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Commensal respiratory bacteria stabilize influenza A virus in exhaled droplets and aerosol particles

Introduction
Aerosol transmission is a major challenge for control of respiratory viruses, particularly those causing recurrent epidemics like influenza A virus (IAV). Besides physical virus removal, inactivation of infectious viruses within exhaled aerosol particles and droplets is a crucial process to limit viral spread. However, we have an incomplete understanding of virus survival within these complex environments. Among the many factors that influence respiratory virus stability, the role of commensal bacteria has received little attention to date. Commensal bacteria colonize the respiratory tract and can co-localize with viruses in exhaled aerosol particles and droplets, yet the impact of virus-bacteria interactions on the stability of exhaled viruses remains understudied. Here, we assessed whether the presence common commensal respiratory bacteria could influence the stability of IAV within droplets deposited on surfaces and within airborne aerosol particles.

Methods
Inactivation kinetics of IAV (strain A/Puerto Rico/8/34) we measured in deposited 1 µL droplets (phosphate-buffered saline solution or artificial saliva) in an environmental chamber at 40 and 75% RH, in the presence and absence of five different species of commensal respiratory bacteria. Commensal bacteria included both Gram-positive and Gram-negative species. Additional experiments in the presence of inactivated, structurally intact or inactivated, lysed bacteria were performed to investigate the mechanism of virus protection. The onset of efflorescence during droplet drying was determined by means of videography. Finally, the effect of commensal bacteria on IAV inactivation in small particles was determined in an aerosol chamber at 40% RH.

Results
Bacterial presence within droplets resulted in persistence of 10- to 100-fold more infectious IAV after 1 h of droplet drying, whereby Gram-positive bacteria offered more stabilization to IAV than Gram-negative bacteria. Among the bacteria tested herein, *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most stabilizing compared to other commensals at equivalent density. Bacterial viability wasn't required for viral stabilization, though maintained bacterial morphology seemed essential. The stabilizing effect of bacteria could partly be explained by their effect on the droplet shape: in the presence of bacteria droplets flattened, which caused faster drying and earlier efflorescence. This, in turn, shortened the time during which the virus was exposed to supersaturated salt molalities, which are known to be inactivating. However, even when no efflorescence occurred at 75% RH, or the bacteria-induced changes in droplet morphology were abolished by aerosolization instead of deposition on a well plate, the bacteria remained protective, albeit to a lesser extent.

Conclusions
The respiratory microbiota stabilizes IAV in exhaled aerosol particles and droplets and therefore may be a previously unconsidered contributing factor toward efficacy of respiratory virus transmission. Given that different bacteria stabilize IAV to different degrees, the composition of a person's respiratory microbiota likely influences the efficacy of expelled viral spread and may help explain differences in virus transmission among individuals. Our findings are also relevant to enteric aerosol particles and droplets, such as those generated from wastewater or toilet facilities, where bacterial loads are significant. Finally, this work highlights the need to examine the stability of expelled pathogens in microbially complex matrices to better understand the variability of pathogen transmission in human populations.

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