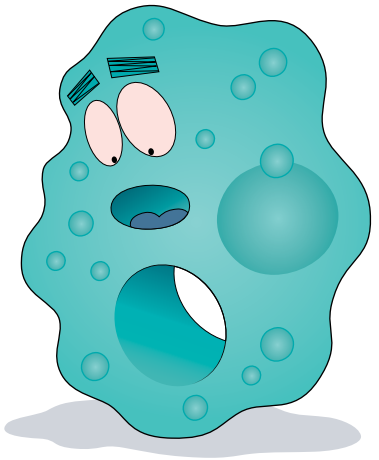
BACTERIAL TOXINS

Emergent *Enterococcus* toxins

Enterococcus is a large genus of bacteria that is found in human and animal microbiomes, and multidrug-resistant enterococci (for instance, *Enterococcus faecalis* and *Enterococcus faecium*) have emerged as important nosocomial pathogens. Unlike many other Gram-positive bacteria, potent protein toxins targeting human and animal cells have not been described in *Enterococcus* species. Now, Xiong et al. uncover a new family of *Enterococcus* pore-forming toxins (Epxs) that use human leukocyte antigen class I (HLA-I) or major histocompatibility complex class I (MHC-I) as receptors.



Credit: Philip Patenall/Springer Nature Limited

The authors collectively identified eight Epxs (Epx1–8) in the genomes of *E. faecalis*, *E. faecium* and *Enterococcus hirae* strains isolated from diverse sources around the world, including animal meat, wastewater, healthy humans, infections and even a 40,000-year-old mammoth gut. Genomic analyses revealed that these toxins are 40–89% identical to each other and are phylogenetically distinct from other pore-forming toxins (PFTs).

Next, focusing on Epx1–4, the authors purified Epxs produced in *Escherichia coli* and confirmed that they are cytotoxic when added to a range of human and animal cell lines. Structural analyses of Epx1 and Epx4 revealed that these toxins form a sub-class of the haemolysin family, with an overall architecture of the toxin pore resembling that of α-haemolysin of *Staphylococcus aureus* and structural features similar to other β-barrel PFTs such as γ-haemolysin and leukocidins. An unexpected feature of Epx pore architecture is the formation of a second β-barrel (termed top domain) that sits on top of the cap region and extends the pore channel by 28 Å. The top domain was found to be crucial for cytotoxicity.

Using genome-wide CRISPR–Cas9 screening and direct binding assays, the authors found that Epx2 and Epx3 recognize the HLA-I or MHC-I complex. Epx2 and Epx3 preferentially recognized human HLA-I and homologous MHC-I of equine, bovine and porcine (but not murine) origin. The authors were able to specifically map the binding site for Epx2 (the region involved in antigen presentation and T cell receptor binding) by switching domains between human HLA-I and murine MHC-I. Treatment with interferon-γ, which upregulates MHC-I expression, significantly increased the sensitivity of human cell lines and intestinal organoids to Epx2- and Epx3-induced cytotoxicity.

Last, the authors co-cultured *E. faecium* DIV0147 harbouring Epx2 with human cell lines, human peripheral blood mononuclear cells (PBMCs) or intestinal organoids. Co-culture with *E. faecium* DIV0147, but not a control strain, led to significant cell line death and damage to PBMCs and intestinal organoid monolayers. The addition of an Epx2 antibody abrogated the toxic effects of *E. faecium* DIV0147.

Altogether, these data demonstrate toxin-mediated virulence by enterococci encoding Epxs with a potential role in immune suppression and epithelial barrier disruption during infection.

Ashley York

ORIGINAL ARTICLE Xiong, X. et al. Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptors. *Cell* https://doi.org/10.1016/j.cell.2022.02.002 (2022)

IN BRIEF

BACTERIAL PATHOGENESIS

Insights into the mechanism of superantigen

Staphylococcus aureus produces the exotoxin superantigen, which binds and activates major histocompatibility complex class II (MHC-II) on host immune cells. Superantigen is a major virulence factor, leading to serious complications including toxic shock syndrome; however, the underlying pathogenic mechanisms are incompletely understood. Using human MHC-II transgenic mice, Tuffs et al. now find that *S. aureus* bloodstream infection stimulates $CD4^+$ T cells to produce interferon-γ (IFNγ) in a superantigen-dependent manner, as shown with superantigen-deletion mutants. IFNγ, in turn, impairs macrophage function and thus increases bacterial loads in the liver. The authors confirmed macrophage dysfunction and increased bacterial replication in human cells.

ORIGINAL ARTICLE Tuffs, S. W. et al. Superantigens promote *Staphylococcus aureus* bloodstream infection by eliciting pathogenic interferon-gamma production. *Proc. Natl Acad. Sci. USA* **119**, e2115987119 (2022)

MICROBIOME

Skewed representation in human microbiome data

To determine the geographical origin of human microbiome studies, Abdill et al. analysed the metadata of human microbiome data deposited in the Sequence Read Archive, DNA Data Bank of Japan and European Nucleotide Archive, which are the three largest genomic databases. Of the 444,829 human microbiome samples, more than 40% came from the United States, followed by China and Western Europe. This over-representation is particularly striking when comparing the sample numbers to the respective population size; for example, the 40% of US samples represents only ~4% of the world population. Countries in central and southern Asia, such as India, Pakistan and Bangladesh, were particularly under-represented and 120 countries had no human microbiome samples in these databases. The authors conclude that a concerted effort is needed to address this disparity and to make microbiome studies more globally representative.

ORIGINAL ARTICLE Abdill, R. J. et al. Public human microbiome data are dominated by highly developed countries. *PLoS Biol.* **20**, e3001536 (2022)

VIRAL INFECTION

Multitude of viruses in game animals

Wild animals that are caught or bred and traded as food are reservoirs of emerging viruses and surveying the virus diversity in such potential reservoir species is a priority. He, Hou, Zhao et al. performed a metatranscriptomic survey in China of 1941 game animals belonging to 18 species, including porcupines, civets, raccoon dogs, pangolins, hedgehogs and rabbits. The authors identified a multitude of viruses in these animals, including 102 vertebrate-associated viruses. The results highlighted the risk of virus spillover; for example, a civet carried the bat coronavirus HKU8 and hedgehogs harboured Middle East Respiratory Syndrome-like viruses. Furthermore, the avian influenza virus H9N2 was present in civets and Asian badgers, with the latter showing symptoms of respiratory disease. The animals also carried multiple zoonotic pathogens as well as human viruses, such as influenza B virus, parainfluenza virus and norovirus. These results highlight that game animals are important conduits of virus spillover, connecting wild animals, domestic animals and humans.

ORIGINAL ARTICLE He, W.-T. et al. Virome characterization of game animals in China reveals a spectrum of emerging pathogens. *Cell* https://doi.org/10.1016/j.cell.2022.02.014 (2022)

RELATED ARTICLE Harvey, E. & Holmes, E. C. Diversity and evolution of the animal virome. *Nat. Rev. Microbiol.* https://doi.org/10.1038/s41579-021-00665-x (2022)