# Airway *Corynebacterium* interfere with *Streptococcus pneumoniae* and *Staphylococcus aureus* infection


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## Introduction

- *Streptococcus pneumoniae* colonizes the nasopharynx of 5-70% of the population worldwide and is an important precursor of lower airway infection.
  - *S. pneumoniae* is a leading infectious cause of death in children under 5 years of age and is the most common cause of community-acquired bacterial pneumonia.
  - Current pneumococcal vaccines target up to 23 serotypes of *S. pneumoniae*, however, with over 100 serotypes in circulation and only 60-70% effectives in covered serotypes, this provides only partial protection.
- *Staphylococcus aureus* asymptomatically colonizes the anterior nares of 20-30% of the population and is associated with an increased risk of distant infection including skin and soft tissue infections, endocarditis, bacteremia, and pneumonia.
  - There is currently no vaccine for *S. aureus* and prevention strategies are limited to hygiene and contact prevention.
- *Corynebacterium* are a commensal bacterium in the airway which are correlated with reduced *S. aureus* and *S. pneumoniae* colonization and promotion of a more stable airway microbiome.
  - Here we investigated the potential of *Corynebacterium* colonization as a preventive strategy against pathogen infection.

## *C. pseudodiphtheriticum* protects against airway pathogen infection in mice

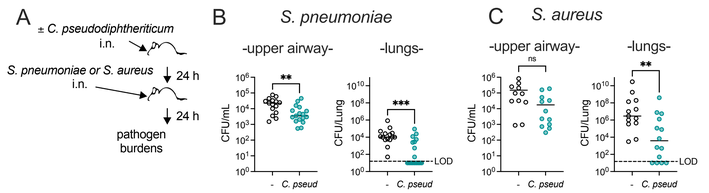


**Figure 1. Exposure to *C. pseudodiphtheriticum* reduces *S. pneumoniae* and *S. aureus* respiratory tract infection.**
(A) Pathogen burdens detected in mice at 24 hours post infection with or without pre-exposure to *C. pseudodiphtheriticum* (*C. pseud*) i.n. Mice were treated with antibiotics for two weeks prior to bacterial exposures. **$p$<.01, ***$p$<.001, Mann-Whitney *U* test. Data are pooled from three independent experiments. LOD indicates the limit of detection for CFUs.

## *C. pseudodiphtheriticum* reduces pathogen adherence to human respiratory tract epithelial cells

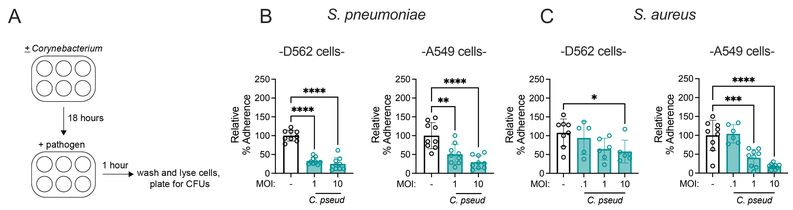


**Figure 3. *C. pseudodiphtheriticum* colonization reduces adherence of *S. pneumoniae* and *S. aureus* to human respiratory tract epithelial cells.** (A) Cell adherence assay schematic. (B) Percent adherence of *S. pneumoniae* on D526 and A549 cells at 1 hour post-infection with or without pre-colonization of epithelial cells with *C. pseudodiphtheriticum* for 18 hours at the indicated MOI. (D) Percent adherence of *S. aureus* as for (C). *$p$<.05, **$p$<.01, ****$p$<.0001, one-way ANOVA with Dunnett's post hoc analysis. Data are pooled from two independent experiments (A) or three independent experiments (C-E) with 3 replicates per condition.

## *C. accolens* reduces pathogen adherence to epithelial cells in a lipase-independent manner

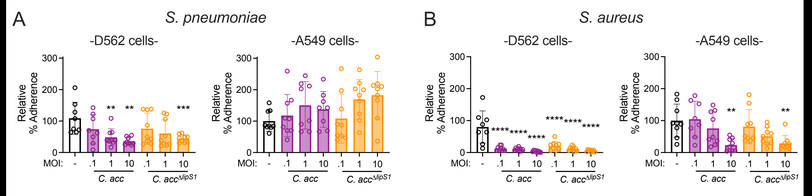


**Figure 4. *C. accolens* colonization reduces pathogen adherence to human respiratory tract epithelial cells in a lipase-independent manner.** (A) Percent adherence of *S. pneumoniae* on D526 and A549 cells at 1 hour post-infection with or without pre-colonization of epithelial cells with *C. accolens* WT (*C. acc*) or *lipS1* deficient *C. accolens* (*C. acc*$^{\Delta lipS1}$) for 18 hours at the indicated MOI. (B) Percent adherence of *S. aureus* as for (A). (**$p$<.01, ***$p$<.001, ****$p$<.0001, one-way ANOVA with Dunnett's post hoc analysis. Data are pooled from two independent experiments (A) or three independent experiments (B-D) with 3 replicates per condition.

## Novel *Corynebacterium* secreted factor inhibits *S. aureus* hemolysis

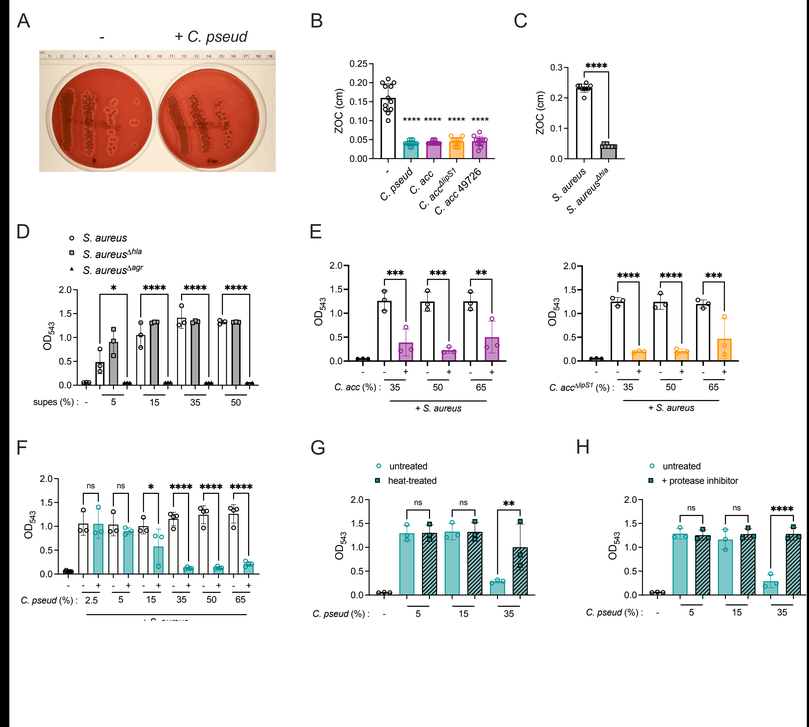


**Figure 6. *Corynebacterium* secreted factor directly inhibits *S. aureus* hemolysin activity.** (A) *S. aureus* colonies on sheep blood agar plates with or without pre-spreading plates with filtered supernatants (CFCM) from *Corynebacterium*, with hemolysis visualized as cleared zones (ZOC) surrounding colonies. (B) Zone of clearance (ZOC) quantified for *S. aureus* as tested as in (A). (C) ZOC quantified for WT *S. aureus* compared with *hla* deficient *S. aureus* as for (B). (D) Hemolysis detected as $OD_{543}$ of human red blood cells combined with the indicated percentage of filtered *S. aureus* supernatants. (E,F) Hemolysis as for (D) with addition of filtered supernatants (CFCM) from *Corynebacterium* at indicated percentages. (G,H) Hemolysis of human red blood cells as in (F) for *S. aureus* supernatants combined with untreated, heat-treated, or protease inhibited CFCM from *C. pseudodiphtheriticum*. *$p$<.05, **$p$<.01, ***$p$<.001, ****$p$<.0001, one-way ANOVA with Dunnett's post-hoc analysis (A), Tukey's post-hoc analysis (D), or Sidak's post-hoc analysis (E-H), or unpaired t test (B). Data are representative of three experiments (A), pooled from three independent experiments with three replicates per group (B) or pooled from 3-4 independent experiments with one replicate per group (E-H).

## *Corynebacterium* secreted lipase selectively inhibits *S. pneumoniae* growth

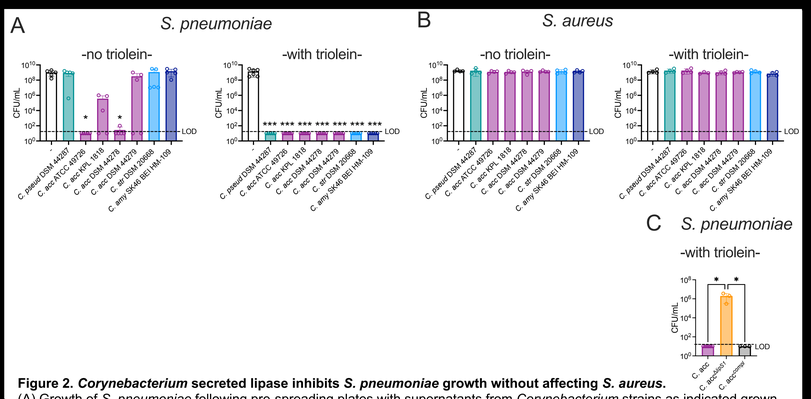


**Figure 2. *Corynebacterium* secreted lipase inhibits *S. pneumoniae* growth without affecting *S. aureus*.**
(A) Growth of *S. pneumoniae* following pre-spreading plates with supernatants from *Corynebacterium* strains as indicated grown with 1% Tween 80 alone (no triolein) or supplemented with 180 mg/mL triolein (with triolein). (B) Growth of *S. aureus* as in (A). (C) Growth of *S. pneumoniae* as for (A) following pre-spreading with supernatants from *C. accolens* WT (*C. acc*), a *C. accolens* mutant deficient in *lipS1 (C. acc*$^{\Delta lipS1}$), or a *lipS1* complemented strain (*C. acc*$^{compl}$), *$p$<.05, ***$p$<.001, Kruskal-Wallis test with Dunn's post hoc analysis. Data are pooled from 3-4 independent experiments. LOD indicates the limit of detection.

## Epithelial cell colonization inhibition requires live *Corynebacterium*

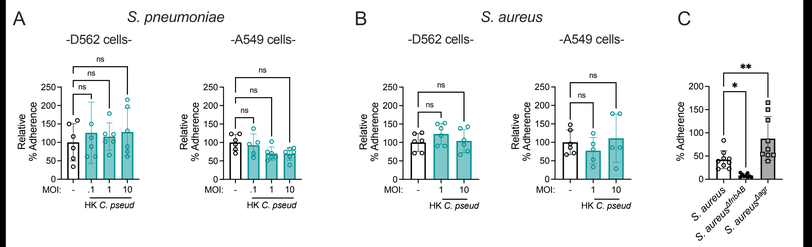


**Figure 5. *Corynebacterium* colonization interference requires live bacteria and is sensitive to *S. aureus* adherence capacity.** (A) Percent adherence of *S. pneumoniae* on D526 and A549 cells 1 hour post-infection with or without 18-hour pre-exposure to heat-killed *C. pseudodiphtheriticum* (HK *C. pseud*) at the indicated MOI. (B) Percent adherence of *S. aureus* MRSA strain USA300 as for (A). (C) Percent adherence of WT *S. aureus*, *fnbAB* deficient *S. aureus*, and *agr* deficient *S. aureus* to A549 cells 1 hour post-infection. *$p$<.05, **$p$<.01, one-way ANOVA with Dunnett's post-hoc analysis. Data are pooled from two (A-B) or three (C-E) independent experiments with three replicates per condition.

## Conclusions and Future Directions

### Conclusions

- Pre-exposure to *C. pseudodiphtheriticum* displays dual protective effects against respiratory tract infection with *S. pnuemoniae* and *S. aureus*.
- Corynebacterium exhibit free fatty acid dependent inhibition of *S. pneumoniae,* highlighting the importance of including exogenous lipids in future testing models.
- *C. pseudodiphtheriticum* and *C. accolens* pre-colonization reduces *S. aureus* and *S. pneumoniae* adherence to epithelial cells in a lipase-independent manner which requires live bacteria, suggesting that a resident microbial population with *Corynebacterium* may offer protective effects against bacterial pathogen infection.
- *Corynebacterium* may reduce *S. aureus* virulence by blocking hemolytic activity through secretion of an unidentified novel factor.

### Future Directions

- Future experimentation using protein purification techniques seeks to further categorize the secreted factor which *Corynebacterium* use to inhibit *S. aureus* hemolytic activity.

## Acknowledgments


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