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MINIREVIEW

Polymicrobial Interactions Operative during Pathogen Transmission

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ABSTRACT Pathogen transmission is a key point not only for infection control and public health interventions but also for understanding the selective pressures in pathogen evolution. The "success" of a pathogen lies not in its ability to cause signs and symptoms of illness but in its ability to be shed from the initial hosts, survive between hosts, and then establish infection in a new host. Recent insights have shown the importance of the interaction between the pathogen and both the commensal microbiome and coinfecting pathogens on shedding, environmental survival, and acquisition of infection. Pathogens have evolved in the context of cooperation and competition with other microbes, and the roles of these cooperations and competitions in transmission can inform novel preventative and therapeutic strategies.

IMPORTANCE Transmission of pathogens from one host to another is an essential event in pathogenesis. Transmission is driven by factors intrinsic to the host and to the pathogen. In addition, transmission is altered by interactions of the pathogen with the commensal microbiota of the host and coinfecting pathogens. Recent insights into these interactions have shown both enhanced and reduced transmission efficiencies depending on the makeup of the polymicrobial community. This review will discuss polymicrobial interactions during shedding from the initial host, time in the environment, and acquisition by the new host.

KEYWORDS coinfection, host-pathogen interactions, pathogenesis, transmission

Transmission of pathogens is a multifactorial process by which a pathogen must be shed from an infected host, survive its transit between hosts, and then establish an infection in a new host. Understanding transmission dynamics of pathogens is key to control of endemic and epidemic infections. In addition, transmission is a point at which pathogens are under selective pressures, since the ultimate "success" of a pathogen is related not only to its ability to cause disease in its hosts but also to its ability to establish productive infections in new hosts. Pathogen transmission can be direct or indirect, it can involve many host species for a pathogen to undergo a complex life cycle, or it can be confined to a single host species. Transmission can occur over short distances of space or time, or a pathogen can spend a long time or distance in the air or associated with a biotic or abiotic surface between hosts.

Transmission factors can be pathogen-associated or host-associated. Some of the best understood pathogen transmission factors are bacterial toxins involved in inducing pathogen shedding, including the AB_5 cholera (1) and pertussis (2) toxins, the cholesterol-dependent pore-forming toxin pneumolysin (3), and the viral enterotoxin nsP4 of rotavirus (4). Host-associated transmission factors can be immune or behavioral. Immune factors can alter both shedding and susceptibility. Naturally acquired or vaccine-induced immunity is mainly thought of as a way to prevent acquisition but can also alter shedding dynamics in the vaccinated host (5). Behavioral factors for humans are typically recreational and occupational exposures but can also be from direct

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pathogen control of behaviors, such as rabies' induction of aggression and salivation and *Toxoplasma gondii*'s reduction of rodents' fear responses (6, 7).

Using a more expansive definition of host, we can include the microbial members of the microbiome, mycobiome, and virome of the host and the roles that these play in shedding, environmental survival, and acquisition of pathogens. In addition, we explore recent insights into the roles of coinfecting pathogens on transmission. Beneficial and antagonistic interactions between microbes can occur in the infected host to alter shedding in the environment, in intermediate hosts to alter pathogen survival between hosts, and in the new host, changing susceptibility to acquisition.

POLYMICROBIAL IMPACTS ON SHEDDING

The microbial community is typically considered to consist of the benign commensal occupants of the surfaces and mucosal sites of humans, plants, and animals, but it can also encompass pathogenic species. These pathogens could be asymptomatically colonizing or causing symptoms while infecting an organism. In addition to more intense signs and symptoms from coinfection, which can enhance shedding, infection by multiple infectious agents can increase the pathogen load at the mucosal site and therefore increase the likelihood and magnitude of shedding. Inflammatory molecules and signs and symptoms, such as coughing, sneezing, mucosal discharge, and diarrhea, induced by one pathogen can cause increased colonization density and shedding of other pathogens infecting the same mucosal site.

Infection with human influenza viruses (8, 9), respiratory syncytial virus (RSV) (10–13), or human rhinovirus (12) can increase the nasopharyngeal load of bacterial pathogens, including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, and *Haemophilus influenzae*. This effect is also seen in murine models (14, 15) and in humans (16–18) receiving live, attenuated influenza vaccine, with higher density and duration of colonization with pathogenic bacteria. Upper respiratory viral illnesses have been implicated in shedding of *S. aureus* in neonates (19) and in adults (20–22). The ability of influenza virus infection to facilitate increased transmission of *S. pneumoniae* has been seen in murine (23, 24) and ferret (25–27) models. Influenza infection can enhance inflammation and nasal secretions (28), impacting pneumococcal colonization density, shedding, and transmission (29, 30). Recent insights have shown that the coinfection of pneumococcus and influenza virus triggers shared interferon responses, enhancing shedding of both pathogens (31). Influenza A virus (IAV) infection can also trigger *S. pneumoniae*'s biofilm to planktonic transition (32, 33), which increases pathogenesis and could be a mechanism for enhanced shedding from the host.

The impact of bacterial colonization density on IAV transmission is mixed. Ferret models of depletion of respiratory flora or restoration of respiratory flora with *S. pneumoniae* did not alter viral load in the respiratory secretions of coinfected animals (34). However, in other studies, coinfection of ferrets with *S. pneumoniae* and IAV reduced shedding of IAV, instead causing pneumonia and bacteremia (27). Infant mouse models of IAV transmission have shown a reduced shedding of IAV in pups colonized with *S. pneumoniae* (35) in a sialidase-dependent manner. However, in humans naturally infected with IAV, the diversity and presence of *Neisseria* were shown to increase the duration of IAV shedding (36). Together, these studies support a role for the human upper respiratory microbiome in shedding of pathogenic bacteria and viruses and suggest differential contributions of the microbiome in alterations of pathogen shedding. However, the roles of the microbiota and coinfecting pathogens in the upper respiratory tract on IAV transmission are complicated; they may be host dependent and specific to certain regions of the upper respiratory tract, requiring further study.

Coinfection with multiple respiratory viruses can alter shedding dynamics of each virus. A prior infection with another respiratory virus decreased duration of RSV shedding, but simultaneous infection increased the duration of RSV shedding in a household transmission study (37). In addition, simultaneous coinfection with both RSV-A and RSV-B subtypes or with either RSV-A or RSV-B subtype and another respiratory

virus enhances the quantity of RSV shed (38). Coinfection with other respiratory viruses was suggested in SARS superspreading events in 2003 (39) and has been suggested as a mode of enhanced transmission for SARS-CoV2 (40). The mechanism behind respiratory virus coinfection and shedding of each virus is presumably multifactorial, since the virus infection can be immunomodulatory, causing increased mucus production, coughing, or sneezing that could physically expel coinfecting pathogens, or could be coincidental due to common exposure conditions for several respiratory viruses.

Respiratory coinfection in domesticated animals enhances pathogen colonization density (41, 42) and shedding, which has relevance for animal health and welfare, integrity of the food animal supply chain, and control of zoonotic and foodborne infections. Chickens coinfected with low-pathogenicity avian influenza (LPAI) and classical infectious bronchitis virus (a gammacoronavirus) had enhanced shedding of both viruses (43, 44); however, compared to singly infected birds, animals coinfected with variant infectious bronchitis virus had enhanced shedding of only LPAI (43). Mammals with coinfecting respiratory pathogens also had enhanced shedding of IAV, with enhanced shedding of swine influenza seen in piglets coinfected with *Actinobacillus pleuropneumoniae* and swine influenza (45). In addition, mink exhibit higher shedding of IAV when coinfected with *Pseudomonas aeruginosa* (46).

Coinfections, likewise, alter pathogen shedding at the genital mucosa. Human immunodeficiency virus infection can increase *Neisseria gonorrhoeae* and *Chlamydia trachomatis* bacterial loads in the vagina and cervical mucus of infected people (47). Further, epidemiological connections support the connection between coinfection with HIV and bacterial sexually transmitted infections (STIs) (48, 49). Coinfection with *N. gonorrhoeae*, likewise, increases vaginal HIV shedding in humanized mice (50), and bacterial STIs, including infections with *N. gonorrhoeae*, and *C. trachomatis*, and protozoan pathogen *Trichomonas vaginalis* enhance shedding of HIV in infected humans (51, 52). However, both bacterial and viral STI acquisition are also dependent on the vaginal microbial community, which complicates the determination of their respective roles in pathogen shedding. Mechanisms for altered shedding are local immunomodulation by vaginal lactobacilli, which reduces genital shedding of HIV without altering systemic viral load (53), and altering vaginal communities in response to concurrent viral infection. HIV-1/HSV-2-coinfected women have higher diversities in the vaginal microbiome, increased anaerobes, decreased lactobacilli, and increased proinflammatory cytokine levels, suggesting enhancement of HIV shedding potential (54). Further, infection with a bacterial STI or presence of bacterial vaginosis enhanced interleukin-10 expression in cervical secretions, allowing increased viral replication and viral load, thereby providing a mechanism for enhanced shedding of HIV (55).

Shedding of GI pathogens is also altered in both humans and domesticated animals by coinfecting pathogens. Shedding of human noroviruses in asymptomatic children is enhanced by coinfection with other enteric viruses (56). Zoonotic hepatitis E virus has enhanced shedding in pigs coinfected with porcine reproductive and respiratory syndrome virus (PRRSV) (57) or porcine circovirus type 2 (58) with a higher hepatitis E viral load in feces and for a longer time than hepatitis E virus monoinfected pigs. Further, both PRRSV and porcine circovirus also enhance transmissibility of hepatitis E between pigs and lead to a higher liver titer of hepatitis E virus, leading to potential zoonotic foodborne infection from eating contaminated liver (59). Coinfection with *Lawsonia intracellularis* or PRRSV enhances shedding of *Salmonella enterica* subsp. *enterica* (60), another foodborne illness causative agent related to pork consumption and production. Boiler chickens infected with infectious bursal disease virus have enhanced shedding of *Campylobacter jejuni* due to immunosuppression by viral infection (61). Cows with liver fluke *Fasciola hepatica* in their feces have increased likelihood of Shiga toxigenic *E. coli* in their feces (62). These findings provide further examples of agricultural pathogens enhancing the risk of foodborne illness.

The beneficial gut microbiome has direct and indirect roles in blocking pathogen acquisition, so modifying the members of the community can alter shedding of

pathogens. Compared to noncolonized gnotobiotic pigs, gnotobiotic pigs that received a human fecal microbiome transplant have enhanced shedding of human noroviruses (63) and rotaviruses (64), as measured by both more days of shedding and higher titers shed, suggesting a role for the human gut microbial community in viral shedding. Likewise, shedding of live, attenuated rotavirus vaccine in humanized piglets was dependent on microbial community (65), as was shedding of rotavirus vaccine virus in antibiotic-treated adult volunteers (66). Attempts to alter the bacterial community to reduce pathogen shedding have also proven fruitful, as gnotobiotic pigs colonized with probiotic *E. coli* Nissle strain have reduced rotavirus severity and shedding duration (67). However, supplementing cattle feed with beneficial microbes did not reduce the frequency of shedding nor the amount of *E. coli* or *Salmonella* shed (68). Modifying the chicken gut microbiome with live, attenuated *S. enterica* vaccination or dietary supplementation with galacto-oligosaccharides reduced shedding of virulent *S. enterica* (69). Disrupting the gut microbiome with antibiotics enhances *Clostridioides difficile* (70), *Klebsiella pneumoniae* (71), and *Salmonella* (72) shedding in mice, supporting a role for the microbiome in preventing hospital-associated and foodborne infections, respectively. Antibiotic treatment led to changes in resident microbiome community composition, supporting the roles of the gastrointestinal microbiome in alteration of shedding and transmission dynamics. Further, modulating the microbiome by supplementing the diet with *Lactobacillus* prevents *C. difficile* shedding in a mouse model (73).

Pathogen shedding is not consistent across all infected hosts. Some hosts appear to shed pathogen at a much higher frequency, a phenomenon deemed "supershedding"; this, along with other environmental factors, leads to the phenomenon of "superspreading," where 20% of infections lead to 80% of new infections (74). The microbiome has been implicated in supershedding of *Salmonella* (70) and enterohemorrhagic *E. coli* (75) and to immune-mediated supershedding of *E. coli* (76).

Viruses such as herpesviruses that lead to a chronic infection can be shed throughout the host's life span. These reactivation and shedding events are often linked to immunosuppressive events and environmental factors. However, coinfecting HIV, beyond being immunosuppressive, has been shown to enhance salivary shedding of human cytomegalovirus, human herpesvirus 8, and Epstein-Barr virus in patients with high HIV titers more than in those with a suppressed HIV load (77). Finally, certain classes of virus require infection by another virus to compete their infection cycles, and thus, to be shed. These viruses include the satellite viruses of plants, adeno-associated viruses of mammals, hepatitis deltavirus, and the recently recognized related deltaviruses of birds (78) and reptiles (79, 80).

POLYMICROBIAL IMPACTS ON ENVIRONMENTAL SURVIVAL

After being shed, a pathogen must oftentimes survive in the environment until it encounters a new susceptible host to infect. The external environment presents many potential challenges for pathogen survival. In the environment, pathogens interact with other microbes in the air, soil, on biotic and abiotic surfaces, and in and on vector hosts. Interaction with other microbes that are simultaneously shed from the infected host or encountered on biotic and abiotic surfaces can protect the pathogen from desiccation and UV light, as detailed in examples below. Alternatively, inactivation of pathogens can be accelerated by such interactions. For example, peptidoglycan-associated cyclic lipopeptide of *Bacillus subtilis* destabilizes coronaviruses and other enveloped viruses, suggesting that these viruses may be inactivated or their infectivity may be reduced by association with soil environments (81). Likewise, bacterial lipopolysaccharide (LPS) destabilizes influenza A viruses (82). However, for enteric viruses, interactions with bacteria and their products are stabilizing. Picornaviruses (83–85), reoviruses (86), caliciviruses (87, 88), and astroviruses (89) are stabilized by interactions with bacteria or isolated bacterial envelope components such as peptidoglycan, LPS, or lipoteichoic acid (LTA). This suggests that these enteric viruses may be stabilized during their

environmental phase by either the bacterial components of the microbiome of the original host or by environmental bacteria present after shedding.

Recent work has shown similar stabilizing effects from certain members of the human upper respiratory microbial community protecting IAVs from desiccation-mediated viability loss. Again, a component of the bacterial cell envelope—the polysaccharide capsule of both *S. pneumoniae* and *H. influenzae*—was shown to be important for stabilizing IAV (34). Further, depletion of the ferret upper respiratory community with antibiotics prior to infection with IAV inhibited respiratory transmission to either antibiotic-depleted or untreated naive contact ferrets. Reconstitution of the donor ferret bacterial community with *S. pneumoniae* restored respiratory transmission of IAV to naive untreated contact animals (34). This finding supports a role for the host's bacterial community in infection by viral pathogens. Another respiratory pathogen, the opportunistic bacterial pathogen *Legionella pneumophila*, which is acquired through inhalation of contaminated water, has enhanced environmental survival when internalized by fungi, protecting the bacteria from UV light (90).

The microbiome of vector hosts also has a key role in transmission of bacterial, parasitic, and viral pathogens. Best characterized is the role of the mosquito endosymbiont *Wolbachia pipientis* in preventing infection of mosquitoes and therefore the transmission of arboviruses (91) and *Plasmodium* (91). Proposed mechanisms of viral inhibition include modulation of lipid metabolism in the mosquito host (92), direct blockade of viral entry into mosquito cells (93), and degradation of viral RNA (94). Another mosquito commensal, *Chromobacterium* sp. Panama, present in *Aedes aegypti* midgut and the soil, secretes a protease that degrades dengue virus envelope protein, thereby blocking infection of mosquito cells *in vitro* and *in vivo* (95) and transmission of dengue virus to human hosts. The natural microbiome of the *Ixodes scapularis* tick is necessary for the tick's colonization by *Borrelia burgdorferi*, the causative agent of Lyme disease (96). Endosymbionts of *Dermacentor andersoni* ticks can control infection and transmission of *Anaplasma marginale*, a rickettsial pathogen of livestock, and *Francisella novicida*, a bacterial pathogen related to the causative agent of tularemia (97).

Foodborne illness is a leading cause of morbidity and mortality worldwide. In addition to the above-mentioned effects of coinfection and microbiome composition of animal hosts in shedding of pathogens in animals used for food, large outbreaks are increasingly being traced back to animal or human fecal contamination of produce, either directly or through the irrigation water (98). Human and animal feces, where one-quarter to one-half of the dry mass is bacterial in origin (99) stabilize enteric viruses on environmental fomites (100). The endogenous microbiome of the plant and associated soil also stabilize pathogens or promote internalization of the pathogen into the plant cell (101), where it can be protected from decontamination. The most common foodborne viruses are hepatitis A virus and norovirus. Both are nonenveloped and have high environmental stability. Peptidoglycan from *Bacillus subtilis*, found in soil and plant phyllosphere environments, protected a norovirus surrogate from bleach-mediated decontamination (102) even in the absence of direct binding of virus to the bacterial surface. Murine norovirus (103) and human noroviruses (88) directly interact with bacteria (88, 103) and fungi (103) and therefore might be protected from decontamination to a higher degree. Bacterial burden and damage to plant tissues correlated with higher norovirus stability on harvested leaves (104), suggesting that bacterial members of the leaf microbiome contribute to norovirus persistence and that this can be enhanced by harvesting or bacterial-mediated damage to the leaf tissues.

Shellfish are also a common source of foodborne infection. Since they are often eaten raw or undercooked, the role of the normal flora of shellfish on pathogen colonization is critical to shellfish-borne infection because high heat inactivation cannot protect consumers in these cases. Shellfish can concentrate foodborne pathogenic viruses from human and animal fecal contamination of seawater within their tissues, including norovirus (105), hepatitis A virus (106), and hepatitis E virus (107). Human pathogens such as *Vibrio vulnificus* and *V. parahaemolyticus* are normal commensals of oysters

(108, 109). Further, interactions between normal commensals of oysters with *S. enterica in vitro* can activate quorum-sensing virulence regulons (110), providing a further mechanism for foodborne-associated illness from shellfish.

POLYMICROBIAL IMPACTS ON ACQUISITION

Probably the best-characterized role of the microbiome in transmission is that of prevention of infection in the new host. Much effort has gone into understanding these protective microbial communities and modifying them via the ingestion and application of probiotics (111). Characterization of the microbial community in people with or without respiratory infection (36, 112–115) and characterization of beneficial and detrimental vaginal microbial communities for bacterial and viral pathogens (116–121) have been insightful for predicting infection risk. Predicted beneficial interactions are both indirect, through bacterial modulation of immune function and epithelial barrier integrity, and direct, through prevention of pathogen binding to epithelial tissues, depletion of metabolites, and production of antagonistic metabolites. The main method by which polymicrobial interactions can alter acquisition of other pathogens is by changing the local inflammatory state, thereby increasing or decreasing the likelihood of successful infection. In addition, the resident microbial community plays an important role in maintaining the permeability of epithelial barriers, alteration of which can impact the invasive potential of pathogens. Nutrient availability, toxic metabolites, and signaling molecules produced by the microbial community at the site of infection alter the pathogen's ability to grow and to express virulence-associated molecules or the host tissue's ability to alter receptor expression. Together, these factors produced by the infection site's microbial community alter the pathogen's ability to successfully infect its next host.

Immune modulation can act either locally or systemically and can be protective or can enhance infection susceptibility. Induction of interferon (IFN) lambda by gut commensal bacteria can lead to persistent murine norovirus infection (122). IFN lambda can also act in the nasal mucosa, where its induction by IAV can promote *Staphylococcus aureus* and *Streptococcus pneumoniae* colonization and infection (123); however, *Staphylococcus epidermidis* can act on nasal cells to produce IFN lambda and prevent IAV infection (124). Local production of IFN beta in the gut stimulated by *Bacteroidetes* outer membrane vesicle glycolipids can block experimental infection with vesicular stomatitis virus and may act more broadly to provide antiviral resistance in the gut (125). Immunomodulatory signals by the gut microbial community can also alter susceptibility to nonenteric infections. Production of the short-chain fatty acid butyrate by enteric commensals can lead to a systemic suppression of IFN production and interferon responsive gene expression and increase susceptibility to influenza virus, reovirus, HIV-1, human metapneumovirus, and vesicular stomatitis virus (126). Alteration of the gut microbial community during an IAV infection can alter the production of another short-chain fatty acid, acetate, reducing bactericidal activity of alveolar macrophages and enhances susceptibility to bacterial pneumonia (127).

Another beneficial function of the microbiota in preventing infection is the promotion of epithelial barrier integrity (128), with microbial signals promoting healthy tissue function by increasing expression of cell-cell adhesion molecules and modulating inflammatory signals. While typically characterized in the mucosa of the gut (129), the microbiota of the skin is important in maintaining barrier integrity and preventing *Staphylococcus aureus* infection (130). In the vagina, *Lactobacillus crispatus* colonization can promote epithelial cell growth and barrier integrity (131), but *Gardnerella vaginalis*, *Atopobium vaginae*, and *Prevotella bivia*, which are considered "bad" vaginal microbiota, act to disrupt tight junctions and can promote the acquisition of the protozoan pathogen *Trichomonas vaginalis* (132).

Barrier function in the respiratory tract is enhanced by mucociliary clearance. The human disease cystic fibrosis results in altered viscosity of mucus and reduced mucociliary clearance, enhancing susceptibility to bacterial colonization and infection of the

lower respiratory tract. Alterations of mucociliary clearance can also be bacterially mediated. Colonization of porcine lung tissues with *Bordetella bronchiseptica* led to reduced ciliary action and enhanced susceptibility to colonization and infection with *Streptococcus suis* (133).

Production of toxic metabolites is another mechanism by which the commensal microbiota prevent colonization and infection by pathogens. A secreted product of the human commensal *Lactobacillus reuteri*, known as reuterin, leads to production of reactive oxygen species by *Clostridioides difficile* to reduce *C. difficile* outgrowth (134). Microbiota-derived fatty acids, in addition to their roles in immunomodulation described above, can alter pathogen colonization and infection. The intestinal parasite *Cryptosporidium parvum* is inhibited by medium- or long-chain saturated fatty acids derived from microbiota, and yet long-chain unsaturated fatty acids enhanced the invasive potential (135) of the parasite. In addition to microbial modulation of *C. difficile* growth, *Bloutia* and *Clostridium sporogenes* metabolites can reduce toxin expression by *C. difficile* (136, 137). In addition, glycan and sphingolipid metabolism by the gut microbiota can alter susceptibility to human norovirus infection (138).

The microbiota or coinfecting pathogens can alter host cell receptor expression to both enhance and inhibit pathogen infection. Colonizing *Streptococcus pneumoniae* can inhibit IAV infection when bacterial sialidase removes terminal sialic acid residues used for viral attachment and infection (35). *Neisseria gonorrhoeae* infection stimulates cytokine production, increasing both immune cell trafficking to the cervix and expression of viral receptors enhancing HIV infection (139).

Quorum-sensing signals from coinfecting pathogens or cytokine signals from infected hosts—even those infected with a different pathogen—can alter bacterial behavior and lead to enhanced pathogenesis. Nontypeable *Haemophilus influenzae* upregulates pili in virally coinfected cells, increasing colonization and invasive potential (140). Pathogen behavior can also be changed through promotion or inhibition of biofilm formation and by formation of multispecies biofilms in the middle ear (141) or gut (142), which can promote both pathogenesis and antimicrobial treatment failure. Multispecies biofilms can even be trans-kingdom, with *Staphylococcus* and *Candida* (143) and *Enterococcus* and *Candida* (144) forming multispecies biofilms. In addition to biofilm-mediated antimicrobial therapy failure, antimicrobial resistance can be transferred from one pathogen to another, particularly in the multispecies biofilm, but also in other cocolonization scenarios, such as *Streptococcus agalactiae* obtaining antimicrobial resistance from coinfecting sexually transmitted pathogens (145).

CONCLUSIONS

Polymicrobial interactions can enhance or reduce pathogen transmission. Greater understanding of the roles of normal and pathogenic members of the microbiome, mycobiome, and virome on transmission of pathogens can lead to insights into control of both endemic and epidemic transmission. Linking certain natural or altered microbial communities to "supershedding" events could explain spillover events in zoonotic transmission and the asymmetric shedding seen in epidemics when 20% of cases cause 80% of new cases (74). In addition, identifying how the pathogens evolved to exploit each other and the microbiome can lead to greater understanding of host-pathogen interactions. As we better characterize the various human, plant, and animal microbial communities, we can decipher the common mechanisms by which pathogens exploit these communities to both establish infection and to be shed and survive in the environment to establish a new infection. Further, knowing the interactions between pathogens and the microbiome of native and nonnative hosts could lend insights into zoonotic transmission.

REFERENCES

1. Reidl J, Klose KE. 2002. *Vibrio cholerae* and cholera: out of the water and into the host. FEMS Microbiol Rev 26:125–139. https://doi.org/10.1111/j.1574-6976.2002.tb00605.x.
2. Scanlon K, Skerry C, Carbonetti N. 2019. Role of major toxin virulence factors in pertussis infection and disease pathogenesis. Adv Exp Med Biol 1183:35–51. https://doi.org/10.1007/5584_2019_403.

3. Zafar MA, Wang Y, Hamaguchi S, Weiser JN. 2017. Host-to-host transmission of *Streptococcus pneumoniae* is driven by its inflammatory toxin, pneumolysin. Cell Host Microbe 21:73–83. https://doi.org/10.1016/j.chom.2016.12.005.
4. Iosef C, Chang KO, Azevedo MS, Saif LJ. 2002. Systemic and intestinal antibody responses to NSP4 enterotoxin of Wa human rotavirus in a gnotobiotic pig model of human rotavirus disease. J Med Virol 68:119–128. https://doi.org/10.1002/jmv.10178.
5. Maier HE, Nachbagauer R, Kuan G, Ng S, Lopez R, Sanchez N, Stadlbauer D, Gresh L, Schiller A, Rajabhathor A, Ojeda S, Guglia AF, Amanat F, Balmaseda A, Krammer F, Gordon A. 2020. Preexisting antineuraminidase antibodies are associated with shortened duration of influenza A (H1N1)pdm virus shedding and illness in naturally infected adults. Clin Infect Dis 70:2290–2297. https://doi.org/10.1093/cid/ciz639.
6. Ingram WM, Goodrich LM, Robey EA, Eisen MB. 2013. Mice infected with low-virulence strains of *Toxoplasma gondii* lose their innate aversion to cat urine, even after extensive parasite clearance. PLoS One 8:e75246. https://doi.org/10.1371/journal.pone.0075246.
7. Vyas A. 2013. Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*. J Exp Biol 216:120–126. https://doi.org/10.1242/jeb.072983.
8. Wolter N, Tempia S, Cohen C, Madhi SA, Venter M, Moyes J, Walaza S, Malope-Kgokong B, Groome M, Du Plessis M, Magomani V, Pretorius M, Hellferscee O, Dawood H, Kahn K, Variava E, Klugman KP, von Gottberg A. 2014. High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. J Infect Dis 210:1649–1657. https://doi.org/10.1093/infdis/jiu326.
9. Thors V, Christensen H, Morales-Aza B, Oliver E, Sikora P, Vipond I, Muir P, Finn A. 2019. High-density bacterial nasal carriage in children is transient and associated with respiratory viral infections: implications for transmission dynamics. Pediatr Infect Dis J 38:533–538. https://doi.org/10.1097/INF.0000000000002256.
10. Brealey JC, Chappell KJ, Galbraith S, Fantino E, Gaydon J, Tozer S, Young PR, Holt PG, Sly PD. 2018. *Streptococcus pneumoniae* colonization of the nasopharynx is associated with increased severity during respiratory syncytial virus infection in young children. Respirology 23:220–227. https://doi.org/10.1111/resp.13179.
11. Brealey JC, Young PR, Sloots TP, Ware RS, Lambert SB, Sly PD, Grimwood K, Chappell KJ. 2020. Bacterial colonization dynamics associated with respiratory syncytial virus during early childhood. Pediatr Pulmonol 55:1237–1245. https://doi.org/10.1002/ppul.24715.
12. Meyer VMC, Siqueira MM, Costa P, Caetano BC, Oliveira Lopes JC, Folescu TW, Motta FDC. 2020. Clinical impact of respiratory virus in pulmonary exacerbations of children with cystic fibrosis. PLoS One 15:e0240452. https://doi.org/10.1371/journal.pone.0240452.
13. Yan T, Tang X, Sun L, Tian R, Li Z, Liu G. 2020. Co infection of respiratory syncytial viruses (RSV) and streptococcus pneumonia modulates pathogenesis and dependent of serotype and phase variant. Microb Pathog 144:104126. https://doi.org/10.1016/j.micpath.2020.104126.
14. Mina MJ, McCullers JA, Klugman KP. 2014. Live attenuated influenza vaccine enhances colonization of *Streptococcus pneumoniae* and *Staphylococcus aureus* in mice. mBio 5:e01040-13. https://doi.org/10.1128/mBio.01040-13.
15. Hirano T, Kurono Y, Ichimiya I, Suzuki M, Mogi G. 1999. Effects of influenza A virus on lectin-binding patterns in murine nasopharyngeal mucosa and on bacterial colonization. Otolaryngol Head Neck Surg 121:616–621. https://doi.org/10.1016/S0194-5998(99)70068-9.
16. de Steenhuijsen Piters WAA, Jochems SP, Mitsi E, Rylance J, Pojar S, Nikolaou E, German EL, Holloway M, Carniel BF, Chu M, Arp K, Sanders EAM, Ferreira DM, Bogaert D. 2019. Interaction between the nasal microbiota and *Streptococcus pneumoniae* in the context of live-attenuated influenza vaccine. Nat Commun 10:2981. https://doi.org/10.1038/s41467-019-10814-9.
17. Tarabichi Y, Li K, Hu S, Nguyen C, Wang X, Elashoff D, Saira K, Frank B, Bihan M, Ghedin E, Methé BA, Deng JC. 2015. The administration of intranasal live attenuated influenza vaccine induces changes in the nasal microbiota and nasal epithelium gene expression profiles. Microbiome 3:74. https://doi.org/10.1186/s40168-015-0133-2.
18. Thors V, Christensen H, Morales-Aza B, Vipond I, Muir P, Finn A. 2016. The effects of live attenuated influenza vaccine on nasopharyngeal bacteria in healthy 2 to 4 year olds: a randomized controlled trial. Am J Respir Crit Care Med 193:1401–1409. https://doi.org/10.1164/rccm.201510-2000OC.
19. Eichenwald HF, Kotsevalov O, Fasso LA. 1960. The "cloud baby": an example of bacterial-viral interaction. Am J Dis Children 100:161–173.
20. Bassetti S, Bischoff WE, Walter M, Bassetti-Wyss BA, Mason L, Reboussin BA, D'Agostino RB, Jr., Gwaltney JM, Jr., Pfaller MA, Sherertz RJ. 2005. Dispersal of *Staphylococcus aureus* into the air associated with a rhinovirus infection. Infect Control Hosp Epidemiol 26:196–203. https://doi.org/10.1086/502526.
21. Belani A, Sherertz RJ, Sullivan ML, Russell BA, Reumen PD. 1986. Outbreak of staphylococcal infection in two hospital nurseries traced to a single nasal carrier. Infect Control 7:487–490. https://doi.org/10.1017/S0195941700065097.
22. Sherertz RJ, Reagan DR, Hampton KD, Robertson KL, Streed SA, Hoen HM, Thomas R, Gwaltney JM, Jr. 1996. A cloud adult: the *Staphylococcus aureus*-virus interaction revisited. Ann Intern Med 124:539–547. https://doi.org/10.7326/0003-4819-124-6-199603150-00001.
23. Kono M, Zafar MA, Zuniga M, Roche AM, Hamaguchi S, Weiser JN. 2016. Single cell bottlenecks in the pathogenesis of *Streptococcus pneumoniae*. PLoS Pathog 12:e1005887. https://doi.org/10.1371/journal.ppat.1005887.
24. Richard AL, Siegel SJ, Erikson J, Weiser JN. 2014. TLR2 signaling decreases transmission of *Streptococcus pneumoniae* by limiting bacterial shedding in an infant mouse influenza A coinfection model. PLoS Pathog 10:e1004339. https://doi.org/10.1371/journal.ppat.1004339.
25. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B. 2010. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. J Infect Dis 202:1287–1295. https://doi.org/10.1086/656333.
26. Rowe HM, Karlsson E, Echlin H, Chang TC, Wang L, van Opijnen T, Pounds SB, Schultz-Cherry S, Rosch JW. 2019. Bacterial factors required for transmission of *Streptococcus pneumoniae* in mammalian hosts. Cell Host Microbe 25:884–891. https://doi.org/10.1016/j.chom.2019.04.012.
27. Brown KM, Sage VL, French AJ, Jones JE, Padovani GH, Avery AJ, Myerburg MM, Schultz-Cherry S, Rosch JW, Hiller NL, Lakdawala SS. 2020. Coinfection of *Streptococcus pneumoniae* reduces airborne transmission of influenza virus bioRxiv https://doi.org/10.1101/2020.11.10.376442:2020.11.10.376442.
28. Barbier D, Garcia-Verdugo I, Pothlichet J, Khazen R, Descamps D, Rousseau K, Thornton D, Si-Tahar M, Touqui L, Chignard M, Sallenave JM. 2012. Influenza A induces the major secreted airway mucin MUC5AC in a protease-EGFR-extracellular regulated kinase-Sp1-dependent pathway. Am J Respir Cell Mol Biol 47:149–157. https://doi.org/10.1165/rcmb.2011-0405OC.
29. Short KR, Reading PC, Wang N, Diavatopoulos DA, Wijburg OL. 2012. Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of *Streptococcus pneumoniae*. mBio 3:e00255-12. https://doi.org/10.1128/mBio.00255-12.
30. Siegel SJ, Roche AM, Weiser JN. 2014. Influenza promotes pneumococcal growth during coinfection by providing host sialylated substrates as a nutrient source. Cell Host Microbe 16:55–67. https://doi.org/10.1016/j.chom.2014.06.005.
31. Zangari T, Ortigoza MB, Lokken-Toyli KL, Weiser JN. 2021. Type I interferon signaling is a common factor driving *Streptococcus pneumoniae* and influenza A virus shedding and transmission. mBio 12:e03589-20. https://doi.org/10.1128/mBio.03589-20.
32. Marks LR, Davidson BA, Knight PR, Hakansson AP. 2013. Interkingdom signaling induces *Streptococcus pneumoniae* biofilm dispersion and transition from asymptomatic colonization to disease. mBio 4:e00438-13. https://doi.org/10.1128/mBio.00438-13.
33. Pettigrew MM, Marks LR, Kong Y, Gent JF, Roche-Hakansson H, Hakansson AP. 2014. Dynamic changes in the *Streptococcus pneumoniae* transcriptome during transition from biofilm formation to invasive disease upon influenza A virus infection. Infect Immun 82:4607–4619. https://doi.org/10.1128/IAI.02225-14.
34. Rowe HM, Livingston B, Margolis E, Davis A, Meliopoulos VA, Echlin H, Schultz-Cherry S, Rosch JW. 2020. Respiratory bacteria stabilize and promote airborne transmission of influenza A virus. mSystems 5:e00762-20. https://doi.org/10.1128/mSystems.00762-20.
35. Ortigoza MB, Blaser SB, Zafar MA, Hammond AJ, Weiser JN. 2018. An infant mouse model of influenza virus transmission demonstrates the role of virus-specific shedding, humoral immunity, and sialidase expression by colonizing *Streptococcus pneumoniae*. mBio 9:e02359-18. https://doi.org/10.1128/mBio.02359-18.
36. Lee KH, Foxman B, Kuan G, López R, Shedden K, Ng S, Balmaseda A, Gordon A. 2019. The respiratory microbiota: associations with influenza symptomatology and viral shedding. Ann Epidemiol 37:51–56. https://doi.org/10.1016/j.annepidem.2019.07.013.

37. Wathuo M, Medley GF, Nokes DJ, Munywoki PK. 2016. Quantification and determinants of the amount of respiratory syncytial virus (RSV) shed using real time PCR data from a longitudinal household study. Wellcome Open Res 1:27. https://doi.org/10.12688/wellcomeopenres.10284.1.
38. Munywoki PK, Koech DC, Agoti CN, Kibirige N, Kipkoech J, Cane PA, Medley GF, Nokes DJ. 2015. Influence of age, severity of infection, and coinfection on the duration of respiratory syncytial virus (RSV) shedding. Epidemiol Infect 143:804–812. https://doi.org/10.1017/S0950268814001393.
39. Bassetti S, Bischoff WE, Sherertz RJ. 2005. Are SARS superspreaders cloud adults? Emerg Infect Dis 11:637–638. https://doi.org/10.3201/eid1104.040639.
40. Weissberg D, Böni J, Rampini SK, Kufner V, Zaheri M, Schreiber PW, Abela IA, Huber M, Sax H, Wolfensberger A. 2020. Does respiratory coinfection facilitate dispersal of SARS-CoV-2? investigation of a super-spreading event in an open-space office. Antimicrob Resist Infect Control 9:191. https://doi.org/10.1186/s13756-020-00861-z.
41. Loving CL, Brockmeier SL, Vincent AL, Palmer MV, Sacco RE, Nicholson TL. 2010. Influenza virus coinfection with *Bordetella bronchiseptica* enhances bacterial colonization and host responses exacerbating pulmonary lesions. Microb Pathog 49:237–245. https://doi.org/10.1016/j.micpath.2010.06.004.
42. Kalhoro DH, Gao S, Xie X, Liang S, Luo S, Zhao Y, Liu Y. 2016. Canine influenza virus coinfection with *Staphylococcus pseudintermedius* enhances bacterial colonization, virus load and clinical presentation in mice. BMC Vet Res 12:87. https://doi.org/10.1186/s12917-016-0708-6.
43. Hassan KE, Ali A, Shany SAS, El-Kady MF. 2017. Experimental coinfection of infectious bronchitis and low pathogenic avian influenza H9N2 viruses in commercial broiler chickens. Res Vet Sci 115:356–362. https://doi.org/10.1016/j.rvsc.2017.06.024.
44. Mahana O, Arafa AS, Erfan A, Hussein HA, Shalaby MA. 2019. Pathological changes, shedding pattern and cytokines responses in chicks infected with avian influenza-H9N2 and/or infectious bronchitis viruses. Virus Dis 30:279–287. https://doi.org/10.1007/s13337-018-00506-1.
45. Pomorska-Mól M, Dors A, Kwit K, Kowalczyk A, Stasiak E, Pejsak Z. 2017. Kinetics of single and dual infection of pigs with swine influenza virus and *Actinobacillus pleuropneumoniae*. Vet Microbiol 201:113–120. https://doi.org/10.1016/j.vetmic.2017.01.011.
46. Bo-Shun Z, Li LJ, Qian Z, Zhen W, Peng Y, Guo-Dong Z, Wen-Jian S, Xue-Fei C, Jiang S, Zhi-Jing X. 2020. Coinfection of H9N2 influenza virus and *Pseudomonas aeruginosa* contributes to the development of hemorrhagic pneumonia in mink. Vet Microbiol 240:108542. https://doi.org/10.1016/j.vetmic.2019.108542.
47. Low AJ, Konate I, Nagot N, Weiss HA, Mabey D, Segondy M, Vickerman P, Meda N, van de Perre P, Mayaud P, for the Yerelon Cohort Study Group. 2014. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection in HIV-1-infected women taking antiretroviral therapy: a prospective cohort study from Burkina Faso. Sex Transm Infect 90:100–103. https://doi.org/10.1136/sextrans-2013-051233.
48. Rob F, Jůzlová K, Kružicová Z, Vaňousová D, Lásiková Š, Sýkorová B, Machala L, Rozsypal H, Veselý D, Zákoucká H, Hercogová J. 2019. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* coinfections among patients with newly diagnosed syphilis: a single-centre, cross-sectional study. Cent Eur J Public Health 27:285–291. https://doi.org/10.21101/cejph.a5142.
49. Ferré VM, Ekouevi DK, Gbeasor-Komlanvi FA, Collin G, Le Hingrat Q, Tchounga B, Salou M, Descamps D, Charpentier C, Dagnra AC. 2019. Prevalence of human papillomavirus, human immunodeficiency virus, and other sexually transmitted infections among female sex workers in Togo: a national cross-sectional survey. Clin Microbiol Infect 25:1560.e1–1560.e7. https://doi.org/10.1016/j.cmi.2019.04.015.
50. Xu SX, Leontyev D, Kaul R, Gray-Owen SD. 2018. *Neisseria gonorrhoeae* coinfection exacerbates vaginal HIV shedding without affecting systemic viral loads in human $CD34^+$ engrafted mice. PLoS One 13:e0191672. https://doi.org/10.1371/journal.pone.0191672.
51. Tanton C, Weiss HA, Le Goff J, Changalucha J, Rusizoka M, Baisley K, Everett D, Ross DA, Belec L, Hayes RJ, Watson-Jones D. 2011. Correlates of HIV-1 genital shedding in Tanzanian women. PLoS One 6:e17480. https://doi.org/10.1371/journal.pone.0017480.
52. Fastring DR, Amedee A, Gatski M, Clark RA, Mena LA, Levison J, Schmidt N, Rice J, Gustat J, Kissinger P. 2014. Co-occurrence of *Trichomonas vaginalis* and bacterial vaginosis and vaginal shedding of HIV-1 RNA. Sex Transm Dis 41:173–179. https://doi.org/10.1097/OLQ.0000000000000089.
53. Mane A, Angadi M, Vidhate P, Bembalkar S, Khan I, Bichare S, Ghate M, Thakar M. 2017. Characterization of vaginal lactobacilli from HIV-negative and HIV-positive Indian women and their association with genital HIV-1 shedding. J Med Microbiol 66:1471–1475. https://doi.org/10.1099/jmm.0.000599.
54. Keller MJ, Huber A, Espinoza L, Serrano MG, Parikh HI, Buck GA, Gold JA, Wu Y, Wang T, Herold BC. 2019. Impact of herpes simplex virus type 2 and human immunodeficiency virus dual infection on female genital tract mucosal immunity and the vaginal microbiome. J Infect Dis 220:852–861. https://doi.org/10.1093/infdis/jiz203.
55. Cohen CR, Plummer FA, Mugo N, Maclean I, Shen C, Bukusi EA, Irungu E, Sinei S, Bwayo J, Brunham RC. 1999. Increased interleukin-10 in the endocervical secretions of women with non-ulcerative sexually transmitted diseases: a mechanism for enhanced HIV-1 transmission? AIDS 13:327–332. https://doi.org/10.1097/00002030-199902250-00004.
56. Ayukekbong J, Lindh M, Nenonen N, Tah F, Nkuo-Akenji T, Bergström T. 2011. Enteric viruses in healthy children in Cameroon: viral load and genotyping of norovirus strains. J Med Virol83:2135–42. https://doi.org/10.1002/jmv.22243.
57. Salines M, Barnaud E, Andraud M, Eono F, Renson P, Bourry O, Pavio N, Rose N. 2015. Hepatitis E virus chronic infection of swine coinfected with porcine reproductive and respiratory syndrome virus. Vet Res 46:55. https://doi.org/10.1186/s13567-015-0207-y.
58. Salines M, Andraud M, Pellerin M, Bernard C, Grasland B, Pavio N, Rose N. 2019. Impact of porcine circovirus type 2 (PCV2) infection on hepatitis E virus (HEV) infection and transmission under experimental conditions. Vet Microbiol 234:1–7. https://doi.org/10.1016/j.vetmic.2019.05.010.
59. Salines M, Dumarest M, Andraud M, Mahé S, Barnaud E, Cineux M, Eveno E, Eono F, Dorenlor V, Grasland B, Bourry O, Pavio N, Rose N. 2019. Natural viral coinfections in pig herds affect hepatitis E virus (HEV) infection dynamics and increase the risk of contaminated livers at slaughter. Transbound Emerg Dis 66:1930–1945. https://doi.org/10.1111/tbed.13224.
60. Belóil P-A, Fravalo P, Fablet C, Jolly J-P, Eveno E, Hascoet Y, Chauvin C, Salvat G, Madec F. 2004. Risk factors for *Salmonella enterica* subsp. *enterica* shedding by market-age pigs in French farrow-to-finish herds. Prev Vet Med 63:103–120. https://doi.org/10.1016/j.prevetmed.2004.01.010.
61. Li L, Pielsticker C, Han Z, Kubasová T, Rychlik I, Kaspers B, Rautenschlein S. 2018. Infectious bursal disease virus inoculation infection modifies *Campylobacter jejuni*-host interaction in broilers. Gut Pathog 10:13. https://doi.org/10.1186/s13099-018-0241-1.
62. Howell AK, Tongue SC, Currie C, Evans J, Williams DJL, McNeilly TN. 2018. Coinfection with *Fasciola hepatica* may increase the risk of *Escherichia coli* O157 shedding in British cattle destined for the food chain. Prev Vet Med 150:70–76. https://doi.org/10.1016/j.prevetmed.2017.12.007.
63. Lei S, Twitchell EL, Ramesh AK, Bui T, Majette E, Tin CM, Avery R, Arango-Argoty G, Zhang L, Becker-Dreps S, Azcarate-Peril MA, Jiang X, Yuan L. 2019. Enhanced GII.4 human norovirus infection in gnotobiotic pigs transplanted with a human gut microbiota. J Gen Virol 100:1530–1540. https://doi.org/10.1099/jgv.0.001336.
64. Kumar A, Vlasova AN, Deblais L, Huang HC, Wijeratne A, Kandasamy S, Fischer DD, Langel SN, Paim FC, Alhamo MA, Shao L, Saif LJ, Rajashekara G. 2018. Impact of nutrition and rotavirus infection on the infant gut microbiota in a humanized pig model. BMC Gastroenterol 18:93. https://doi.org/10.1186/s12876-018-0810-2.
65. Miyazaki A, Kandasamy S, Michael H, Langel SN, Paim FC, Chepngeno J, Alhamo MA, Fischer DD, Huang HC, Srivastava V, Kathayat D, Deblais L, Rajashekara G, Saif LJ, Vlasova AN. 2018. Protein deficiency reduces efficacy of oral attenuated human rotavirus vaccine in a human infant fecal microbiota transplanted gnotobiotic pig model. Vaccine 36:6270–6281. https://doi.org/10.1016/j.vaccine.2018.09.008.
66. Harris VC, Haak BW, Handley SA, Jiang B, Velasquez DE, Hykes BL, Jr, Droit L, Berbers GAM, Kemper EM, van Leeuwen EMM, Boele van Hensbroek M, Wiersinga WJ. 2018. Effect of antibiotic-mediated microbiome modulation on rotavirus vaccine immunogenicity: a human, randomized-control proof-of-concept trial. Cell Host Microbe 24:197–207. https://doi.org/10.1016/j.chom.2018.07.005.
67. Kandasamy S, Vlasova AN, Fischer D, Kumar A, Chattha KS, Rauf A, Shao L, Langel SN, Rajashekara G, Saif LJ. 2016. Differential effects of *Escherichia coli* Nissle and *Lactobacillus rhamnosus* strain GG on human rotavirus binding, infection, and B cell immunity. J Immunol 196:1780–1789. https://doi.org/10.4049/jimmunol.1501705.
68. Brown TR, Edrington TS, Genovese KJ, He HL, Anderson RC, Nisbet DJ. 2020. Evaluation of the efficacy of three direct fed microbial cocktails to reduce fecal shedding of *Escherichia coli* O157:H7 in naturally colonized cattle and fecal shedding and peripheral lymph node carriage of salmonella in experimentally infected cattle. J Food Prot 83:28–36. https://doi.org/10.4315/0362-028X.JFP-19-208.

69. Azcarate-Peril MA, Butz N, Cadenas MB, Koci M, Ballou A, Mendoza M, Ali R, Hassan H. 2017. An attenuated *Salmonella enterica* serovar Typhimurium strain and galacto-oligosaccharides accelerate clearance of *Salmonella* infections in poultry through modifications to the gut microbiome. Appl Environ Microbiol 84:e02526-17. https://doi.org/10.1128/AEM.02526-17.
70. Lawley TD, Clare S, Walker AW, Goulding D, Stabler RA, Croucher N, Mastroeni P, Scott P, Raisen C, Mottram L, Fairweather NF, Wren BW, Parkhill J, Dougan G. 2009. Antibiotic treatment of *Clostridium difficile* carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts. Infect Immun 77:3661–3669. https://doi.org/10.1128/IAI.00558-09.
71. Young TM, Bray AS, Nagpal RK, Caudell DL, Yadav H, Zafar MA. 2020. Animal model to study *Klebsiella pneumoniae* gastrointestinal colonization and host-to-host transmission. Infect Immun 88:e00071-20. https://doi.org/10.1128/IAI.00071-20.
72. Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA, Monack DM. 2008. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infect Immun 76:403–416. https://doi.org/10.1128/IAI.01189-07.
73. Quigley L, Coakley M, Alemayehu D, Rea MC, Casey PG, O'Sullivan Ó, Murphy E, Kiely B, Cotter PD, Hill C, Ross RP. 2019. *Lactobacillus gasseri* APC 678 reduces shedding of the pathogen *Clostridium difficile* in a murine model. Front Microbiol 10:273. https://doi.org/10.3389/fmicb.2019.00273.
74. Woolhouse ME, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JL, Ndhlovu PD, Quinnell RJ, Watts CH, Chandiwana SK, Anderson RM. 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc Natl Acad Sci U S A 94:338–342. https://doi.org/10.1073/pnas.94.1.338.
75. Zaheer R, Dugat-Bony E, Holman DB, Cousteix E, Xu Y, Munns K, Selinger LJ, Barbieri R, Alexander T, McAllister TA, Selinger LB. 2017. Changes in bacterial community composition of *Escherichia coli* O157:H7 supershedder cattle occur in the lower intestine. PLoS One 12:e0170050. https://doi.org/10.1371/journal.pone.0170050.
76. Wang O, Liang G, McAllister TA, Plastow G, Stanford K, Selinger B, Guan Le L. 2016. Comparative transcriptomic analysis of rectal tissue from beef steers revealed reduced host immunity in *Escherichia coli* O157:H7 super-shedders. PLoS One 11:e0151284. https://doi.org/10.1371/journal.pone.0151284.
77. Basso M, Andreis S, Scaggiante R, Franchin E, Zago D, Biasolo MA, Del Vecchio C, Mengoli C, Sarmati L, Andreoni M, Palù G, Parisi SG. 2018. Cytomegalovirus, Epstein-Barr virus, and human herpesvirus 8 salivary shedding in HIV positive men who have sex with men with controlled and uncontrolled plasma HIV viremia: a 24-month longitudinal study. BMC Infect Dis 18:683. https://doi.org/10.1186/s12879-018-3591-x.
78. Wille M, Netter HJ, Littlejohn M, Yuen L, Shi M, Eden JS, Klaassen M, Holmes EC, Hurt AC. 2018. A divergent hepatitis D-like agent in birds. Viruses 10:720. https://doi.org/10.3390/v10120720.
79. Szirovicza L, Hetzel U, Kipar A, Martinez-Sobrido L, Vapalahti O, Hepojoki J. 2020. Snake deltavirus utilizes envelope proteins of different viruses to generate infectious particles. mBio 11:e03250-19. https://doi.org/10.1128/mBio.03250-19.
80. Hetzel U, Szirovicza L, Smura T, Prähauser B, Vapalahti O, Kipar A, Hepojoki J. 2019. Identification of a novel deltavirus in boa constrictors. mBio 10:e00114-19. https://doi.org/10.1128/mBio.00014-19.
81. Johnson BA, Hage A, Kalveram B, Mears M, Plante JA, Rodriguez SE, Ding Z, Luo X, Bente D, Bradrick SS, Freiberg AN, Popov V, Rajsbaum R, Rossi S, Russell WK, Menachery VD. 2019. Peptidoglycan-associated cyclic lipopeptide disrupts viral infectivity. J Virol 93:e01282-19. https://doi.org/10.1128/JVI.01282-19.
82. Bandoro C, Runstadler JA. 2017. Bacterial lipopolysaccharide destabilizes influenza viruses. mSphere 2:e00267-17. https://doi.org/10.1128/mSphere.00267-17.
83. Aguilera ER, Nguyen Y, Sasaki J, Pfeiffer JK. 2019. Bacterial stabilization of a panel of picornaviruses. mSphere 4:e00183-19. https://doi.org/10.1128/mSphere.00183-19.
84. Lu H, Lehrman MA, Pfeiffer JK. 2019. Use of a glycan library reveals a new model for enteric virus oligosaccharide binding and virion stabilization. J Virol 94:e01894-19. https://doi.org/10.1128/JVI.01894-19.
85. Robinson CM, Jesudhasan PR, Pfeiffer JK. 2014. Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. Cell Host Microbe 15:36–46. https://doi.org/10.1016/j.chom.2013.12.004.
86. Berger AK, Yi H, Kearns DB, Mainou BA. 2017. Bacteria and bacterial envelope components enhance mammalian reovirus thermostability. PLoS Pathog 13:e1006768. https://doi.org/10.1371/journal.ppat.1006768.
87. Robin M, Chassaing M, Loutreul J, de Rougemont A, Belliot G, Majou D, Gantzer C, Boudaud N. 2019. Effect of natural ageing and heat treatments on GII.4 norovirus binding to histo-blood group antigens. Sci Rep 9:15312. https://doi.org/10.1038/s41598-019-51750-4.
88. Li D, Breiman A, Le Pendu J, Uyttendaele M. 2015. Binding to histo-blood group antigen-expressing bacteria protects human norovirus from acute heat stress. Front Microbiol 6:659. https://doi.org/10.3389/fmicb.2015.00659.
89. Pérez-Rodriguez FJ, Vieille G, Turin L, Yildiz S, Tapparel C, Kaiser L. 2019. Fecal components modulate human astrovirus infectivity in cells and reconstituted intestinal tissues. mSphere 4:e00568-19. https://doi.org/10.1128/mSphere.00568-19.
90. Alum A, Isaacs GZ. 2016. Aerobiology of the built environment: synergy between legionella and fungi. Am J Infect Control 44:S138–S143. https://doi.org/10.1016/j.ajic.2016.06.004.
91. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA, O'Neill SL. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. Cell 139:1268–1278. https://doi.org/10.1016/j.cell.2009.11.042.
92. Manokaran G, Flores HA, Dickson CT, Narayana VK, Kanojia K, Dayalan S, Tull D, McConville MJ, Mackenzie JM, Simmons CP. 2020. Modulation of acyl-carnitines, the broad mechanism behind *Wolbachia*-mediated inhibition of medically important flaviviruses in *Aedes aegypti*. Proc Natl Acad Sci U S A 117:24475–24483. https://doi.org/10.1073/pnas.1914814117.
93. Lu P, Sun Q, Fu P, Li K, Liang X, Xi Z. 2020. *Wolbachia* inhibits binding of dengue and Zika viruses to mosquito cells. Front Microbiol 11:1750. https://doi.org/10.3389/fmicb.2020.01750.
94. Bhattacharya T, Newton ILG, Hardy RW. 2020. Viral RNA is a target for *Wolbachia*-mediated pathogen blocking. PLoS Pathog 16:e1008513. https://doi.org/10.1371/journal.ppat.1008513.
95. Saraiva RG, Fang J, Kang S, Angleró-Rodríguez YI, Dong Y, Dimopoulos G. 2018. Aminopeptidase secreted by *Chromobacterium* sp. Panama inhibits dengue virus infection by degrading the E protein. PLoS Negl Trop Dis 12:e0006443. https://doi.org/10.1371/journal.pntd.0006443.
96. Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, Eppler-Epstein R, Deponte K, Fish D, Fikrig E. 2014. Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. Cell Host Microbe 15:58–71. https://doi.org/10.1016/j.chom.2013.12.001.
97. Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, Brayton KA. 2016. The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. ISME J 10:1846–1855. https://doi.org/10.1038/ismej.2015.266.
98. Bintsis T. 2018. Microbial pollution and food safety. AIMS Microbiol 4:377–396. https://doi.org/10.3934/microbiol.2018.3.377.
99. Rose C, Parker A, Jefferson B, Cartmell E. 2015. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. Crit Rev Environ Sci Technol 45:1827–1879. https://doi.org/10.1080/10643389.2014.1000761.
100. Abad FX, Pintó RM, Bosch A. 1994. Survival of enteric viruses on environmental fomites. Appl Environ Microbiol 60:3704–3710. https://doi.org/10.1128/AEM.60.10.3704-3710.1994.
101. Erickson MC, Liao JY, Payton AS, Cook PW, Den Bakker HC, Bautista J, Pérez JCD. 2019. Pre-harvest internalization and surface survival of *Salmonella* and *Escherichia coli* O157:H7 sprayed onto different lettuce cultivars under field and growth chamber conditions. Int J Food Microbiol 291:197–204. https://doi.org/10.1016/j.ijfoodmicro.2018.12.001.
102. Shearer AEH, Kniel KE. 2020. Effect of bacteria and bacterial constituents on recovery and resistance of Tulane virus. J Food Prot 83:661–667. https://doi.org/10.4315/0362-028X.JFP-19-300.
103. Madrigal JL, Bhar S, Hackett S, Engelken H, Joseph R, Keyhani NO, Jones MK. 2020. Attach me if you can: murine norovirus binds to commensal bacteria and fungi. Viruses 12:759. https://doi.org/10.3390/v12070759.
104. Esseili MA, Gao X, Tegtmeier S, Saif LJ, Wang Q. 2016. Abiotic stress and phyllosphere bacteria influence the survival of human norovirus and its surrogates on preharvest leafy greens. Appl Environ Microbiol 82:352–363. https://doi.org/10.1128/AEM.02763-15.
105. Hunt K, Doré B, Keaveney S, Rupnik A, Butler F. 2020. Estimating the distribution of norovirus in individual oysters. Int J Food Microbiol 333:108785. https://doi.org/10.1016/j.ijfoodmicro.2020.108785.

106. Park H, Jung S, Shin H, Ha SD, Park TJ, Park JP, Seo DJ, Choi C. 2019. Localization and persistence of hepatitis A virus in artificially contaminated oysters. Int J Food Microbiol 299:58–63. https://doi.org/10.1016/j.ijfoodmicro.2019.03.017.
107. Santos-Ferreira N, Mesquita JR, Rivadulla E, Inácio ÂS, Martins da Costa P, Romalde JL, Nascimento MSJ. 2020. Hepatitis E virus genotype 3 in echinoderms: first report of sea urchin (*Paracentrotus lividus*) contamination. Food Microbiol 89:103415. https://doi.org/10.1016/j.fm.2020.103415.
108. Baker-Austin C, Oliver JD. 2018. *Vibrio vulnificus*: new insights into a deadly opportunistic pathogen. Environ Microbiol 20:423–430. https://doi.org/10.1111/1462-2920.13955.
109. Lee MJ, Lee JJ, Chung HY, Choi SH, Kim BS. 2016. Analysis of microbiota on abalone (*Haliotis discus hannai*) in South Korea for improved product management. Int J Food Microbiol 234:45–52. https://doi.org/10.1016/j.ijfoodmicro.2016.06.032.
110. Cox CE, Wright AC, McClelland M, Teplitski M. 2016. Influence of *Salmonella enterica* serovar Typhimurium *ssrB* on colonization of eastern oysters (*Crassostrea virginica*) as revealed by a promoter probe screen. Appl Environ Microbiol 82:328–39. https://doi.org/10.1128/AEM.02870-15.
111. Leshem A, Liwinski T, Elinav E. 2020. Immune-microbiota interplay and colonization resistance in infection. Mol Cell 78:597–613. https://doi.org/10.1016/j.molcel.2020.03.001.
112. Chapman TJ, Morris MC, Xu L, Pichichero ME. 2020. Nasopharyngeal colonization with pathobionts is associated with susceptibility to respiratory illnesses in young children. PLoS One 15:e0243942. https://doi.org/10.1371/journal.pone.0243942.
113. Yildiz S, Pereira Bonifacio Lopes JP, Bergé M, González-Ruiz V, Baud D, Kloehn J, Boal-Carvalho I, Schaeren OP, Schotsaert M, Hathaway LJ, Rudaz S, Viollier PH, Hapfelmeier S, Francois P, Schmolke M. 2020. Respiratory tissue-associated commensal bacteria offer therapeutic potential against pneumococcal colonization. Elife 9:e53581. https://doi.org/10.7554/eLife.53581.
114. Fanos V, Pintus MC, Pintus R, Marcialis MA. 2020. Lung microbiota in the acute respiratory disease: from coronavirus to metabolomics. J Pediatr Neonat Individualized Med 9:e090139.
115. Tsang TK, Lee KH, Foxman B, Balmaseda A, Gresh L, Sanchez N, Ojeda S, Lopez R, Yang Y, Kuan G, Gordon A. 2020. Association between the respiratory microbiome and susceptibility to influenza virus infection. Clin Infect Dis 71:1195–1203. https://doi.org/10.1093/cid/ciz968.
116. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. 2011. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 108:4680–4687. https://doi.org/10.1073/pnas.1002611107.
117. Torrone EA, Morrison CS, Chen PL, Kwok C, Francis SC, Hayes RJ, Looker KJ, McCormack S, McGrath N, van de Wijgert J, Watson-Jones D, Low N, Gottlieb SL, on behalf of the STIMA Working Group. 2018. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: an individual participant data meta-analysis of 18 HIV prevention studies. PLoS Med 15:e1002511. https://doi.org/10.1371/journal.pmed.1002511.
118. Tamarelle J, de Barbeyrac B, Le Hen I, Thiébaut A, Bébéar C, Ravel J, Delarocque-Astagneau E. 2018. Vaginal microbiota composition and association with prevalent *Chlamydia trachomatis* infection: a cross-sectional study of young women attending a STI clinic in France. Sex Transm Infect 94:616–618. https://doi.org/10.1136/sextrans-2017-053346.
119. Mungati M, Machiha A, Mugurungi O, Tshimanga M, Kilmarx PH, Nyakura J, Shambira G, Kupara V, Lewis DA, Gonese E, Tippett Barr BA, Handsfield HH, Rietmeijer CA. 2018. The etiology of genital ulcer disease and coinfections With *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Zimbabwe: results from the Zimbabwe STI Etiology Study. Sex Transm Dis 45:61–68. https://doi.org/10.1097/OLQ.0000000000000694.
120. Borgogna JC, Shardell MD, Santori EK, Nelson TM, Rath JM, Glover ED, Ravel J, Gravitt PE, Yeoman CJ, Brotman RM. 2020. The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis. Int J Obstet Gynecol 127:182–192. https://doi.org/10.1111/1471-0528.15981.
121. Campisciano G, Gheit T, De Seta F, Cason C, Zanotta N, Delbue S, Ricci G, Ferrante P, Tommasino M, Comar M. 2019. Oncogenic virome benefits from the different vaginal microbiome-immune axes. Microorganisms 7:414. https://doi.org/10.3390/microorganisms7100414.
122. Baldridge MT, Nice TJ, McCune BT, Yokoyama CC, Kambal A, Wheadon M, Diamond MS, Ivanova Y, Artyomov M, Virgin HW. 2015. Commensal microbes and interferon-λ determine persistence of enteric murine norovirus infection. Science 347:266–269. https://doi.org/10.1126/science.1258025.
123. Planet PJ, Parker D, Cohen TS, Smith H, Leon JD, Ryan C, Hammer TJ, Fierer N, Chen EI, Prince AS. 2016. Lambda interferon restructures the nasal microbiome and increases susceptibility to *Staphylococcus aureus* superinfection. mBio 7:e01939-15. https://doi.org/10.1128/mBio.01939-15.
124. Kim HJ, Jo A, Jeon YJ, An S, Lee KM, Yoon SS, Choi JY. 2019. Nasal commensal *Staphylococcus epidermidis* enhances interferon-λ-dependent immunity against influenza virus. Microbiome 7:80. https://doi.org/10.1186/s40168-019-0691-9.
125. Stefan KL, Kim MV, Iwasaki A, Kasper DL. 2020. Commensal microbiota modulation of natural resistance to virus infection. Cell 183:1312–1324. https://doi.org/10.1016/j.cell.2020.10.047.
126. Chemudupati M, Kenney AD, Smith AC, Fillinger RJ, Zhang L, Zani A, Liu SL, Anderson MZ, Sharma A, Yount JS. 2020. Butyrate reprograms expression of specific interferon-stimulated genes. J Virol 94:e00326-20. https://doi.org/10.1128/JVI.00326-20.
127. Sencio V, Barthelemy A, Tavares LP, Machado MG, Soulard D, Cuinat C, Queiroz-Junior CM, Noordine ML, Salomé-Desnoulez S, Deryuter L, Foligné B, Wahl C, Frisch B, Vieira AT, Paget C, Milligan G, Ulven T, Wolowczuk I, Faveeuw C, Le Goffic R, Thomas M, Ferreira S, Teixeira MM, Trottein F. 2020. Gut dysbiosis during influenza contributes to pulmonary pneumococcal superinfection through altered short-chain fatty acid production. Cell Rep 30:2934–2947. https://doi.org/10.1016/j.celrep.2020.02.013.
128. Nowarski R, Jackson R, Flavell RA. 2017. The stromal intervention: regulation of immunity and inflammation at the epithelial-mesenchymal barrier. Cell 168:362–375. https://doi.org/10.1016/j.cell.2016.11.040.
129. Honda K, Littman DR. 2016. The microbiota in adaptive immune homeostasis and disease. Nature 535:75–84. https://doi.org/10.1038/nature18848.
130. Burian M, Bitschar K, Dylus B, Peschel A, Schittek B. 2017. The protective effect of microbiota on *Staphylococcus aureus* skin colonization depends on the integrity of the epithelial barrier. J Invest Dermatol 137:976–979. https://doi.org/10.1016/j.jid.2016.11.024.
131. Takada K, Komine-Aizawa S, Kuramochi T, Ito S, Trinh QD, Pham NTK, Sasano M, Hayakawa S. 2018. *Lactobacillus crispatus* accelerates re-epithelialization in vaginal epithelial cell line MS74. Am J Reprod Immunol 80:e13027. https://doi.org/10.1111/aji.13027.
132. Hinderfeld AS, Phukan N, Bär AK, Roberton AM, Simoes-Barbosa A. 2019. Cooperative interactions between *Trichomonas vaginalis* and associated bacteria enhance paracellular permeability of the cervicovaginal epithelium by dysregulating tight junctions. Infect Immun 87:e00141-19. https://doi.org/10.1128/IAI.00141-19.
133. Vötsch D, Willenborg M, Baumgärtner W, Rohde M, Valentin-Weigand P. 2021. *Bordetella bronchiseptica* promotes adherence, colonization, and cytotoxicity of *Streptococcus suis* in a porcine precision-cut lung slice model. Virulence 12:84–95. https://doi.org/10.1080/21505594.2020.1858604.
134. Engevik MA, Danhof HA, Shrestha R, Chang-Graham AL, Hyser JM, Haag AM, Mohammad MA, Britton RA, Versalovic J, Sorg JA, Spinler JK. 2020. Reuterin disrupts *Clostridioides difficile* metabolism and pathogenicity through reactive oxygen species generation. Gut Microbes 12:1795388. https://doi.org/10.1080/19490976.2020.1795388.
135. VanDussen KL, Funkhouser-Jones LJ, Akey ME, Schaefer DA, Ackman K, Riggs MW, Stappenbeck TS, Sibley LD. 2020. Neonatal mouse gut metabolites influence *Cryptosporidium parvum* infection in intestinal epithelial cells. mBio 11:e02582-20. https://doi.org/10.1128/mBio.02582-20.
136. Mahnic A, Auchtung JM, Poklar Ulrih N, Britton RA, Rupnik M. 2020. Microbiota *in vitro* modulated with polyphenols shows decreased colonization resistance against *Clostridioides difficile* but can neutralize cytotoxicity. Sci Rep 10:8358. https://doi.org/10.1038/s41598-020-65253-0.
137. Abbas A, Zackular JP. 2020. Microbe-microbe interactions during *Clostridioides difficile* infection. Curr Opin Microbiol 53:19–25. https://doi.org/10.1016/j.mib.2020.01.016.
138. Patin NV, Peña-Gonzalez A, Hatt JK, Moe C, Kirby A, Konstantinidis KT. 2020. The role of the gut microbiome in resisting norovirus infection as revealed by a human challenge study. mBio 11:e02634-20. https://doi.org/10.1128/mBio.02634-20.
139. Sanyal A, Shen C, Ding M, Reinhart TA, Chen Y, Sankapal S, Gupta P. 2019. *Neisseria gonorrhoeae* uses cellular proteins CXCL10 and IL8 to enhance HIV-1 transmission across cervical mucosa. Am J Reprod Immunol 81:e13111. https://doi.org/10.1111/aji.13111.
140. Mokrzan EM, Dairo KA, Novotny LA, Bakaletz LO. 2020. Nontypeable *Haemophilus influenzae* responds to virus-infected cells with a

significant increase in type IV pilus expression. mSphere 5:e00384-20. https://doi.org/10.1128/mSphere.00384-20.
141. Bair KL, Campagnari AA. 2019. *Moraxella catarrhalis* promotes stable polymicrobial biofilms with the major otopathogens. Front Microbiol 10:3006. https://doi.org/10.3389/fmicb.2019.03006.
142. Engevik M, Danhof HA, Auchtung J, Endres BT, Ruan W, Bassères E, Engevik AC, Wu Q, Nicholson M, Luna RA, Garey KW, Crawford SE, Estes MK, Lux R, Yacyshyn MB, Yacyshyn B, Savidge T, Britton RA, Versalovic J. 2021. *Fusobacterium nucleatum* adheres to *Clostridioides difficile* via the RadD adhesin to enhance biofilm formation in intestinal mucus. Gastroenterology 160:1301–1314. https://doi.org/10.1053/j.gastro.2020.11.034.
143. Peters BM, Jabra-Rizk MA, Scheper MA, Leid JG, Costerton JW, Shirtliff ME. 2010. Microbial interactions and differential protein expression in *Staphylococcus aureus* -*Candida albicans* dual-species biofilms. FEMS Immunol Med Microbiol 59:493–503. https://doi.org/10.1111/j.1574-695X.2010.00710.x.
144. Krishnamoorthy AL, Lemus AA, Solomon AP, Valm AM, Neelakantan P. 2020. Interactions between *Candida albicans* and *Enterococcus faecalis* in an organotypic oral epithelial model. Microorganisms 8:1771. https://doi.org/10.3390/microorganisms8111771.
145. Capraro GA, Lala S, Khaled K, Gosciniak E, Saadat B, Alvarez SM, Kumar S, Calhoun T, Landry E, Caldito G, Bocchini JA, Jr, Vanchiere JA. 2020. Association of sexually-transmitted infection and African-American race with *Streptococcus agalactiae* colonization in pregnancy. Antimicrob Resist Infect Control 9:174. https://doi.org/10.1186/s13756-020-00827-1.