecium (<10% 16S relative abundance), and were detected in half of patients (Extended Data Fig. 10). In Lan high and Lan low settings, 25% and 21%, respectively, had high microbiota diversity (inverse Simpson index ≥ 8)

Fig. 3 | Lantibiotic genes are present in human microbiomes of healthy individuals and gut resident, lantibiotic-producing species inhibit VRE. a. Microbiota-derived Blautia species were whole-genome sequenced, assembled, annotated and mined for lantibiotic precursor sequences. VRE was inoculated in conditioned media from 39 strains (n = 4 biologically independent samples from four independent experiments) and monitored for growth. b. Lantibiotic detection from shotgun sequencing of human faecal samples (n = 15 faecal samples). c. In total 421 commensal biobank isolates were whole-genome sequenced, assembled, annotated and mined for lantibiotic precursor sequences to identify a strain of R. faecis that encoded a homologous lantibiotic. VRE was inoculated in conditioned media from three strains of R. faecis cultures (n = 4 biologically independent samples from four independent experiments) with or without detected lantibiotic genes, and VRE growth was monitored 8 h after inoculation. VRE (ATCC 700221) was used in experiments in a and c. *P = 0.0286, two-tailed Mann–Whitney U-test for comparisons with negative control. Data are mean ± s.d. (a, c).

22 patients with a range of E. faecium densities and found lantibiotic gene abundance inversely correlated with the relative abundance of E. faecium (Spearman correlation coefficient = −0.43, P = 2.08 × 10⁻¹⁰) (Fig. 4a). Samples with high lantibiotic abundance (Lan high > 85th percentile) consistently had low abundance of E. faecium (<10% 16S relative abundance), and were detected in half of patients (Extended Data Fig. 10). In Lan high and Lan low settings, 25% and 21%, respectively, had high microbiota diversity (inverse Simpson index ≥ 8)

Fig. 4 | Enrichment of lantibiotic genes correlates with reduced E. faecium in patient faecal samples. a, b. Longitudinally collected faecal samples (n = 238 biologically independent samples) from 22 patients undergoing allogeneic haematopoietic cell transplantation were shotgun sequenced. a. The relative abundance of E. faecium determined by 16S rRNA was plotted against lantibiotic gene abundance (Spearman correlation coefficient = −0.43, P = 2.08 × 10⁻¹⁰). b. Samples were then stratified by abundance of the lantibiotic, and the relative abundance of E. faecium was plotted against microbiota α diversity. The percentage of sample distribution is shown in each quadrant. c. Faecal microbiota transplants were performed on germ-free mice using diversity-matched microbiomes containing either high or low lantibiotic gene abundance. One week after FMT administration, mice were orally gavaged with VRE and colonization was monitored by quantifying VRE from faecal samples. VRE (ATCC 700221) was used for experiments in c. Low lantibiotic abundance ≤ 2τ < high lantibiotic abundance (RPKM); low E. faecium abundance ≤ 10 < high E. faecium abundance (% relative 16S); low α diversity ≤ 8 < high α diversity (inverse Simpson index). *P < 0.05, ****P < 0.0001, two-tailed Mann–Whitney U-test.
and low *E. faecium* abundance, which suggests that diversity compensates for low lantibiotic-gene abundance by parallel, lantibiotic-independent inhibitory mechanisms (Fig. 4b). However, nearly half of the *Lan* samples with low diversity (inverse Simpson index < 8) had high *E. faecium* abundance (≥10% 16S relative abundance); low diversity decreases the likelihood, but some commensal species still provide lantibiotic-independent colonization resistance against *E. faecium*. By contrast, *Lan* samples had low *E. faecium* abundance (*P* < 1 × 10−6) despite low diversity, consistent with the notion that lantibiotic gene abundance in the microbiome contributes to colonization resistance against *E. faecium*.

To determine whether low-diversity *Lan* microbiomes can resist VRE colonization, we identified diversity-matched *Lan* and *Lan* samples and colonized germ-free mice before VRE challenge (Fig. 4c, Supplementary Table 8). Regardless of diversity, *Lan* samples consistently reduced VRE colonization compared with *Lan* samples, which suggests that lantibiotics in the gastrointestinal tract provide colonization resistance.

Microbiota-mediated colonization resistance remains incompletely defined and restoring resistance during antibiotic-induced dysbiosis remains an important goal. BP SCSK belongs to a small subset of commensals that secrete lantibiotics, and therefore can influence the community structure of the microbiota. A potential clinical role for lantibiotics is supported by a previous report that uses lantibiotic-producing commensal *Staphylococcus* species on the skin to provide lantibiotic-independent colonization resistance against *Staphylococcus aureus*. Understanding the mechanisms by which the microbiota confers colonization resistance may lead to the development of therapies to repair dysbiosis, thereby reducing susceptible patients’ risk of colonization by antibiotic-resistant pathogens.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1501-z.

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METHODS

Bacterial strains. Vancomycin-resistant *E. faecium* purchased from ATCC (stock number 700221) was used for all experiments unless otherwise stated. Vancomycin-resistant *E. faecalis* strains used were V583 and MHLSK1. *Listeria monocytogenes* strains used were 10403S and 13932. *Salmonella Typhimurium* strains used were SL1344 and LT2. The following strains were isolated from patients at Memorial Sloan Kettering Cancer Center: vancomycin-resistant *E. faecium* strain MH.0139G, MH.0151F, MH.1107; vancomycin-resistant *E. faecalis* strain MH.SKI; C. difficile strain MH.BBL2; methicillin-resistant *S. aureus* strains MH.SKI and MH.SK2; Klebsiella pneumoniae strains MH.189 and MH.258; *E. coli* strains MH.T18 and MH.X43; and *Proteus mirabilis* strains MH.42F and MH.43A. All gut commensal strains used were isolated from faecal samples of healthy donors and are listed in Supplementary Table 7.

Mouse husbandry. All experiments using wild-type mice were performed with C57BL/6 female mice that were 6–8 weeks old; mice were purchased from Jackson Laboratories. Rag2−/−H2−/− mice were purchased from Taconic Farms, and subsequently bred in-house. Germ-free mice were bred in-house in germ-free isolators. All mice were housed in sterile, autoclaved cages with irradiated food and autoclaved water. Mouse handling and weekly cage changes were performed by investigators wearing sterile gowns, masks and gloves in a sterile biosafety hood. All animals were maintained in a specific-pathogen-free facility at Memorial Sloan Kettering Cancer Center Animal Resource Center. After cohousing for at least two weeks, mice were individually housed and randomly assigned to experimental groups. All animal experiments were performed at least three times unless otherwise noted. Experiments were performed in compliance with Memorial Sloan-Kettering Cancer Center institutional guidelines and approved by the institution’s Institutional Animal Care and Use Committee.

Mouse antibiotic administration. Mice were administered ampicillin (0.5 g l−1); Fisher Scientific) in the drinking water for 5 days. Ampicillin was changed every 3 days. Antibiotic administration ceased after the initial administration of commensal bacteria (after 5 days) unless stated otherwise.

Bacterial in vitro broth culture conditions. The culture broth used for all cultures was pre-reduced brain heart infusion broth supplemented with yeast extract (5 g l−1) and l-cysteine (1 g l−1). The culture conditions were 37 °C and anaerobic unless otherwise stated.

VRE CFU enumeration. VRE CFUs were enumerated from samples by serial dilution in PBS and plating on BD Enterococcus agar supplemented with vancomycin (8 μg ml−1; Novagen) and streptomycin (100 μg ml−1; Fisher Scientific).

VRE in vitro co-culture inhibition experiments. A frozen aliquot of each bacterial strain was inoculated and cultured in broth for 24 h. The resulting cultures were plated as lawns on pre-reduced Columbia agar supplemented with 5% sheep meniscal bacteria (after 5 days) unless stated otherwise.

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chromatography system at 4°C. The lysate supernatant was loaded onto a HiTrap HP nickel affinity column. The column was washed with 75 ml (start buffer plus 30 mM imidazole) and recombinant protein eluted in 25 ml (start buffer plus 1 M imidazole). The His-tagged lantibiotic eluate was loaded on a Luma 10 μm C8(2) 100 Å, LC Column 250 × 4.6 mm and separated with 80 min linear gradient of 0–80% Buffer A. Buffer A was 0.1% TFA in H2O and buffer B was 90% acetonitrile, 10% Buffer A. The LanA–LanA peptide itself and its hydration + 18 Da series eluted in fractions 40–50 (Extended Data Fig. 6b) with the maximum for fully-dehydrated product at 45%. Fractions 43–46 were lyophilized and the concentration of the solution was measured by BCA assay. We obtained approximately 1 mg of product from bacteria in 1.3 l of medium. The His tag and leader sequence were removed by trypsin digestion for 2 h at 25°C. The digestion was stopped by adding formic acid to 1% and the product was separated by reverse phase chromatography on a 0–80% linear gradient as described above. The resulting product was checked by electrospray ionization—mass spectrometry and the spectrum was desiopted and deconvoluted by Xtract algorithm in Xcalibur. The proteolytic fragment corresponding to mature LanA–LanA was observed: 3,152.4. VRE was inoculated in culture broth supplemented with the purified lantibiotic (100 μM) and cultured for 24 h. VRE CFUs were subsequently enumerated.

DNA extraction. DNA was extracted using a phenol–chloroform extraction technique with mechanical disruption ( bead beating). In brief, a frozen aliquot of approximately 100 mg per sample was suspended, while frozen, in a solution containing 500 μl of extraction buffer (200 mM Tris, pH 8.0; 200 mM NaCl and 20 mM EDTA), 210 μl of 20% SDS, 500 μl of phenol:chloroform:Isoamyl alcohol (25:24:1), and 500 μl of 0.1-mm-diameter zirconia/silica beads (BioSpec Products). Microbial cells were lysed by mechanical disruption with a bead beater (BioSpec Products) for 2 min, followed by two rounds of phenol:chloroform:Isoamyl alcohol extraction. After extraction, DNA was precipitated in ethanol, resuspended in 200 μl of TE buffer with RNase (100 mg ml⁻¹), and further purified with QiAamp min prep columns (Qiagen).

Microbial composition by 16S rRNA gene sequencing. Universal bacterial primers—563F (5'-nunnunnn-NNNNNNUU-AYTTGGYDTADDAA-GNG-3') and 926R (5'-nunnunnn-NNNNNNNNN-CGCTCAATYHT-TTRAGT-3'), in which 'N' represents unique 12-base-pair Golay barcodes and 'n' represents additional nucleotides to offset the sequencing of the primers—were used to PCR-amplify the V4–V5 hypervariable region of the 16S rRNA gene. The V4–V5 amplicons were purified, quantified, and pooled at equimolar concentrations before ligating Illumina barcodes and adaptors using the Illumina TruSeq Sample Preparation protocol. The completed library was sequenced using the MiSeq Illumina platform³⁵. Paired-end reads were merged and demultiplexed. The UPARSE pipeline⁶⁸ was used for error filtering using the maximum expected error (εmax = 1)⁴⁸, clustering sequences into operational taxonomic units (OTUs) of 97% distance-based similarity, and identifying and removing potential chimeric sequences using both de novo and reference-based methods. Singleton sequences were removed, and a custom Python script incorporating the de novo clustering algorithm BLAST with NCBI RefSeq as reference training set, was used to perform taxonomic assignment to the species level (E ≤ 1 × 10⁻¹⁰) using representative sequences from each OTU.

Whole-genome sequencing, assembly and annotation. An overnight culture grown from a single colony in culture broth was DNA extracted and sequenced using the Illumina MiSeq platform. Purified DNA was sheared using a Covaris ultrasonicator and prepared for sequencing with a Kapa library preparation kit with Illumina TruSeq adapters to create 300 ± 300 bp nonoverlapping paired-end reads. Raw sequence reads were filtered (Phred score ≥ 30, 4 bp sliding window) using Trimmomatic (v.0.36). Trimmed reads were assembled into contigs and annotated with putative open reading frames using the assembly and annotation services in PATRIC (v.3.5.25).

Metagenomic sequencing. DNA was extracted, sheared, and libraries were prepared as described for whole-genome sequencing. Sequencing was performed using the Illumina HiSeq platform (Illumina) with a paired-end 100 × 100 bp kit in pools targeting 20–30 million reads per sample.

RNA extraction. Samples were extracted using an acidic phenol–chloroform protocol. In brief, approximately 100 μl per sample was suspended in 700 μl of RNA. The suspension was homogenized using a sterile RNAase-free spatula and incubated at 4°C overnight. Samples were pelleted by centrifugation at 13,000g for 10 min and resuspended in 200 μl of RNA extraction buffer supplemented with proteinase K (1 mg ml⁻¹) that was heat-activated at 50°C for 10 min. Samples were incubated at room temperature for 10 min and vortexed every 2 min. Then, 300 μl of Qiagen RLT Plus Buffer (Qiagen) with 3-Mercaptoethanol (1%) was added to each sample, vortexed and incubated for 5 min at room temperature. Samples were then transferred to a sterile bead beating tube with 500 μl of 0.1 mm glass beads. Then, 100 μl of 95% isopropanol was added by bead beating the samples for 3 min (BioSpec Products), followed by one round of acidic phenol–chloroform extraction and one round of chloroform extraction. RNA was precipitated with 50 μl of 3 M ammonium acetate and 500 μl of 100% isopropanol and incubated at −20°C overnight. RNA was pelleted by centrifugation at 13,000g for 20 min at 4°C and washed with 450 μl of 70% ethanol. Ethanol wash was repeated, and the pellet was allowed to air dry at room temperature for 5 min. The pellet was then dissolved in 50 μl of RNAse-free water.

RNA samples were purified using RNAClean XP (Agencourt). DNA contaminants were removed using Turbo DNA-Free kit (Life Technologies), and ribosomal RNA removed using Ribo-Zero rRNA Removal Kit (Illumina). Following ribosomal RNA depletion, RNA clean PX purification was repeated.

RNA sequencing and analysis. RNA sample libraries were prepared using the TruSeq Stranded mRNA protocol (Illumina) and sequenced using the Illumina Miseq platform (Illumina). Raw sequence reads were filtered using Trimomatic (v.0.36), aligned to the genome of BPSCG by using bowtie2 (v.2.3.4.1), assigned to genes using featureCounts (v.1.6.1), and converted to normalized gene counts using DeSeq2 (v.1.2.0).

Oral administration of BPSCG protein precipitate. Antibiotic-treated mice were orally gavaged with BPSCG or BPCont protein precipitate (400 μg). Three hours later, VRE (10⁸ CFUs in 200 μl PBS) was orally gavaged, followed by oral administrations of BPSCG or BPCont protein precipitate every 3 h for 12 h. VRE colonization was monitored by enumerating VRE CFUs from faecal pellets 12 h post-VRE gavage. Faecal pellets were resuspended in PBS to a normalized concentration (100 mg ml⁻¹) for VRE CFU enumeration. Mice were screened for pre-existing VRE colonization by selective plating before proceeding forward with all experiments.

Healthy-donor faecal isolate collection. Faecal samples were collected from healthy human donors (n = 15) and transferred to an anaerobic chamber within 1 h of collection. All culture conditions were performed anaerobically on pre-reduced agar supplemented with CO₂ at 37°C. Faecal samples were resuspended in pre-reduced PBS and serially diluted with three tenfold serial dilutions. The dilutions were streaked on plates and cultured for 72 h. Individual colonies were selected and streaked onto fresh plates and cultured for 48 h. Single colonies were then resuspended in 50 μl of pre-reduced PBS and 10 μl was streaked as a lawn on a fresh plate and cultured for 48 h. Each isolate was obtained from culture and stocks were stored in pre-reduced PBS with 10% glycerol at 80°C. Colony PCR was performed using 2 μl of the above 50 μl single-colony suspension in PBS as a template. The 16S rDNA gene was amplified with primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GTTTACCTTGTTACGACTT-3'), and 1492R (5'-GTTTACCTTGTTACGACTT-3'), 519R (5'-GATTACCGCGGCGGTG-3'). Sanger sequences were quality filtered and aligned into a consensus sequence using custom Python scripts. Species identification was performed with nucleotide BLAST against the NCBI database.

Patient stool collection. Patients were enrolled in a prospective faecal collection protocol, in which faecal samples were routinely collected during the initial transplant hospitalization and stored in a biospecimen bank, as described previously⁴¹. Patients were part of a study consisting of adult patients (≥18 years) undergoing allogeneic haematopoietic stem-cell transplantation at Memorial Sloan Kettering Cancer Center (MSKCC). The study was approved by the Institutional Review Board at MSKCC. All study patients provided written informed consent for IRB-approved biospecimen collection and analysis (protocols 09-141, 06-107). The study was conducted in accordance with the Declaration of Helsinki.

Lantibiotic gene mining. The lantibiotic genes were discovered in the genome of BPSCG using antiSMASH⁴¹ and BAGEL³⁵ and confirmed to be homologous to known lantibiotic gene sequences using BLASTp alignment (Supplementary Table 5). Lantibiotic sequences were identified from metagenomic sequences using DIAMOND (v.0.9.22)⁴⁵ to align reads (E < 0.0001) to a custom database derived from the RefSeq nonredundant database (accessed August 2018), filtering only for lantibiotic genes containing the gallidermin superfamily domain. To identify RefSeq entries containing the gallidermin superfamily domain, a hidden Markov model profile was built according to the NCBI Conserved Protein Domain Family entry for the gallidermin superfamily domain (accession c50420) by using pfam2052 and TIGR03731 hidden Markov model files and searching for RefSeq entries with these sequence patterns using HMMP (3.1b2) (E ≤ 1 × 10⁻³). Lantibiotic sequences were identified from whole-genome-sequence genomes by assembling and annotating genomes as described previously. All open reading frames were searched for homology to the gallidermin superfamily domain using HMMP (3.1b2)⁴¹.

Detected lantibiotic sequence assembly from metagenomic sequencing. Translated sequencing reads aligning to a RefSeq database entry were retrieved using DIAMOND (v.0.9.22) with an identity ≥ 0.9 (e-value ≤ 1 × 10⁻³). Sequences aligning to each RefSeq entry were aligned. All sequencing reads within a sorted group were multiple sequence aligned to each other using MUSCLE (v.3.8.31) and the consensus sequence was used as the assembled, detected lantibiotic sequence.
Statistics. Statistical analyses were performed using R (v.3.3.1) and GraphPad Prism (v.7.0a) software packages. The two-tailed Mann–Whitney U-test was used for comparisons of continuous variables between two groups with similar variances. No statistical methods were used to predetermine sample size. When possible, investigators were blinded during group allocation and outcome assessment (16S and metagenomic shotgun sequence collection, extraction, quantification and analysis; enumeration of VRE in animal, ex vivo and in vitro experiments).

Data were visualized using bar plots with centre values representing the geometric mean and error bars representing the geometric s.d.; line graphs with points representing the geometric mean and error bars representing the geometric s.d.; box plots with the centre line representing the median, box limits representing the upper and lower quartiles and whiskers representing 1.5 times the interquartile range; and heat maps with individual values contained in a matrix representing the mean. Spearman rank correlation tests (two-tailed) were used to find significant correlations between two continuous variables.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
Microbiome sequencing data are available from Bioproject with the accession number 394877.

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Author contributions S.G.K. and E.G.P. designed the experiments and wrote the manuscript. S.G.K. performed and analysed most experiments. S.B. helped to design experiments, performed and analysed fluorescence in situ hybridization and RNA-sequencing analysis on caecal content. T.U.M., S.C. and V.E. cultured bacterial isolates from faecal samples and analysed whole-genome sequences of isolates. P.V.S. and R.C.H. performed peptide purifications and subsequent characterization by mass spectrometry. E.R.L. performed bioinformatic analyses and metagenomic sequence data. R.S. assisted in bacterial culturing and animal experiments. I.M.L. and R.S. maintained and screened mouse strains. M.G. generated and characterized bacterial isolates from faecal samples. W.Q., R.J.J., F.R. and J.R.C. contributed to the development of methods to purify bacterial lantibiotics for biochemical analyses. E.F., L.A. and R.W. performed DNA extractions, 16S MiSeq Illumina sequencing and analysed microbiome sequence data. Z.-M.X.W. assisted in ileal homogenization, western blot, and RT-qPCR analyses. H.-J.J. contributed to the cloning and expression of the lantibiotic gene. S.M.M. and Y.T. enrolled patients undergoing allogeneic haematopoietic cell transplantation in the prospective faecal collection protocol and contributed to the analysis of sequence data. S.N. and K.H. contributed human-derived commensal bacterial strains that were included in this study. J.U.P. and M.R.M.v.d.B. contributed to the analyses of patient-derived faecal samples.

Competing interests K.H. is co-founder and scientific advisor to Vedanta Biosciences. M.R.M.v.d.B. is on the advisory board of and has financial holdings in Seres Therapeutics Inc., serves on the DKMS medical council, has received speaker honoraria from Merck and Acute Leukemia Forum, holds patents that derive royalties from Seres Therapeutics Inc., has received honorarium and research support (1 January 2017 to present) from Seres Therapeutic Inc., and IP licensing with Seres Therapeutics Inc. and Juno. J.U.P. reports research funding, intellectual property fees, and travel reimbursement from Seres Therapeutics. E.G.P. has received speaker honoraria from Bristol-Myer Squibb, Celgene, Seres Therapeutics, Medimmune, Novartis, and Ferring Pharmaceuticals; is an inventor on patent application no. WPO2015179437A1, entitled ‘Methods and compositions for reducing Clostridium difficile infection’ and no. WPO2017091753A1, entitled ‘Methods and compositions for reducing vancomycin-resistant Enterococci infection or colonization’; and holds patents that receive royalties from Seres Therapeutics Inc.

Additional information
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Correspondence and requests for materials should be addressed to E.G.P.
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Extended Data Fig. 1 | BP\textsubscript{SCSK} directly inhibits VRE through a contact-independent mechanism. a–d, VRE was co-cultured with each CBBP\textsubscript{SCSK} isolate (n = 15 biologically independent samples from three independent experiments) and growth was monitored. e, VRE was inoculated in conditioned media from each CBBP\textsubscript{SCSK} isolate culture (n = 15 biologically independent samples from three independent experiments). f–i, VRE was inoculated in conditioned media from each CBBP\textsubscript{SCSK} isolate culture (−VRE), or each CBBP\textsubscript{SCSK} isolate co-cultured with VRE (+VRE) (n = 5 biologically independent samples from five independent experiments) and growth was monitored. j, VRE was inoculated in conditioned media from \textit{Blautila} species cultures (n = 6 strains, 15 biologically independent samples from three independent experiments). VRE (ATCC 700221) was used in all experiments shown. Data are median ± range (a–e, j) or mean ± s.d. (f–i). ****P < 0.0001, two-tailed Mann–Whitney U-test for comparisons with a negative control.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | BP\textsubscript{SCSK} but not BP\textsubscript{control} reduces VRE colonization in vivo. a, b, Faecal samples collected from antibiotic-treated, VRE-dominated mice (n = 4 mice from one independent experiment) orally gavaged with CBBP\textsubscript{SCSK} (a) or CBBP\textsubscript{control} (b) were shotgun sequenced and the relative abundance of each species was determined by 16S rRNA. c, d, Antibiotic-treated (c) or germ-free (d) mice (n = 8 mice from two independent experiments) were orally gavaged with VRE. Three days later, VRE-dominated mice received an oral gavage of CBBP\textsubscript{SCSK} or CBBP\textsubscript{control} and VRE colonization was monitored by quantifying VRE in faecal samples. e–g, Antibiotic-treated mice (n = 4 mice from one independent experiment) were orally gavaged with different strains of clinical VRE isolates. Three days later, VRE-dominated mice received an oral gavage of CBBP\textsubscript{SCSK} or CBBP\textsubscript{control} and VRE colonization was monitored by quantifying VRE in faecal samples. The following VRE strains were used: strain 0151F is an \textit{E. faecium} MLST type ST80 (e); strain 1107 is an \textit{E. faecium} MLST type ST412 (f); strain V583 is an \textit{E. faecalis} strain (g). VRE strains used were VRE (ATCC 700221) (a–d), VRE (0151F) (e), VRE (1107) (f), and VRE (V583) (g). Data are mean ± s.d. *P < 0.05, ***P < 0.001, two-tailed Mann–Whitney U-test.
Extended Data Fig. 3 | \( \text{BP}_{\text{SCSK}} \) colonizes the large intestine. Antibiotic-treated mice (\( n = 5 \) mice from one independent experiment) were orally administered CBBP\textsubscript{SCSK}. Two weeks later, BP\textsubscript{SCSK} localization around the mucosal epithelium (top) and lumen (bottom) of the caecum were visualized by fluorescence in situ hybridization. Entire caecum cross-sections were hybridized with a probe specific for BP\textsubscript{SCSK}. Sections were counterstained with Hoechst dye to visualize the nuclei. Representative images are shown. Scale bars, 25 \( \mu \)m.
Extended Data Fig. 4 | CBBP<sub>SCSK</sub> mediates VRE colonization resistance by producing an inhibitor. **a,** Antibiotic-treated mice (n = 8 mice from two independent experiments) received treatment by oral gavage containing CBBP<sub>SCSK</sub>, CBBP<sub>control</sub>, PBS or VRE. One week later, VRE was inoculated into the caecal content and growth was monitored 6 h after inoculation. **b–i,** Antibiotic-treated mice received an oral gavage containing CBBP<sub>SCSK</sub> (n = 4 mice from one independent experiment) or PBS (n = 3 mice from one independent experiment). Wild-type mice (n = 4 mice from one independent experiment) were untreated and received no antibiotics. Four days later, RNA and proteins were extracted from the distal ileum, and RegIIIγ was measured by RT–qPCR (b) and western blot (c). Other genes involved in host-derived antimicrobial peptide production, including angiogenin-4 (ang4) (d), defensin-1 (def1) (e), amphiregulin (areg) (f), and deleted in malignant brain tumours 1 (dmbt1) (g); or inflammatory mediators including cytochrome b beta (cybb) (h) and calgranulin A (s100a8) (i) were measured by RT–qPCR. **j,** Rag2<sup>−/−</sup> Il2rg<sup>−/−</sup> mice were treated with antibiotics, and orally gavaged with VRE. Three days later, VRE-dominated mice received CBBP<sub>SCSK</sub> or CBBP<sub>control</sub> by oral gavage and VRE colonization was monitored by quantifying VRE in faecal samples. VRE (ATCC 700221) was used in experiments in **a** and **j.** *P < 0.05 (0.0286), **P < 0.001, ***P < 0.0001,****P < 0.0001, two-tailed Mann–Whitney U-test. Data are median ± range (a) or mean ± s.d. (b–i).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | BPSCSK encodes a lantibiotic. a, VRE was inoculated in media conditioned with BPSCSK or BPcontrol culture protein precipitate fractions (n = 8 biologically independent samples from two independent experiments), and monitored for growth. b, c, BPSCSK was whole-genome sequenced, assembled and annotated. b, Schematic comparing the lantibiotic operon discovered in the genome of BPSCSK to the nisA operon from L. lactis. Gene functions are based on the characterization of homologous genes in the nis operon. c, Amino acid sequence alignment comparing the BPSCSK lantibiotic precursor (LanA1–LanA4) and the nisin-A precursor (NisA). Sequence features are based on the characterization of nisin. d, The molecular formula for the mature, post-translationally modified BPSCSK LanA1–LanA4 lantibiotic with a predicted mass of 3152.45 Da. Abu, alpha-aminobutyric acid; Dha, dehydroalanine; Dhb, dehydrobutyrine. e, Media conditioned with BPSCSK or BPcontrol culture protein precipitates, or commercial nisin-A, were incubated with proteinase K for 3 h at 37 °C, boiled at 100 °C, or left untreated. The treated protein precipitate (n = 8 biologically independent samples from four independent experiments) was serially diluted and VRE was inoculated and cultured for 24 h. The MIC value was the highest mean dilution in which VRE inhibition was observed. f, Proteins were precipitated from BPSCSK or BPcontrol, or nisin-A spiked cultures and applied to a SP sepharose column. Each fraction was serially diluted and VRE was inoculated and cultured for 24 h to determine the MIC (n = 4 biologically independent samples from four independent experiments). VRE (ATCC 700221) was used in experiments in a, e and f. ***P < 0.001, ****P < 0.0001, two-tailed Mann–Whitney U-test for comparisons with a negative control. Data are mean ± s.d. (a, f) or mean values (e).
Extended Data Fig. 6 | Heterologous expression of BPSCSK LanA1–LanA4 lantibiotic. a, Genes involved in biosynthesis of the BPSCSK lantibiotic (His-tagged-LanA, LanB and LanC) were cloned into expression vectors (pRSFDuet-1/LanA+LanB, pCDFDuet-1/LanC) and heterologously expressed in E. coli. Schematic map indicating where each lantibiotic gene was inserted into the respective expression vectors. b, c, The His-tagged LanA1–LanA4 lantibiotic was purified from E. coli lysates by HiTrap HP nickel affinity chromatography and subsequently purified to homogeneity by reversed-phase high-performance liquid chromatography. The leader sequence and His tag were removed by trypsin digestion to yield the mature lantibiotic. The purified His-tag product (b) and the purified mature lantibiotic (c) were analysed by electrospray ionization–mass spectrometry (ESI–MS) and the spectrum was deisotoped and deconvoluted using the Xtract algorithm in Xcalibur. The signals with labels correspond to the predicted mass of the His-tagged lantibiotic (M) and its incomplete forms that did not dehydrate all nine residues (for example, M + 1·H2O and M + 2·H2O).
Extended Data Fig. 7 | Oral administrations of \( \text{BP}_{\text{SCSK}} \) protein precipitate reduce VRE colonization in vivo. Antibiotic-treated mice (\( n = 9 \) mice from three independent experiments) were administered \( \text{BP}_{\text{SCSK}} \) or \( \text{BP}_{\text{control}} \) protein precipitate. Three hours later, VRE was orally gavaged, followed by oral administrations of \( \text{BP}_{\text{SCSK}} \) or \( \text{BP}_{\text{control}} \) protein precipitate every 3 h for 12 h and VRE colonization was monitored by quantifying VRE in faecal samples. VRE (ATCC 700221) was used. *\( P = 0.0232 \), two-tailed Mann–Whitney \( U \)-test. Data are mean ± s.d.
Extended Data Fig. 8 | The BPSCSK lantibiotic has a narrower spectrum of activity against Gram-positive commensal strains. a, VRE was inoculated in media conditioned with BPSCSK, *L. lactis* or BPcontrol culture protein precipitate (*n* = 4 biologically independent samples from four independent experiments) and growth was monitored 24 h after inoculation. b, c, Culture broth was conditioned with proteins precipitated from BPSCSK, BPcontrol or commercial nisin-A and serially diluted. The MIC value was determined for common nosocomial pathogens (b) or 158 strains from a commensal biobank (*n* = 2 biologically independent samples from two independent experiments) (c) by calculating the highest dilution factor that inhibited growth. The resistance index is a ratio between MIC of BPcontrol-conditioned media over the MIC of BPSCSK or nisin-A-conditioned media (b). The lantibiotic sensitivity ratio was calculated as the MIC of nisin-A to the MIC of the BPSCSK lantibiotic for each strain (c). *P* < 0.05, **** *P* < 0.0001, two-tailed Mann–Whitney U-test for comparisons with a negative control (a) or between two experimental conditions (b, c). Box plots are as defined in Fig. 2.
Extended Data Fig. 9 | Identification of lantibiotic sequences from metagenomic sequencing of healthy human faecal samples. a, The profile hidden Markov model used to identify the gallidermin superfamily domain, illustrated as a logo. b, Multiple sequence alignment of lantibiotic precursor sequences identified from shotgun sequencing of healthy-donor faecal samples. Detected lantibiotic sequences are the assembly of lantibiotic reads from shotgun metagenomic faecal samples. c, A total of 421 species were individually isolated from healthy human faecal samples, whole-genome sequenced, assembled, annotated and mined for lantibiotic precursor sequences to identify a strain of *R. faecis* encoding a homologous lantibiotic. The precursor lantibiotic sequence is compared to the sequences of BPSCsk_LanA1–LanA4 lantibiotic and nisin-A by multiple alignment.
Extended Data Fig. 10 | Lantibiotic sequences identified from metagenomic sequencing of hospitalized patient faecal samples.

a, Stacked heat map matrices represent a single patient. The top row illustrates abundance of the lantibiotic gene (RPKM). The bottom row illustrates relative abundance of *E. faecium* (percentage of 16S). Columns represent the sample collection day relative to transplant.
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| Data collection | antiSMASH and BAGEL3 were used to screen for bacteriocin genes. To identify RefSeq entries containing the gallidermin superfamily domain, a hidden Markov model profile was built according to NCBI’s Conserved Protein Domain Family entry for gallidermin superfamily domain (accession # cl03420) by using pfam02052 and TIGR03731 hidden Markov model files and searching for RefSeq entries with these sequence patterns using HMMER (3.1b2)(e-value <10^-5). Translated sequencing reads were retrieved from the DIAMOND (v0.9.22) alignment output. Sequencing reads within a sorted group were multiple sequence aligned using MUSCLE (v3.8.31). |
| Data analysis | R (v.3.3.1) and GraphPad Prism (version 7.0a) software packages were used for statistical analyses. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](https://nature.com) for further information.

Data

Policy information about [availability of data](https://nature.com)

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Microbiome sequencing data are available from Bioproject with the accession number 394877.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size for mouse experiments was determined by feasibility and the magnitude of the interventions impact. For the studies included in the letter, the impact of intestinal reconstitution with lantibiotic-producing Blautia producta was large, on the order of over 1000 fold reduction in VRE colonization, enabling comparison groups to consist of 3 or more mice. All experiments were repeated at least two times (for a total of at least 3 separate experiments). |
| Data exclusions | No data was excluded. |
| Replication | Experiments were repeated at least two times to demonstrate reproducibility. |
| Randomization | We selected samples for analyses by determining microbiota composition and also diversity. Thus, in germ free mice reconstituted with patient derived microbiota, samples differing in lantibiotic gene abundance were matched in terms of microbiota diversity. |
| Blinding | Blinding was not feasible for these experiments since mice receiving different treatments had to be clearly labeled and segregated during the course of the experiment. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---|---|
| n/a | Involved in the study |
| [x] Antibodies | [ ] ChIP-seq |
| [x] Eukaryotic cell lines | [ ] Flow cytometry |
| [x] Palaeontology | [ ] MRI-based neuroimaging |
| [ ] Animals and other organisms | |
| [ ] Human research participants | |
| [ ] Clinical data | |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

We used C57BL/6 mice from Jackson laboratories. Mice were female and 6 to 8 weeks of age. Rag2-/IL2rg-/ mice were purchased from Taconic Laboratories.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.
Human research participants

| Policy information about studies involving human research participants |
|---------------------------------------------------|
| **Population characteristics** | Patients undergoing allogeneic hematopoietic stem cell transplantation were receiving care at Memorial Hospital. Healthy human donors were employees of Memorial Sloan Kettering who consented to providing fecal samples for sequence analyses and for culture. |
| **Recruitment** | Patients were informed of the fecal collection study and consent was obtained prior to initiation of hematopoietic stem cell transplantation. Healthy donors were informed of the study and consent was obtained to perform sequencing studies of fecal samples and to culture bacterial species from the fecal sample. |
| **Ethics oversight** | The fecal collection protocol and the healthy human donor protocol were approved by the Memorial Sloan Kettering Institutional Review Board. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.