Polymicrobial-Host Interactions During Infection

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Abstract (250 words or less)

Microbial pathogenesis research has, historically, focused on the study of infections as monomicrobial events. However, the advent of next generation sequencing and culture-independent identification methods has revealed that many, if not most, infections are polymicrobial either in origin or in manifestation. Polymicrobial infections are often associated with increased infection severity and poorer patient outcome. Multiple infecting microbes can interact synergistically to induce virulence traits, to alter the infected niche, or to modulate the host immune response, all of which can promote polymicrobial infection. Importantly, a polymicrobial environment at the time of inoculation, consisting of multiple pathogens or pathogens in combination with the native microbiota, can contribute to the pathogenic progression of a single predominant organism at the time of diagnosis. Hence, in order to completely understand and elucidate the impact of these polymicrobial interactions on infection outcomes, a thorough examination of the entire microbial community present throughout the pathogenic cascade is required: from the time of inoculation to symptomology to resolution. In this review, we highlight themes of metabolite exploitation, immune modulation, niche optimization, and virulence induction that contribute to polymicrobial infections. We focus on recent literature about microbe-microbe and microbe-host interactions that promote polymicrobial infections with an emphasis on understanding these interactions to identify better interventions for these sometimes complex infections.

Abbreviations:

AI, autoinducer; CAUTI, catheter-associated urinary tract infection; CFU, colony forming units; CF, cystic fibrosis; DSS, dextran sodium sulfate; ED, Entner–Doudoroff; GI, gastrointestinal; IBD, inflammatory bowel disease; KGF, keratinocyte growth factor; miRNA, microRNA; MPO, myeloperoxidase; PVL, Panton-Valentine leucocidin; QS, quorum sensing; ROS, reactive oxygen species; Siglecs, sialic acid binding immunoglobulin-like lectins; UTI, urinary tract infection; WLM, wound-like media; ZO, zona-occludens

Keywords (5 words or less, not present in title): UTI, wound infection, SMG, immune modulation, microbial synergy

Introduction

Historically, identifying the causative agent of an infectious disease involved fulfilling Koch's postulates, whereby the causative microbe must be isolated from the infection site, must be culturable in the lab, and must recapitulate the original infection after reintroduction of the organism into the host. While these criteria have been powerful in understanding the etiologies of many infectious diseases, the postulates have several limitations. First, these criteria favor the identification of the most abundant microbial species present at the time of symptomatic infection, ignoring the contribution of other organisms that may have been present at earlier stages of disease or that are simply not culturable. Second, these postulates may assume that secondary microbial species, present at low abundance, are not dominant contributors to disease progression. Yet it is increasingly clear that many or most infections are not monomicrobial, either in origin or in manifestation [1-3]. We now appreciate that many infections either initiate from a polymicrobial inoculation into the infection site, as is the case of the introduction of a bolus of gastrointestinal (GI) tract microbes into the urinary tract prior to urinary tract infection (UTI), or they persist as a polymicrobial community as in the case in catheter-associated urinary tract infections (CAUTI) and in many wound infections. Hence, while the discoveries arising from Koch's postulates gave rise to the field of microbial pathogenesis where the study of monomicrobial infections predominated, the era of sequencing and culture-independent identification of infection-associated microbes have greatly expanded our approach and application of these criteria to the study of infectious diseases [4].

The importance of polymicrobial bacterial communities in the development of some disease states, such as periodontitis and otitis media, have been appreciated for decades and are therefore relatively well-understood [5-9]. However, there is a growing appreciation that some infections that have long been considered as predominantly monomicrobial, such as

UTI, can actually be influenced by both the microbial consortium at the time of infection, as well as other organisms that may be present at lower titers during active infection. Furthermore, evolving populations of microbial organisms, within a protected niche in the form of biofilms, is a common theme in chronic and/or recurrent infections. In addition, the burgeoning appreciation of niche-specific microbiomes has necessitated a reconsideration of microbial community contributions, and effects of altering those communities, to infection in general.

Here, we review the most recent literature on polymicrobial interactions during infection and dissect the latest mechanistic insights into host-polymicrobial interactions leading to disease. While polymicrobial infections often include viral and/or fungal constituents [1], here we focus only polybacterial interactions. Hence, for the purposes of this review, we define polymicrobial infection as two or more bacterial species present at any titer at an infection site. We emphasize niches that have not historically been associated with polymicrobial infection such as the urinary tract, as well as organisms not historically thought to contribute to infection. Several organizing principles have been described by others in considering polymicrobial interactions, including the ecological bases of interactions as well as the various type of interactions (direct bacteria-bacteria, bacteria-host, and indirect interactions via small molecule) [10-12]. In this review, we highlight common functional organizing principles that are emerging across common polymicrobial infection sites, including metabolite exploitation, immune modulation, niche optimization, and virulence induction. Finally, we identify gaps within this field of research where additional research, and/or novel approaches are necessary in order to fully comprehend the pathogenesis and extent of polymicrobial infections.

Gastrointestinal Infections

It is well-established that species and functional diversity within the gastrointestinal (GI) microbiome influences host metabolism, physiology, and immune system development [13-16]. Alteration of microbial communities in the gut can lead to development of diarrheal diseases in the host. The epidemiology, biogeography, host inflammatory responses, and other risk factors of these diarrheal diseases have been recently reviewed [17-20]. Here, we will focus solely on how polymicrobial bacterial interactions impact the development of inflammation of the GI tract, which can, in turn, impact the development of inflammatory bowel disease (IBD) and acute bacterial gastroenteritis [18, 20, 21].

IBD is a chronic inflammatory intestinal disorder that can be subdivided into two distinct types: Crohn's disease and ulcerative colitis [17, 22]. Acute bacterial gastroenteritis, on the other hand, is the result of inflammation of any part of the gastrointestinal tract as a consequence of acute infection [23, 24].

Etiological agents of acute bacterial gastroenteritis have been determined with both culture-dependent and culture-independent methods, and include *Escherichia coli*, *Shigella spp*, *Salmonella spp*, *Vibrio spp*, *Campylobacter spp*, *Yersinia enterocolitica*, *Aeromonas spp*, *Clostridium spp*, *Bacillus spp* and *Listeria spp*, with the three most common being *Campylobacter jejuni*, *Salmonella spp* and *Shigella spp* [25-27]. Hence, infection by any of these microbial species can lead to bacterial-driven inflammation in the host, which can result in acute gastroenteritis. Unlike bacterial gastroenteritis, there is no single defining cause for IBD and may include factors such as the external environment, genetics of the host, and, more recently, infecting microbes [17]. For example, individuals asymptomatically colonized with *Clostridium difficile* are at greater risk of developing IBD [28-30].

Colonization resistance, mediated by both the host and host microbiota, plays an important role in preventing infection by enteric pathogens [31-34]. Numerous studies have shown that IBD development is associated with both displacement of microbiota and increased colonization of enteric pathogens in the ileum [35-39]. In addition, host factors contribute to colonization resistance via immune modulation through the Th1/Th17 and Treg/Th17 axis, as well as by IgA secretion by M cells in Peyer's patches, all of which have been extensively reviewed elsewhere [12, 18, 40-43]. Recently, in a murine model of GI infection, intestinal epithelial cells were shown to promote colonization resistance against opportunistic pathogens through a novel mechanism whereby epithelial interleukin-22 receptor IL-22RA1 mediate fucosylation of glycans in the gut through upregulation of *Fut2* [44-46]. Type 3 innate lymphoid cells drive this process by secreting IL-22 in response to the resident microbiota or invading *S. typhimurium*, resulting in stimulation of IL-22RA1 on intestinal epithelial cells [46]. Fucosylation of glycans is associated with increased abundance of the obligate anaerobes Ruminococcaceae and *Bacteroides spp*., compared to saline controls, driving fucose metabolism by these species. Outgrowth by these anaerobes ultimately drives colonization resistance against *Citrobacter rodentium* and *E. faecalis* [44]. Using a GFP-reporter fused to the promoter of a fucose metabolism gene in *E. coli*, only co-infection of both *E. coli* and *Bacteroides acidifaciens*, which is a microbiota member encoding α(1,2) fucosidase activity, resulted in significant GFP induction in the gut [45]. Importantly, LPS-induced fucosylation of intestinal epithelium in mice protected the animals against infecting *C. rodentium* [45]. Hence, the host has the capability to mediate colonization resistance by supporting the growth of resident microbiota to prevent infection by opportunistic pathogens. Together, these studies demonstrate that a complex interplay exists, where growth promotion of the resident microbiota is mediated not only by the host immune response, but also by nutrient availability.

Nutrient availability is another key determinant in the composition of microbial communities in the gut. The ability of microbes to acquire nutrients through a variety of utilization and competition strategies (metabolite exploitation) can determine their ability to colonize and persist. Infecting bacteria that are able to use more varied energy sources can potentially outcompete the resident microbiota, thereby appropriating the niche for subsequent colonization and infection. The ability to sense and acquire nutrients such as iron, fatty acids and carbohydrate sources can result in differential regulation of virulence factors, influencing the pathogenicity of the infecting bacteria [12, 47-49]. Studies using murine models have shown that the intestinal mucosa converts microbiota-generated toxic hydrogen sulphide to thiosulfate, which limits the build-up of toxic reactive species in the gut [50]. During *Salmonella typhimurium* infection, neutrophils infiltrate the GI tract and release large amounts of reactive oxygen species (ROS) to combat the infection [51]. However, *Salmonella*-invoked ROS release has an unintended consequence of oxidative conversion of thiosulfate to tetrathionate, which *S. typhimurium* is able to utilize as an alternative electron acceptor, allowing the organism to grow better and outcompete the resident microbiota **(Figure 1A)** [51]. Similar to *S. typhimurium*, *Yersinia enterocolitica* has a tetrathionate respiration gene cluster (*ttrBCA*) and is able to utilize tetrathionate as an alternative electron acceptor [52]. Infection with a *Y. enterocolitica* Δ*ttrBCA* mutant resulted in 60% increased survival of TLR1$^{-/-}$ mice as compared to infection with wild type *Y. enterocolitica* in TLR1$^{-/-}$ mice. Interestingly, infection by the Δ*ttrBCA* resulted in the increase in tetrathionate levels but a decrease in the δ--proteobacteria, *Desulfovibrio desulfuricans*, suggesting that the tetrathionate gene cluster is important in outgrowth of δ--proteobacteria in TLR1$^{-/-}$ mice [52]. Collectively, metabolite exploitation by enteric pathogens provides a competitive advantage by allowing them to outcompete the intestinal microbiota and promote infection.

In addition to the example of metabolite exploitation described above, modulation of the immune system is another strategy that different microbial species can exploit during infection of the gut. Invading microbes can stimulate the host inflammatory response to dislodge resident microbiota which facilitates colonization. *C. rodentium* infection results in inflammation with a subsequent decrease in diversity of the intestinal microbiota and a shift to increasing proportions of γ-proteobacteria, which correlates with the development of IBD [33, 53]. In contrast, *C. jejuni* infection does not lead to an inflammatory response in mice, hence the displacement of the microbiota is not observed [54, 55]. In short, microbial-driven inflammation by infecting microbes can induce displacement of the microbiota, disrupting colonization resistance. Using a dextran sodium sulfate (DSS)-induced model of colitis in IL-10 knockout mice, which promotes inflammation, non-pathogenic *E. coli* were able to colonize at levels similar to that of *C. rodentium* in wild type mice, demonstrating that the inflammatory process is crucial for both *E. coli* and *C. rodentium* infection [33, 53]. In addition, members of the microbiota constitutively produce nitric oxide which contributes to resistance to infection by enteric pathogens. *B. subtilis* nitric oxide production promotes colonization resistance by stimulating the innate immune response via increased phosphorylation of p38 MAPK production, an effect that is not observed with the *B. subtilis* Δ*nos* mutant [56]. In addition to immune stimulation, excessive levels of nitric oxide from opportunistic pathogens or the resident microbiota can cause tissue damage in the gut, resulting in inflammation [57, 58]. *Lactobacillus plantarum* has been shown to immunosuppress the host through induction of the anti-inflammatory IL-10 cytokine which is postulated to help co-infecting enteric pathogens to establish infection [59-61]. In a mixed infection of peritoneal exudate cells by both pathogenic *E. coli* and non-pathogenic *Lactobacillus plantarum*, levels of NO and IL-10 increased additively, culminating in both immunosuppression and tissue damage [62]. Moreover, in a trinitrobenzenesulfonic acid

(TNBS)-induced colitis mouse model, co-inoculation of heat-killed *E. coli* and live *L. plantarum* resulted in similar levels of myeloperoxidase (MPO) production as heat-killed *E. coli* monomicrobial inoculation. Mixed infection also resulted in three times less MPO compared to *L. plantarum* monomicrobial infection and was associated with the more frequent occurrence of acute colitis after co-infection compared to *E. coli* monomicrobial infection [62]. Together, these findings suggest that enteric pathogen-mediated immune modulation can drive the disease state of acute colitis to that of a chronic nature.

In addition to inducing the displacement of the resident microbiota, some infecting microbes can synergize with the host or host microbiota to cause inflammatory diarrheal disease. The presence of both *K. pneumoniae* and *P. mirabilis* in the colonic mucus has been correlated with colitis in an murine model lacking an adaptive immune system [63]. Individually, *K. pneumoniae* and *P. mirabilis* can stimulate the production of the pro-inflammatory cytokine, TNF-α, in dendritic cells. Interestingly, these two organisms are unable to cause colitis in the absence of other resident microbiota members, suggesting either synergistic immune modulation by both the infecting and resident microbes, or induction of virulence factors in both organisms that drive inflammation and subsequent colitis [63]. Although the mechanisms of *K. pneumoniae-P. mirabilis*-microbiota synergy are not yet elucidated, it would be unsurprising if there is induction of virulence factors between co-infecting bacteria in the gut based on the synergistic virulence observed in other niches.

Recently, a novel host-microbe interaction was found to be mediated through small non-coding microRNAs (miRNAs) that are produced by the host [64]. Some miRNAs constitutively produced by the GI epithelium share sequence homology with *Fusobacterium nucleatum* and *E. coli* genomes, and these miRNAs can enter *Fusobacterium nucleatum* and *E. coli* cells and co-localize with the bacterial DNA, resulting in augmented bacterial growth

[64]. Exposure to these miRNAs results in increased transcription of *F. nucleatum* rRNA genes and of *E. coli yegH*, suggesting that these host miRNAs are able to regulate bacteria transcription and ultimately affect bacterial growth in the gut. Taken together, synergy between colitis-inducing microbes, including host factors and host microbiota, can result in virulence induction and are important in determining the pathophysiology of a gut infection.

Much research on inflammatory diseases in the gut focuses on the interplay between the resident microbiota, the host, and opportunistic pathogens. Unlike other niches described in this review, the contribution of the microbiota to both protection and infection is better characterized in the GI tract. The majority of these underlying mechanisms involve polymicrobial interactions that affect the fine-tuning of inflammation and nutrient accessibility. Future work requires expanding our understanding of how host factors and host genetics may predispose individuals to infection by enteric pathogens that lead to inflammatory diseases in the gut.

Urinary Tract Infections

Urinary tract infections (UTI) can be acquired from both community and hospital settings, and are clinically differentiated into two broad categories: uncomplicated and complicated. Uncomplicated UTI typically involves healthy individuals without any structural or functional urinary tract abnormalities [65]. Complicated UTI involves individuals with a compromised urinary tract as a result of obstruction or indwelling medical devices such as catheters [66].

The main etiological agent for both uncomplicated and complicated UTI is uropathogenic *Escherichia coli* (UPEC) [67-69]. Unlike uncomplicated UTIs which are usually monomicrobial at the time of diagnosis, complicated UTIs tend to be polymicrobial, with

multiple Gram-negative and Gram-positive organisms including *Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Providencia stuartii*, and *Morganella morganii* [67, 70-72]. Polymicrobial CAUTI can involve up to 5 different culturable bacteria species, with at least one species having CFU counts of $>10^5$ per ml [72, 73]. Approximately 95% of individuals with polymicrobial UTI are infected with two to four different species [74]. Epidemiological studies in the elderly have found that up to 33% of all complicated UTIs are polymicrobial in nature [8, 9]. Culture-independent, nucleic acid-based studies have reported up to 68 species present on Foley catheters, including fastidious and anaerobic microbial species that were routinely missed from traditional culture based studies [75, 76]. Importantly, polymicrobial UTIs have been associated with a poorer disease outcome, highlighting the explicit need to better understand these infection modalities [10].

Despite the prevalence of polymicrobial UTI, there have been few mechanistic studies to investigate the pathogenic strategies or consequences of polymicrobial infections in this niche. In one of the earliest studies, Tsuchimori and colleagues [77] co-infected *E. faecalis* and *P. aeruginosa* in a mouse model of ascending UTI. They demonstrated a synergistic effect between *E. faecalis* and *P. aeruginosa* where co-infection resulted in pyelonephritis which was absent after infection by either species alone [77]. Co-infection with *E. faecalis* also resulted in increased *P. aeruginosa* resistance to clearance by β-lactam antibiotics which was consistent with earlier studies involving co-infection with *P. mirabilis* and *E. faecalis* where eradication of *P. mirabilis* required twice the dose of antibiotics as compared to monomicrobial infections [77, 78]. Although the mechanism leading to enhanced antibiotic resistance after co-infection has not been elucidated in this study, other studies have shown that antibiotic resistance can arise as a result of horizontal gene transfer between the bacteria population [79-81]. Moreover, biofilms can also impede the influx of antibiotics and increase

antibiotic tolerance [82], leading to subsequent long term exposure to sub-inhibitory concentrations of antibiotics [83]. Such exposure eventually select for a rapidly evolving population that eventually becomes resistant to antibiotics [83]. These findings indicate that the presence of Gram-positive bacteria within a polymicrobial UTI is of clinical relevance and could potentially alter disease or treatment outcomes. Moreover, while the presence of Gram-positive bacteria at titers of $<10^5$ CFU/ml in urine have been historically dismissed as contaminants during clinical diagnosis of UTI when another dominant uropathogen is present [84], it is clear from these studies that even low levels of Gram-positive bacteria can have significant effects on UTI outcomes.

More recently, GBS and UPEC co-infection studies demonstrated that UPEC titers were significantly higher in both acute and chronic UTI after co-infection with GBS, compared to monomicrobial UPEC infection, despite the fact that GBS is largely eliminated from the bladder by 24 hours after infection [85]. This finding suggested that GBS impacts the host-pathogen dynamic early in infection, prior to GBS clearance, giving rise to long-term effects on chronic UPEC UTI. GBS monomicrobial UTI studies showed that GBS capsular sialic acids suppress leukocyte recruitment to the infected bladder and were associated with decreased levels of proinflammatory cytokines and increased levels of anti-inflammatory IL-10 in the bladder [86]. GBS surface sialic acids can interact with host sialic acid binding immunoglobulin-like lectins (Siglecs) on neutrophils to limit immune cell function and promote GBS survival [87, 88]. GBS sialic acids also mediate immune suppression during coinfection with UPEC [85]. Taken together, these studies suggest that the presence of GBS during polymicrobial inoculation of the urinary tract, despite its rapid clearance and therefore low titers during acute infection **(Figure 1B)**, impacts UPEC UTI outcomes and may therefore be an overlooked risk factor for chronic UTI in some populations.

In the same study, co-infections with a FimH deficient UPEC strain also resulted in increased survival within the bladder lumen despite being unable to invade the bladder epithelium [85]. Monomicrobial UPEC studies have shown that the Type 1 pilus tip adhesin (FimH) is essential for binding and invasion of the bladder epithelium and a FimH mutant exhibit a 10,000-fold decrease in colonization as compared to the wild type [89]. However, in co-infection studies with GBS, the same FimH UPEC mutant was not attenuated, suggesting that GBS is able to promote UPEC colonization. These studies show that bacteria capable of modulating the host immune response are important contributors to polymicrobial infections in the urinary tract, and other less virulent species may take advantage of this property.

In addition to host immune modulation, inter-species interactions can directly enhance virulence. Among bacterial species of the Proteeae family that are found associated with polymicrobial CAUTI, an overwhelming 86% were urease positive [70]. Urease is a key virulence factor for *P. mirabilis* and *ureC* mutants deficient for urea hydrolysis exhibit attenuated virulence and a reduction in bacterial colonization by >100-fold in both the kidneys and bladder [90, 91]. Urease catalyzes the hydrolysis of urea to ammonia and increases the local pH environment, which causes the subsequent precipitation of cations and anions [92]. This precipitation can lead to the formation of urinary stones and encrustation of catheters leading to catheter obstruction. In one polymicrobial CAUTI study involving urease positive *P. mirabilis* and *P. stuartii* strains, co-infection resulted in increased frequencies of urolithiasis (urinary stones) and bacteremia, and was achieved through the synergistic action of urease activity from each organism [93]. These findings demonstrate how the local environment plays an important role in polymicrobial host-pathogen interaction where synergy in the form of enhanced enzymatic activity can occur, resulting in virulence induction.

More recently, Alteri and colleagues demonstrated that both UPEC and *P. mirabilis*, despite possessing all the necessary genes and enzymes for central metabolic pathways, utilized a distinct set of carbon metabolism pathways *in-vivo* [94]. Differential utilization of central carbon metabolism pathways facilitated enhanced colonization and persistence of both organisms during co-infection in the urinary tract, compared to monomicrobial infections. In a mouse model of ascending UTI, the glycolysis, pentose phosphate, and Entner–Doudoroff (ED) pathways were required for full *P. mirabilis* fitness, while the gluconeogenesis cycle was dispensable. In contrast, the gluconeogenesis and oxidative TCA cycle was required for UPEC, and the glycolysis, pentose phosphate, and ED pathways were dispensable for UPEC [94]. This partitioning of metabolic pathways effectively prevents the two species from directly competing against one another, allowing cooperative growth in a nutrient limited niche. In addition, UPEC CFU were 100-fold greater and *P. mirabilis* were 10-fold higher in both bladder and kidneys following co-infection [94]. Strikingly, unlike UPEC monomicrobial infections where levels of pro-inflammatory cytokines were minimal, monomicrobial *P. mirabilis* infection induced higher levels of the pro-inflammatory cytokines IL-1α, IL-1β, IL-6, and G-CSF and caused more tissue damage [94]. However, during co-infection studies, pro-inflammatory cytokine levels were similar to monomicrobial UPEC infection, demonstrating that host immune modulation by UPEC, or resulting from the combination UPEC and *P. mirabilis*, occurs during co-infection [94].

Together, these recent studies present compelling evidence that the presence of multiple species within the urinary tract can cause profound effects in the host, ranging from immune modulation to changes in the local niche environment. Such synergistic effects can result in enhanced colonization, persistence, increased antimicrobial tolerance, and exacerbated UTIs. All of these factors can make polymicrobial UTIs more difficult to treat. This is further exacerbated by the fact that a large number of polymicrobial UTIs are biofilm-associated

CAUTI [73, 75] which are inherently more tolerant to antibiotic and immune clearance [73]. Numerous studies have shown that biofilms are critical to the pathogenesis of CAUTI and recurrent UTI, where a majority of clinical isolates were found to be capable of biofilm production [95-97]. Biofilm producing microbial species produce extracellular polymeric substance (EPS) that encases the entire community, protecting the microbial organisms from the host immune response, antibiotic penetration, and from mechanical shear stress [82]. As the most common hospital-associated infections [98] and the likelihood that the prevalence of UTI and CAUTI is likely to increase as the global population ages [99], it is increasingly important to understand how bacterial diversity and biofilms affect disease outcomes in the urinary tract.

Wound Infections

Acute wound infections can occur after a breach of the epithelial barrier [100]. While most acute wound infections heal and resolve with time, underlying or existing pathologies such as diabetes mellitus and obesity can result in the eventual development of a chronic wound where the epithelial barrier remains compromised. A majority of chronic wounds involve biofilms [101-103] that are usually polymicrobial in nature [104]. Biofilms are attributed to the non-healing nature of chronic wounds, where their presence not only delays the wound healing process, but also complicates treatment outcomes [101-103, 105-107]. Moreover, polymicrobial wound infections that involve biofilms exhibit increased tolerance to antibiotic treatment as compared to monomicrobial infections [108, 109]. Common etiological agents of chronic wound infections include *P. aeruginosa*, *S. aureus*, *Streptococcus spp.*, *Enterococcus spp.*, and *E. coli* [110, 111]. Here, we focus specifically on how polymicrobial

interactions negatively affect the normal wound healing processes, which can lead to subsequent development of chronic wounds.

In the first polymicrobial experimental wound infection study, performed in guinea pigs, co-infection with both *E. coli* and *Bacteriodes fragilis* resulted in a >100-fold increase in bacterial CFU for each species after 7 dpi, compared to monomicrobial infection. Moreover, compared to monomicrobial infection, co-infection with *B. fragilis* and *E. coli* resulted in increased inflammation and purulent wounds with impaired healing developed, demonstrating pathogenic synergy [112]. Similarly, wounds infected with both *P. aeruginosa* and *S. aureus* have been associated with a chronic non-healing state [113-116]. Interestingly, co-infection studies of *P. aeruginosa* and *S. aureus* have shown that the *P. aeruginosa* limits *S. aureus* growth [109, 117-121]. Using a murine wound excisional model, peptidoglycan sensing by *P. aeruginosa* is important for its competitive advantage in the presence of other Gram-positive species. *P. aeruginosa* is able to respond to *N*-acetylglucosamine or peptidoglycan fragments via a putative two-component response regulator PA0601, resulting in the induction and secretion of elastase and pyocyanin [117]. Upon co-infection with equal numbers of *P. aeruginosa* and *S. aureus*, *P. aeruginosa* outnumbered *S. aureus* by >100-fold at 4 dpi in the wound. However, a *P. aeruginosa* PA0601 deletion mutant was not able to outcompete *S. aureus* in the same manner, suggesting that *P. aeruginosa* specifically senses *S. aureus* peptidoglycan leading to virulence factor induction that allow it to outcompete *S. aureus* in co-infected wounds [117]. However, during co-infection in porcine wounds with *P. aeruginosa*, the *S. aureus* virulence factor protein A was significantly downregulated by >3-fold at both 2 and 4 dpi, whereas Panton-Valentine leukocidin (PVL) and α-hemolysin (hla) were significantly upregulated at 4 dpi compared to monomicrobial infection [109]. PVL and hla are toxic to leukocytes and result in necrosis within wounds [122-125]. These findings therefore suggest that *S. aureus* mounts countermeasures that promote its virulence within

polymicrobial wounds [109, 117]. Moreover, co-infection with *P. aeruginosa* and *S. aureus* together resulted in decreased production of pro-inflammatory cytokines IL-1α, IL-1β, IL-6 and IL-8 compared to either monomicrobial infection [109]. Taken together, these studies show that competition between microbes can result in altered virulence factor expression, as well as host immune response modulation, thus providing a competitive edge to a subset of species within a polymicrobial infection.

Polymicrobial infections can contribute to the chronic non-healing state of wounds by impairing the integrity of the epithelial barrier. *P. aeruginosa* and *Acinetobacter baumanii* co-infection in a porcine burn wound model results in the significant down-regulation of mammalian tight junction proteins zona-occludens-1 (ZO-1) and zona-occludens-2 (ZO-2) compared to uninfected controls, resulting in a functionally leaky epidermis [126]. Having a compromised epithelial barrier can further predispose individuals to other opportunistic microbial infections. Decreased ZO expression occurs via *P. aeruginosa*-mediated induction of host microRNA-(miRNA)-146a and miRNA-106b that silence the genes encoding ZO-1 and ZO-2, as well as via *A. baumanii*-mediated induction of miRNA-106b, leading to an additive increase in miRNA-106b induction in human keratinocytes that is absent during monomicrobial infection. Induction of miRNA-146a and -106b was biofilm-specific because neither a *P. aeruginosa Δpsl* biofilm mutant or planktonic conditioned media could induce the host miRNAs [126]. Similarly, *P. aeruginosa* and *S. aureus* co-infection studies in a porcine wound infection model showed significantly impaired wound re-epithelization through suppression of keratinocyte growth factor 1 (KGF-1), which was absent in monomicrobial infections [109]. These findings provide mechanistic evidence that polymicrobial infections involving biofilms can disrupt the wound healing process and further predisposes the host to other opportunistic microbial infections.

In addition to synergistic virulence gene induction and impairment of the host epithelial barrier function, polymicrobial biofilms in wounds confer enhanced tolerance to antimicrobials. In a study using murine wound excisional model, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *Finegoldia magna*, biofilms were grown *in-vitro* and transplanted onto wounded mice where they remained as a heterogeneous mix of these 4 species for up to 12 dpi [108]. In contrast, in planktonic co-cultures, *P. aeruginosa* outcompeted the other three microbes. Bacterial communities in the transplanted biofilm did not outcompete one another, but instead became spatially separated, where *P. aeruginosa* localized at the wound margin and extended into the dermis while the other three species remained superficial. Differential spatial distribution was speculated to be important for virulence in chronic wounds wherein *P. aeruginosa* projections into the dermis may represent a more hyper-virulent population as compared to the *P. aeruginosa* present in the superficial layer. The hyper-virulent population was associated with an increasing ability to form biofilm infection through the expression of extracellular proteases and iron-scavenging proteins [10, 108, 127]. Moreover, the rate of wound closure in polymicrobial infected mice was 10% slower compared to monomicrobial *P. aeruginosa*-infected mice at 8 dpi, and *P. aeruginosa* from the polymicrobial biofilm wound were 2-fold more tolerant to gentamycin as compared to planktonic cultures [108]. In wound-like media (WLM), where coagulated plasma serves as a scaffold for biofilm formation, polymicrobial *P. aeruginosa* and *S. aureus* biofilms displayed increased gentamicin tolerance which was enhanced by both the host-derived matrix and bacterial extracellular polymeric substances, compared to planktonic co-cultures [128]. Strikingly, upon physical disruption of the community via homogenization, *S. aureus* was >400-fold more sensitive to gentamycin-killing as compared to the intact biofilm, suggesting that the polymicrobial biofilm matrix alone conferred antimicrobial tolerance. Moreover, co-inoculation of *P. aeruginosa algD* and *S. aureus ica* biofilm deficient mutants in either the *in-*

vitro WLM or *in-vivo* murine wound excisional model resulted in increased susceptibility to gentamycin killing. Collectively these findings show that the polymicrobial biofilm matrix, in combination with host matrix proteins, can impede antimicrobial penetration into the biofilm resulting in increased antimicrobial tolerance and greater bacterial persistence within the wound [108, 129].

The majority of studies on polymicrobial wound infections have focused on elucidating interactions between the infecting microbial species, but contributions from the host microbiota are often ignored or disregarded. However, the presence of microbiota-associated organisms in wounds can contribute to lower wound healing efficiency [130, 131]. Recently, the composition of the skin microbiota has been demonstrated to be an indicator of wound healing efficiency [130, 131]. In a comparative study of murine diabetic models, wounds of *db*/*db* diabetic mice contained a greater abundance of Staphylococci compared to *db*/+ mice, which negatively correlated with the efficiency of wound healing in the diabetic mice [130]. Moreover, genes associated with an acute pro-inflammatory response were most strongly induced in *db*/*db* diabetic mice where Staphylococci were abundant, and this prolonged inflammation correlated with non-healing wounds [132-135]. Our current understanding regarding how the resident skin microbiota affects both the propensity for and the severity of wound infections is still limited. Future studies should address whether the resident microbiota has the capability to influence either the host response or other opportunistic pathogen(s) during infection which can ultimately impact the efficiency of wound healing resulting in a poorer disease outcome.

Lung Infections

Polymicrobial lung infections have been most extensively studied in cystic fibrosis (CF) patients. CF is an autosomal recessive disorder [136]. No cure exists for CF patients and they eventually succumb to pulmonary failure as a consequence of microbial infection [137]. Unlike healthy individuals where the lower respiratory tract is asymptomatically colonized by commensals from the oropharynx [138], the lower respiratory tract of individuals with CF are symptomatically colonized with a variety of bacterial species. The dominant infecting organism in the lower respiratory tract of adult CF patients is *P. aeruginosa*, while *Streptococcus pneumonia*, *Haemophilus influenza*, and *S. aureus* are more frequently isolated in juvenile CF patients [139]. However, the exploitation of culture-independent techniques in the last decade has revealed a greater bacterial diversity in the CF lung than was previously appreciated, including numerous anaerobes of the genera *Fusobacterium, Prevotella*, *Rothia*, *Streptococcus*, and *Veilonella* [140, 141]. Mounting evidence suggests that the microbiome of the CF lung is more complex than previously thought, with obligate anaerobes, facultative anaerobes, and microaerophiles playing key roles in disease pathogenesis [140, 142, 143]. Because a number of recent reviews have covered polymicrobial infection in the lung caused by the traditionally-studied infecting bacterial, fungal, and viral organisms [144-146], here we will specifically focus on polymicrobial interactions between aerobic and anaerobic microbial species.

Much recent work in polymicrobial CF involves elucidating the roles of the "*Streptococcus milleri* group" (SMG). The SMG are emerging opportunistic pathogens that have been increasingly associated with polymicrobial infections in which strict anaerobes are of a significant burden to CF patients [147, 148] and are also commonly co-isolated with *P. aeruginosa* [148]. This group is made up of the viridians streptococci: *S. constellatus*, *S. intermedius*, and *S. anginosus*. The SMG are usually facultative anaerobes with some isolates requiring and/or exhibiting enhanced growth under high CO_2 conditions while some isolates

are only able to grow under anaerobic conditions [149, 150]. SMG are usually present only in the upper respiratory tract of healthy individuals, but have been found to exist in both the upper and lower respiratory tracts of CF patients [151].

In a murine model of *S. constellatus* and *Prevotella intermedia* co-infection, acute pneumonia with a 60% mortality rate was observed, whereas monomicrobial infections resulted only in mild pneumonia with a 10% mortality rate [147]. Extended *S. constellatus* and *P. intermedia* persistence was also observed during co-infections, with lung CFU at 60 hours post infection (hpi) of up to 10^6 and 10^3, respectively. In monomicrobial infections, no viable CFU were recovered for either species 60 hpi [147]. *In vitro* experiments demonstrated that *P. intermedia*, or *P. intermedia* culture supernatants alone, enhanced the growth of *S. constellatus* by 10-fold [147, 152]. In addition, *P. intermedia* cell free culture filtrates impaired phagocytic killing of bacteria by host polymorphonuclear leukocytes, and monomicrobial infections of *S. constellatus* supplemented with *P. intermedia* culture filtrates also resulted in longer survival of *S. constellatus* [147, 152].

In one of the first mechanistic studies of interspecies interactions between *P. aeruginosa* and the oropharyngeal microbiota, Duan and colleagues demonstrated that the viridians streptococci are capable of enhancing *P. aeruginosa* pathogenicity through modulation of virulence gene expression [151]. Key *P. aeruginosa* virulence genes involved in the synthesis and production of rhamnolipids, flagellin, exotoxin, elastase, and phenazine were all upregulated during co-culture with *Streptococcus* strain CF004 (species not indicated). This modulation was achieved through production of the autoinducer 2 (AI-2) metabolite by *Streptococcus* strain CF004, which *P. aeruginosa* is unable to produce but is capable of responding to **(Figure 1D)** [151, 153]. Subsequent studies showed that *S. anginosus*, *S. constellatus*, and *S. intermedius* clinical isolates from CF patients were also able to produce AI-2 individually [154]. However, the ability of *P. aeruginosa* to respond to AI-2 is not

confined to the SMG, and modulation can occur with other AI-2 producing microbial species [151]. Numerous studies have shown that AI-2 is responsible for facilitating interspecies communication [155-158] and AI-2 antagonists have been shown to successfully interfere with both intra- and inter-species communication *in-vitro* [159-162].

The clinical significance of SMG in polymicrobial CF was further demonstrated when ceftriaxone antibiotic treatment to specifically eliminate *S. constellatus* and *S. intermedius* resolved acute pulmonary exacerbation in CF patients. Surprisingly, clinical improvement correlated with a corresponding decrease in *S. constellatus* and *S. intermedius*, but not *P. aeruginosa* [163]. Recent studies have shown that treatment of *P. aeruginosa* with tobramycin in CF patients has unintended consequences of increasing the CFU of other microbial species [164]. During *S. constellatus* and *P. aeruginosa* co-infection studies, *P. aeruginosa* produce rhamnolipids that directly kills *S. constellatus*. However, tobramycin treatment to target *P. aeruginosa* reduces *P. aeruginosa* CFU, which correlates with lower rhamnolipid production and, hence, higher *S. constellatus* CFU [164]. Interactions between the viridians Streptococcus and *P. aeruginosa* is complex, because while *P. aeruginosa* is able to produce rhamnolipids which kills the viridians Streptococcus, the viridians Streptococcus are also able to produce AI-2 which can upregulate rhamnolipid production in *P. aeruginosa* **(Figure 1D)** [151] or can be used as a metabolite by *P. aeruginosa* [165] thus providing a competitive advantage to *P. aeruginosa*. Together, these findings suggest that the infection dynamics are constantly in flux, where antibiotic administration against traditional CF pathogens can result in non-traditional CF pathogens such as the SMG either having a growth advantage, increased persistence, or exacerbate clinical symptoms.

Conflicting reports have emerged in which the presence of SMG were also found to correlate with better disease outcome and patient stability [141] instead of acute exacerbation [148]. Recent studies have identified SMG intra-species diversity as the main driver for anti- or pro-

inflammatory responses, where an initial acute infection elicits a strong immune response from the host, which selects for immunomodulatory strains that elicit a weaker pro-inflammatory cytokine response [166]. These findings resolve the conundrum and suggest that species diversity, which can be influenced by multiple factors ranging from antibiotic treatment to microbial diversity to the state of the host, is important for patient outcome.

The unexpected discovery of numerous non-traditional CF bacterial species through culture independent techniques has expanded the field of CF lung polymicrobial research. The studies discussed here show that formerly under-appreciated CF-associated bacterial species like SMG are also important in polymicrobial infections, functioning through immune modulation, virulence induction and in niche optimization whereby QS molecules from one species can influence other non-producing species. Importantly, studies have shown that the lung environment can be anaerobic and oxygen levels within the mucus of the CF lung decrease with disease progression [167]. Varying oxygen levels within the mucus can thus support or facilitate the growth of various aerobes, facultative anaerobes, and obligate anaerobes within these localized niches where inter- and intra-species interactions can occur [168, 169]. These findings therefore necessitate investigating the interactions between traditional CF and non-traditional CF pathogens with special attention to the environment within the CF lung, especially since certain areas within this niche can be anoxic and would favor growth of SMG and/or other anaerobes.

Conclusions

Determining the etiologic agents of infection have long relied on isolation of culturable bacteria from the site of infection, often focusing on the predominant isolated organism during the height of infection. The widespread application of next-generation sequencing

552 technologies to the study of infectious disease has resulted in a greater appreciation that many
553 infections are polymicrobial in nature, both in time and space, rendering inadequate the
554 application of Koch's postulates to some polymicrobial infections, especially those involving
555 non-culturable organisms. Moreover, our growing understanding of the delicate interplay
556 between the native microbiome, invading pathogens, and the host response to each, has given
557 rise to many new questions about the fundamental basis of bacterial pathogenesis.

558 In this review, we highlight several themes central to polymicrobial-host interactions that
559 promote infectious diseases: 1) metabolite exploitation, 2) immune modulation, 3) niche
560 optimization, and 4) virulence induction. First, while a diverse GI microbiota aids in the
561 resistance to pathogens, metabolic by-products of the same microbiota may be converted to a
562 molecule that can be exploited by *Salmonella* for its own growth in an inflammatory
563 environment (**Fig 1A**). Second, it is increasingly clear that differential temporal presence of
564 multiple organisms can also affect infection progression or outcome. This is exemplified by
565 the case of polymicrobial inoculation of GI-associated bacteria into the urinary tract, wherein
566 the presence of GBS can suppress the acute immune response and, despite its own rapid
567 clearance, can augment chronic UPEC infection (**Fig 1B**). The infection niche can be
568 differentially exploited for optimal polymicrobial persistence via metabolic
569 compartmentalization to prevent inter-species competition, such as occurs with *P. aeruginosa*
570 and *S. aureus* in wounds (**Fig 1C**). This means that there may be an important role for the
571 polymicrobial community at the time of, or preceding, pathogen introduction, even if that
572 same community is not present at the time of symptomology or diagnosis. Therefore, a
573 thorough investigation into the organisms present at an infection site before, during, and after
574 pathogen introduction may provide insight into communities that promote infection versus
575 those that do not. Finally, there are a growing number of examples in which one species (or
576 group of species) can alter the virulence gene expression profiles of another, as is the case for

induction of a biofilm transcriptional program in *P. aeruginosa* when cued by SMG production of AI-2 in the CF lung (**Fig 1D**). A summary of the polybacterial interactions in different host niches can be found in **Table 1**. Polymicrobial biofilms further provide an often ideal environment for co-opting or sharing of nutrients, exchange of genetic determinants that promote virulence, and provide a protective niche to withstand the onslaught of host immune defenses [170, 171]. Taken together, it is clear that the traditional reductionist approach taken in the identification of etiological agents associated with diseases is fraught with various inadequacies. Moving forward, a systems based approach should be adopted as polymicrobial diseases often involve a number of microbial species, in which their specific interactions with each other as well as with the host and environment can contribute to the disease outcome.

Future challenges

Given the broadening landscape of how microbial consortia can work together to cause infection, careful attention must be paid to the entire community present during the pathogenic cascade: from the time of inoculation to symptomology to resolution. Yet, due to the complexity of bacteria-host interactions and the difficulty in extricating the influences of one bacteria species on another *in vivo*, most polymicrobial infection studies to date have been limited to co-infection with two species. Future work should move beyond the current two species co-infection models to integrate investigation of the molecular, spatial, and temporal dynamics after polymicrobial inoculation of the infection niche. The fields of oral microbiology and microbial ecology lead the way in mechanistic studies of these polymicrobial interactions, where lessons of how interspecies diversity promotes stress tolerance, how individual members of the community contribute to biofilm development and resilience, and how diversity promotes human health have been demonstrated [172-178].

Furthermore, the contribution of the host microbiome to infection dynamics is poorly understood. While it is increasingly clear that a diverse GI tract microbiome can limit infection, our understanding of the molecular interactions between the host and microbiota or between the microbiota and invading pathogens is poorly described. The native microbiomes of the skin, urinary tract, and lungs are even less well understood, and our appreciation of the role of these microbial communities during infection is its infancy [131, 179-184].

A more complete understanding of all of the microbial contributors to infection may ultimately impact diagnosis or treatment, wherein the presence of a subdominant or non-traditional organism at the site of infection, and/or in hosts with predisposing risk factors, might indicate a different course of treatment. This in turn paves way for personalized medicine, as there may be cases where specific targeting of microbial subsets, such as those that modulate the host response or augment the virulence of other pathogens, may drastically improve the disease outcome.

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Figure Legend:

Figure 1. **Central themes of polymicrobial interactions during infections across various niches.**

A. Metabolite Exploitation. Opportunistic pathogens capable of utilizing an alternative source of electron acceptor are able to establish an infection. Using a classical example, the resident gut microbiota secretes hydrogen sulphide which is converted to thiosulfate by host epithelial cells. During *Salmonella typhimurium* infection (labelled in dark blue with flagella), neutrophils are recruited to the site of the infection and respond by secreting reactive oxygen species (ROS), which in turn converts thiosulfate to tetrathionate. *S. typhimurium* can then utilize the tetrathionate as an alternate electron acceptor to outcompete the resident microbiota and establish an infection.

B. Immune Modulation. Infecting microbial species can modulate the host inflammatory response and decrease the response to establish an infection or persist within the niche. For example, capsular sialic acids from *Group B Streptococcus* (labelled in purple) can suppress leukocyte recruitment while suppressing pro-inflammatory cytokines and increasing anti-inflammatory cytokines, despite being cleared 24 hours post infection. *Escherichia coli* (depicted in green), can then take advantage of this predisposed state of the host to cause a more severe infection in the urinary tract where it can also invade bladder epithelium cells and persist as intracellular bacterial communities (IBCs).

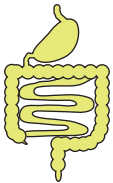
C. Niche Optimization. Different microbial species can establish synergistic infections allowing for the persistence and survival within the host. For example, *Pseudomonas aeruginosa* (labelled in purple) and *Staphylococcus aureus* (labelled in green) are

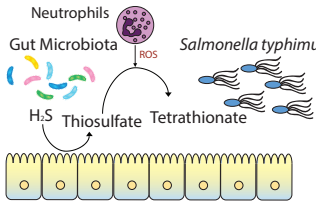
commonly co-isolated from chronic wounds with biofilms resulting in a chronic non-healing state with increased tolerance to various antimicrobials.

D. Virulence Induction. Infecting microbial species can upregulate certain virulence factors through sensing of the resident microbiota and/or other pathogenic microbial species. *Streptococcus viridans* (depicted in white) from the lower airway of the CF lung can produce the metabolite, autoinducer-2 (AI-2), which facilitates interspecies communication. Co-infecting *Pseudomonas aeruginosa* is able to sense the AI-2 system and upregulate virulence genes leading to prolonged inflammatory states within the CF lung.

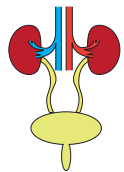
Polymicrobial Interactions

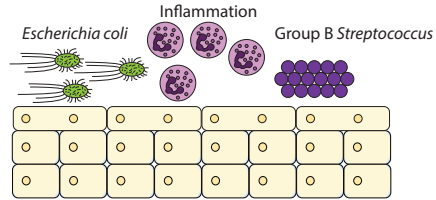
Metabolite Exploitation



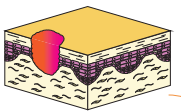


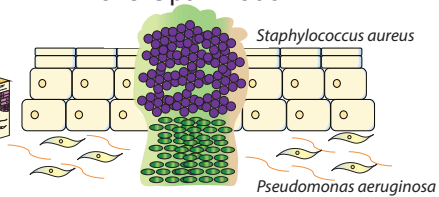
Immune Modulation



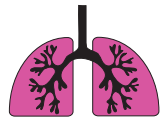


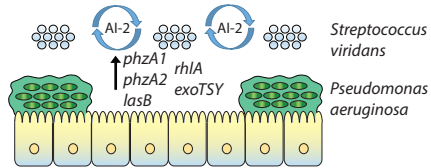
Niche Optimization



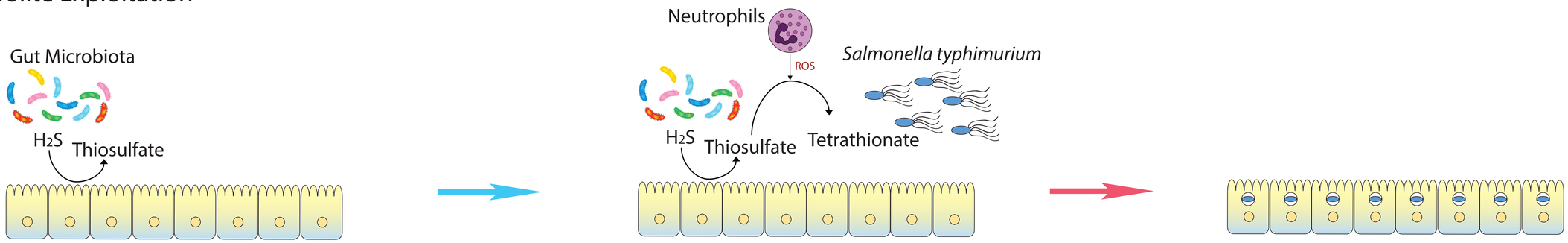


Virulence Induction



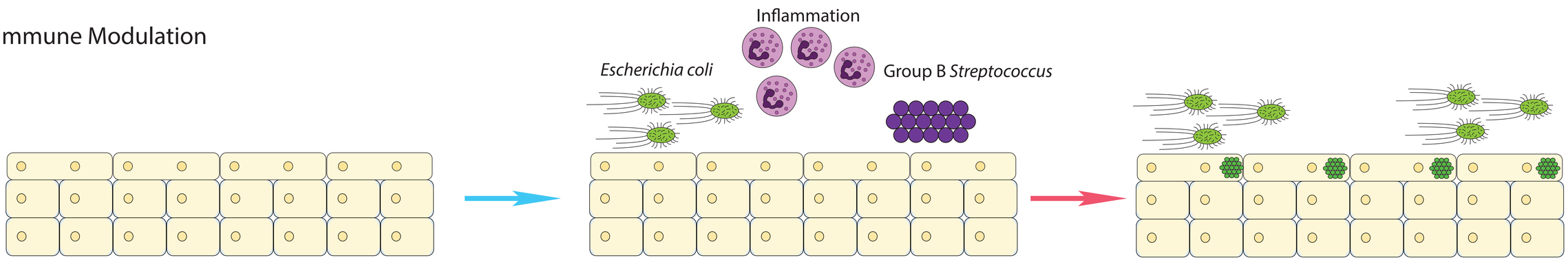


A) Metabolite Exploitation



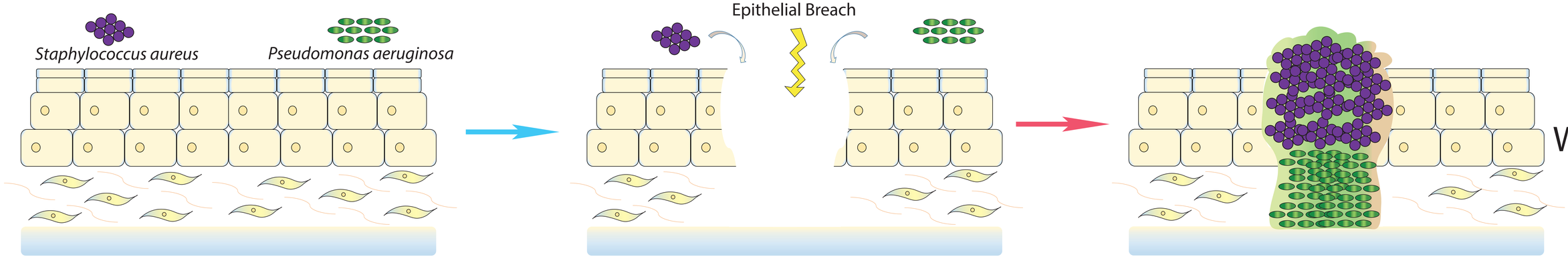
Gut

B) Immune Modulation



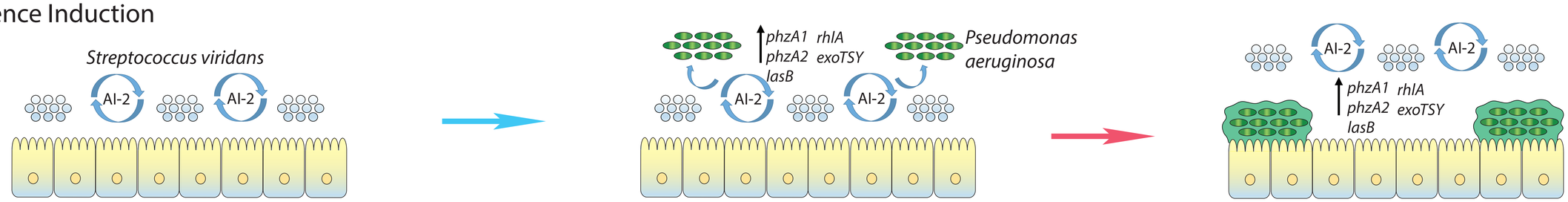
Urinary Tract

C) Niche Optimization



Wound

D) Virulence Induction



Lung

Naive

Infection

Table 1: **Summary of polybacteria-host interactions and the outcomes**

	Host Niche	Polymicrobial Interaction	Outcome(s)	Reference(s)
Metabolite Exploitation	Gut	*S. typhimurium*-resident microbiota	• Utilization of neutrophil derived tetrathionate to outcompete resident microbiota	[51]
	Gut	*Y. enterocolitica*-δ- Proteobacteria	• Utilization of neutrophil derived tetrathionate to outcompete δ-Proteobacteria in *TLR1*$^{-/-}$ mice	[52]
Niche Optimization	Gut	Ruminococcaceae and *Bacteroides spp*.	• Host epithelial interleukin-22 receptor IL-22RA1 mediates fucosylation of glycans in the gut to promote Ruminococcaceae and *Bacteroides spp*. growth • Colonization resistance against *C. rodentium* and *E. faecalis*	[44-46]
	Urinary Tract	*E. faecalis* and *P. aeruginosa*	• More severe infection (pyelonephritis) • Increased *P. aeruginosa* resistance to clearance by β-lactam antibiotics	[77]
	Wound	*P. aeruginosa* and *S. aureus*	• Chronic non-healing wound • Impaired *S. aureus* growth • Impaired wound re-epithelization through suppression of keratinocyte growth factor 1 (KGF-1) • Increased gentamicin tolerance that is dependent on both the polymicrobial biofilm matrix and host matrix proteins	[113-116] [109, 117-121] [109] [128]
	Wound	*P. aeruginosa*, *S. aureus*, *E. faecalis*, and *Finegoldia magna*	• Impaired wound closure Spatial partitioning, in which *P. aeruginosa* localizes at the wound margin and extends into the dermis while the other three species remain superficial	[108]
Virulence Induction	Gut	*F. nucleatum* and *E. coli*	• Host miRNAs enter bacterial cells, co-localize with chromosomal DNA, and regulate bacterial transcription	[64]
	Urinary Tract	*P. mirabilis* and *P. stuartii*	• Increased frequencies of urinary stones and bacteremia via the synergistic action of urease activity from each organism	[93]

	Wound	*P. aeruginosa* and *S. aureus*	• Induction and secretion of elastase and pyocyanin by *P. aeruginosa*, inhibiting growth of other Gram-positives • Upregulation of the virulence factors Panton-Valentine leukocidin (PVL) and α-hemolysin (hla) in *S. aureus*	[117] [109]
	Wound	*P. aeruginosa* and *A. baumanii*	• Additive increase in miRNA-106b induction that down-regulates mammalian tight junction proteins zona-occludens-1 (ZO-1) and zona-occludens-2 (ZO-2) • Functionally leaky epidermis	[126]
	Lung	*P. aeruginosa* and viridians streptococci	• Upregulation of key *P. aeruginosa* virulence genes during co-culture such as rhamnolipids, flagellin, exotoxin, elastase, and phenazine • Upregulation achieved through production of the autoinducer 2 (AI-2) metabolite by *Streptococcus*	[151]
Immune Modulation	Gut	*C. rodentium* and resident microbiota	• Inflammation-dependent decrease in diversity of the intestinal microbiota • Increased proportions of γ-proteobacteria	[33, 53]
	Gut	*E. coli* and *L. plantarum*	• Increased levels of NO and IL-10, culminating in both immunosuppression and tissue damage	[62]
	Gut	*K. pneumoniae* and *P. mirabilis*	• Increased incidence of colitis in a murine model lacking an adaptive immune system • Only increase colitis incidence in the presence of other resident microbiota	[63]
	Urinary Tract	Group B *Streptococcus* and uropathogenic *E. coli*	• Increased titers of UPEC during co-infection with GBS via, in part, GBS sialic acid-mediated immune suppression	[85]
	Urinary Tract	*P. mirabilis* and uropathogenic *E. coli*	• Increased titers of both UPEC and *P. mirabilis* via metabolic partitioning • Lowered pro-inflammatory cytokine production	[94]
	Wound	*P. aeruginosa* and *S. aureus*	• Decreased production of pro-inflammatory cytokines IL-1α, IL-1β, IL-6 and IL-8	[109]
	Lung	*S. constellatus* and *P. intermedia*	• Extended persistence of both species	[147, 152]