Enterococcus faecalis suppresses *Staphylococcus aureus*-induced NETosis and promotes bacterial survival in polymicrobial infections

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INTRODUCTION

Polymicrobial infections are often correlated with poorer prognoses such as higher mortality. *Enterococcus faecalis* is notorious for its antibiotic resistance and mechanisms to avoid immunosurveillance. Worryingly, *Staphyloccoccus aureus* is found to be increasingly co-isolated with *E. faecalis*, together increasing their overall virulence and pathogenicity in a host.

Neutrophils is fundamental to the immune response of a host. However, recent studies showed that neutrophil responses towards *E. faecalis* is not only lacking, *E. faecalis* also possesses the ability to reduce *S. aureus*-induced neutrophil extracellular trap formation (NETosis). This commensalistic relationship has profound implication for the treatment of these polymicrobial infections.

This study investigates the mechanisms by which *E. faecalis* modulates neutrophil-mediated immune responses in co-infections and explores its impact on the survival of *S. aureus*, highlighting a novel aspect of immune subversion in polymicrobial infections.

MATERIALS AND METHODS

S. aureus (*USA300LAC*) and ***E. faecalis*** (*OG1RF*) in standard culture media to specific optical densities

Mice bone marrow neutrophils (*C57BL/6 mice*) via magnetic-activated cell sorting

In-Vitro Assays

Neutrophil-Bacteria Interaction & Survival

Neutrophils infected with single or mixed species with bacterial survival assessed

Phagocytosis	NETosis
Reactive oxygen species (ROS) production was measured using diphenyleneiodonium and fluorescence assay	Chromatin decondensation, citrullinated histone H3, neutrophil elastase assessed by immunofluorescence microscopy Extracellular DNA release quantified using Sytox Orange stains Neutrophil death via ATP detection (assay)
Degranulation	
Flow Cytometry analysis of surface markers (*CD63, CD15, CD14, CD16*)	

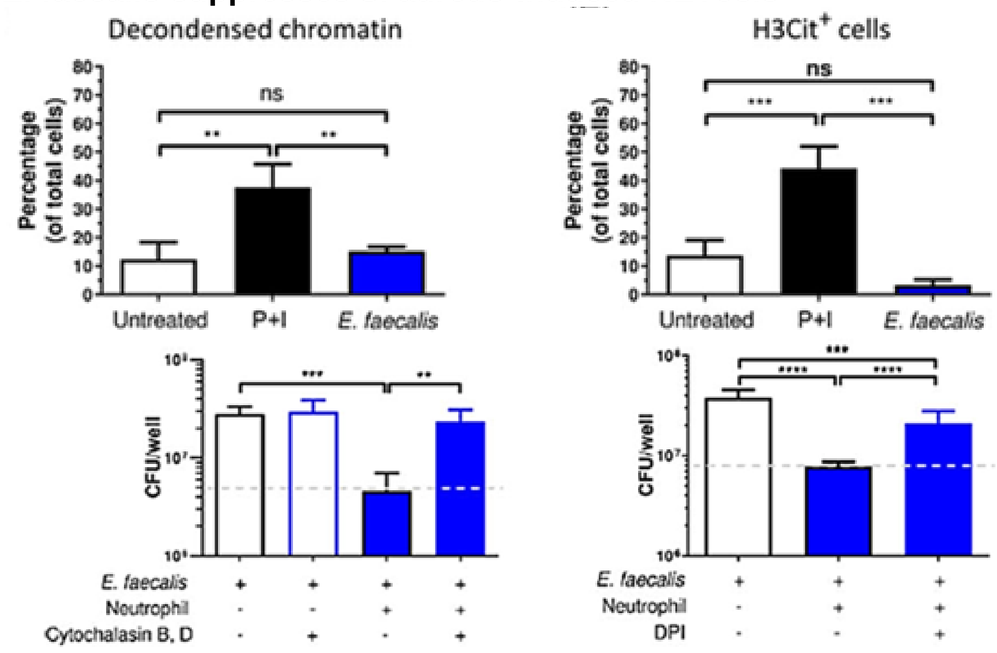
Mouse Wound Infection Model

Wounds made by punch biopsy on dorsal skin and inoculated with bacterial suspensions (single or mixed species)

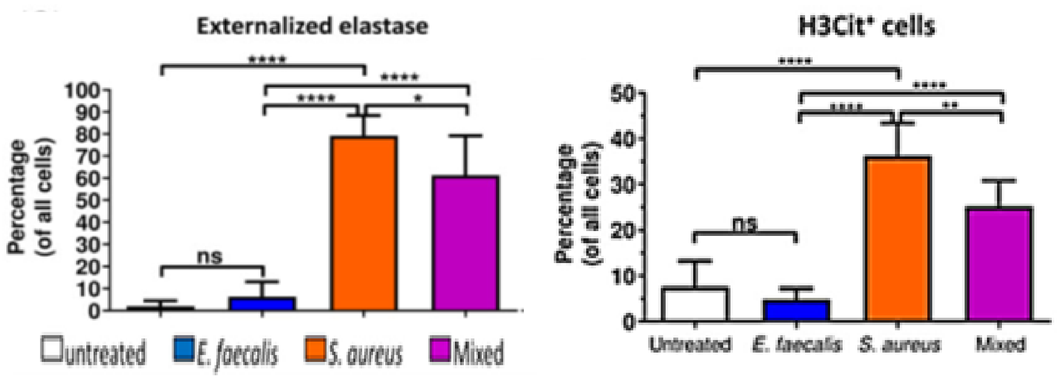
Tissue samples were collected at 24 hrs with bacterial CFUs enumerated

RESULTS

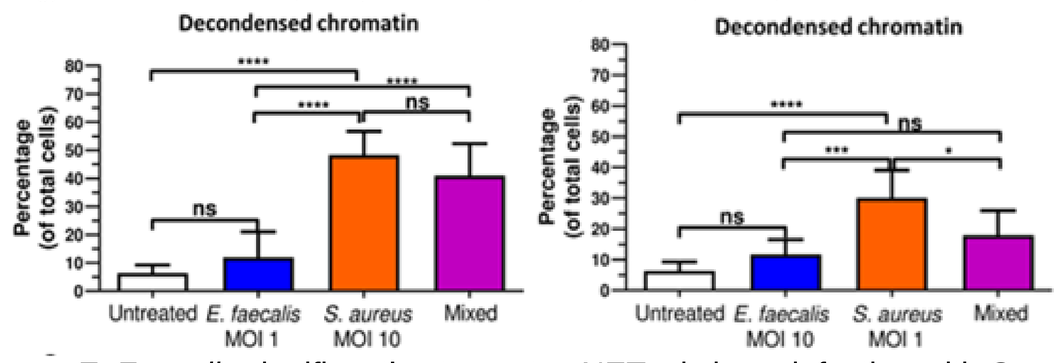
E. faecalis suppresses *S. aureus*-Induced NETosis



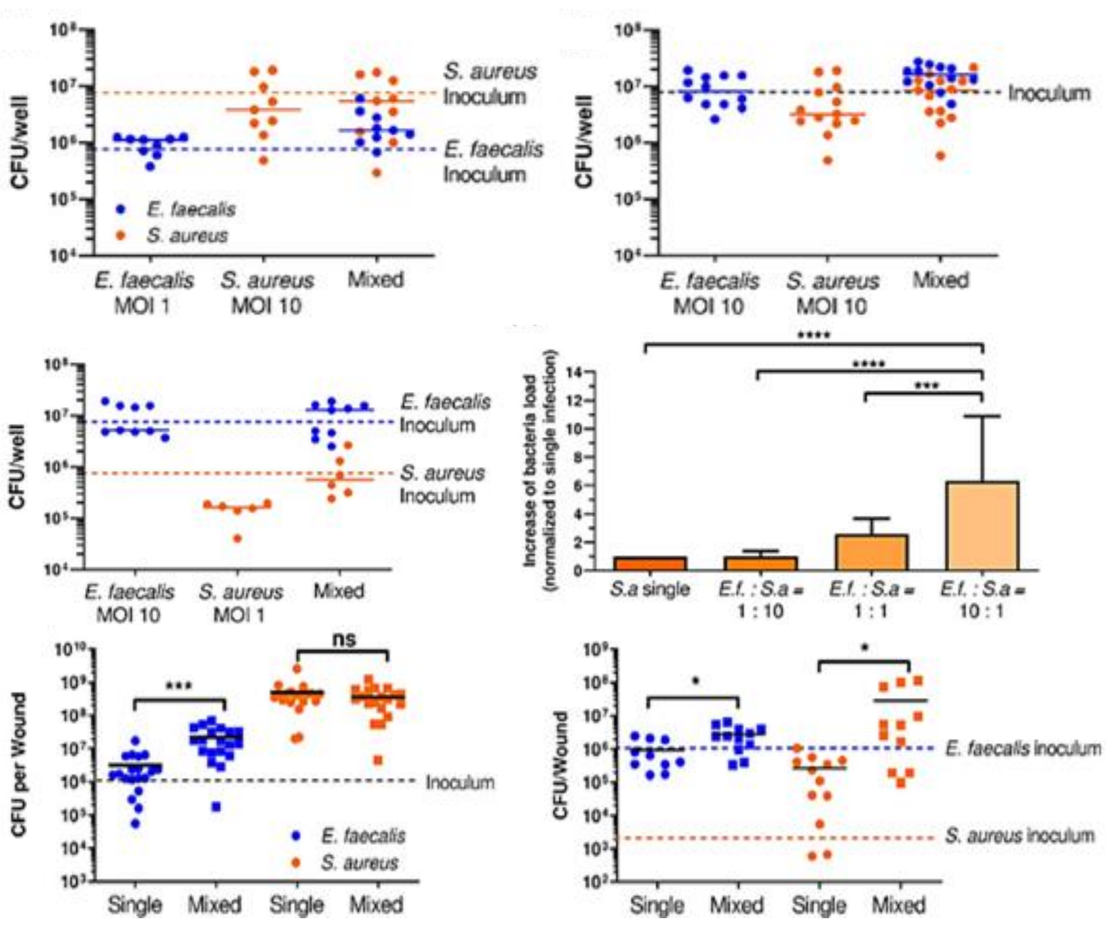
- *E. faecalis* does not induce strong NETosis via PAD-4 mediated (H3Cit+) pathway, with neutrophil response consisting primarily of phagocytosis, ROS-mediated killing, and azurophilic degranulation



- *S. aureus* strongly promotes NETosis - This is partially attenuated by co-infection of *E. faecalis*.



- *E. Faecalis* significantly attenuates NETosis in co-infection with *S. aureus*, with a 10:1 (*E. faecalis:S. aureus*) ratio demonstrating complete attenuation.



- In vitro assays and In vivo wound infection models showed higher inoculum of *E. faecalis* protects *S. aureus* from neutrophil-mediated antimicrobial functions during co-infection.

DISCUSSION AND CONCLUSION

Key findings on *E. faecalis and S. aureus* interactions

- Polymicrobial infections, such as those that involving *S. aureus* and *E. faecalis* are often found in chronic infections, and they complicate treatment due to increased antibiotic resistance and unique survival mechanisms.
- *E. faecalis* alone fails to induce NETosis and actively suppresses NET formation by *S. aureus* in a dose dependent manner in coinfections, promoting the survival of *S. aureus.*
- Coinfection decreases level of citrullinated histones which inhibits NETosis.
- *E. faecalis* is able to evade NETosis-mediated killing and proliferate in coinfections. This is due to virulence factors to avoid NETosis-mediated killing and degrading NET structures.

Limitations of study

- While *E. faecalis* is able to reduce citrullination, it is unclear if *E. faecalis* directly inhibits PAD-4 or if the reduction is due to other indirect mechanisms.
- It is unclear whether the increased in vivo population of *S. aureus* is a result of impaired NETosis or other external factors. Bacteria metabolite exchange and other host cells may have contributed to the colonisation of *S. aureus*.

FUTURE DIRECTIONS

By investigating the specific pathways through which *E. faecalis* attenuates histone citrullination could possibly lead us to discovery of therapeutic targets for treatment.

Clinically. we could collect wound samples from diabetic patients to analyse the interactions between *E. faecalis* and *S. aureus* in vivo. This could allow us to produce more targeted interventions for these co-infections.