Candida–Bacteria Interactions: Their Impact on Human Disease

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ABSTRACT Candida species are the most common infectious fungal species in humans; out of the approximately 150 known species, Candida albicans is the leading pathogenic species, largely affecting immunocompromised individuals. Apart from its role as the primary etiology for various types of candidiasis, C. albicans is known to contribute to polymicrobial infections. Polymicrobial interactions, particularly between C. albicans and bacterial species, have gained recent interest in which polymicrobial biofilm virulence mechanisms have been studied including adhesion, invasion, quorum sensing, and development of antimicrobial resistance. These trans-kingdom interactions, either synergistic or antagonistic, may help modulate the virulence and pathogenicity of both Candida and bacteria while uniquely impacting the pathogen–host immune response. As antibiotic and antifungal resistance increases, there is a great need to explore the intermicrobial cross-talk with a focus on the treatment of Candida-associated polymicrobial infections. This article explores the current literature on the interactions between Candida and clinically important bacteria and evaluates these interactions in the context of pathogenesis, diagnosis, and disease management.

INTRODUCTION

Candida species are the most common commensal fungus that coexists with hundreds of species of bacteria in the human body. Between 24 and 70% of humans harbor Candida species in various body niches, including the oral and vaginal mucosa and the skin (1). Out of over 150 Candida species, Candida albicans is the principal pathogenic species that causes infections, especially in patient populations with immune dysfunction due to HIV infection, malignancy, immunosuppressive therapy, and organ transplantation. Therefore, these opportunistic infections of Candida in topical or systemic forms have become widespread and account for 8 to 10% of bloodstream infections in hospitals (2). Nearly 70% of denture wearers experience denture stomatitis, or inflammation of oral mucosa covered by denture prostheses, with C. albicans being a primary etiological factor (3, 4). Almost 75% of the female population has experienced an episode of vulvovaginal candidiasis at least once in their lifetime, and many have recurring episodes (5). In many of these conditions, there is a phenotypic change for Candida from harmless commensal to invasive pathogen. Adhesion to...
various surfaces, morphogenesis, phenotypic and genotypic switching, and production of lytic enzymes are major virulence mechanisms facilitating this conversion (6). However, properties of the host are also complicit in enabling Candida to act as an invasive pathogen since compromise in the interleukin-17 (IL-17)/Th17 arm of the host immune response (e.g., AIDS, Job’s syndrome, etc.) or an imbalance in the host microbiome (7) both can contribute to candidiasis (7). During this shift, commensal or transient organisms living with Candida species in various locations may play diverse roles in the process of pathogenesis; environmental bacteria may also be introduced via catheters, cannulae, and prosthetic appliances and interact with the already present Candida. Such interactions may be detrimental to the health of the human host, leading to mortality.

Currently, fungal-bacterial relations have gained attention due to their impact on human health, the environment, and the health care economy (8). However, polymicrobial infections associated with Candida have been reported in the past at variable levels and in a variety of locations. A previous study that analyzed both veteran’s affair and university hospital patients concluded that 27% of candidemic infections had a polymicrobial composition (9). Candida species have been found to coexist frequently with Staphylococcus aureus and Streptococcus mutans on denture surfaces and oral mucosa of denture users (10). Polymicrobial infective endocarditis cases among intravenous drug abusers have increased in frequency, with mixtures of Candida species and bacteria becoming common etiologies (11). Alarmingly, Candida-associated polymicrobial infections have often resulted in high mortality and morbidity in both adults and children because of their increased dissemination behavior and the current lack of diagnostic sensitivity (9, 12). The interplay between resident microbes, the host, and the compartment environment contributes toward virulence seen in the host and must be taken into account when discussing Candida, especially in a biofilm mode of growth (see Fig. 1). Here we review some of the important interactions between Candida species, with a focus on C. albicans, and clinically relevant bacteria with reference to their regulation of virulence and pathogenicity as well as the current diagnostic and management strategies for these polymicrobial infections.

**Candida Biofilms**

Biofilms are comprised of heterogeneous communities of microorganisms that attach to biotic or abiotic surfaces and/or to one another and are embedded within a host- and/or microbe-derived hydrated extracellular matrix, with a complex three-dimensional architecture (13–15) (see Fig. 2). Biofilm-associated infections have unique clinical significance because of the tendency of embedded microbes to harbor resistance against antimicrobials and host defenses. Biofilms are known to utilize multiple strategies to withstand antimicrobial agents, such as physical barriers, dramatically down-regulated metabolic rates, and persister phenotypes (16). These complex communities develop both on mucosa and on the surfaces of indwelling medical devices, incorporating endogenous and exogenous microorganisms, thereby creating polymicrobial environments. Many common infections such as dental caries, periodontitis, otitis media, and diabetic foot wound infections are associated with polymicrobial biofilms (17). With the help of advanced culture-independent molecular techniques (e.g., next-generation sequencing), interspecies interactions have been demonstrated to play a significant role in colonization, survival, infection dynamics, and resistance to antimicrobials and host defenses (18, 19). Recently, dual-species transcriptomics has been utilized to examine gene expression of C. albicans in the presence of other organisms. Dutton and colleagues used RNA-sequencing to analyze the transcriptomes of Streptococcus gordonii and C. albicans during coculture and found that C. albicans genes contributing to hyphal development and arginine bio-synthesis were highly upregulated in the presence of S. gordonii (20). The presence of multiple and differing organisms within the biofilm community may also provide growth advantages to pathogens and increase the ability to share genetic information encoding antimicrobial resistance (17). In particular, Staphylococcus epidermidis and C. albicans form thicker biofilms in the presence of extracellular DNA (eDNA) (21).

*C. albicans* is the leading pathogenic biofilm former among Candida species, and its ability to form biofilms is dependent on the morphogenetic switch from yeast to filamentous hyphae (22). Through multiple knockouts and genetic manipulation, genes governing the transition between yeast and hyphae as well as those factors affected downstream have been determined to create “yeast-locked” and “hyphae-locked” mutants (23, 24). In particular, *EFG1* and *CPH1* have been implicated in the phenotypic change of yeast to hyphae; *EFG1* is considered the master transcription regulator for hyphal transition and is required in most conditions found in a human host, including neutral pH, carbon dioxide, and sera presence (25); while many transcriptional factors affect hyphal formation, *EFG1* has been
shown repeatedly to be important and is commonly used in genetic knock-outs to force *C. albicans* to stay in the yeast form in most environmental situations. *In vivo* infection studies with *C. albicans* strains harboring homozygous deletions of *EFG1* and *CPH1* have demonstrated that hyphal morphogenesis is required for the development of oropharyngeal, vulvovaginal, and hematogenously disseminated candidiasis (26–28). Hyphae facilitate intermicrobial interactions and adhesion to surfaces, knitting the complex biofilm architecture together. One of the earliest *in vitro* studies demonstrated that certain piliated strains of bacteria enhanced *C. albicans* attachment to epithelial cells, showing how bacteria could assist fungi (29). Several subsequent studies have focused on these bacterial interactions with *C. albicans* biofilms in the context of disease. *Pseudomonas aeruginosa*, another potent former of biofilms and a pathogen in immunocompromised cystic fibrosis patients, has been shown to create a thick biofilm on *C. albicans* hyphae which results in killing of the fungus (30). *S. aureus* also favors binding to *C. albicans* hyphae; however, both bacteria and fungus coexist in a live, mature

**FIGURE 1** Schematic showing the interdependent relationships required for development of human disease. Infection is influenced by microbe–microbe interactions, microbe–host interactions, antimicrobial host defenses, and environmental factors. Significant changes in any of these factors can lead to the development of or predisposition to infection. For example, microbes lacking virulence factors may become apathogenic. Similarly, host immunodeficiencies will encourage infectious processes. It is now becoming increasingly appreciated that intermicrobial interactions and environmental cues also determine infection outcomes such that specific microbial populations under certain conditions may enhance or predict disease progression (184).
biofilm on biomedical surfaces and oral epithelium (31, 32).

Several species of bacteria have been shown to alter the expression of hyphae-associated genes in C. albicans, such as CDR4, when grown in coculture (33). S. mutans, a major bacterial player in the formation of dental caries, possesses specific glycosyltransferase enzymes that allow it to tightly bind to C. albicans, providing a possible explanation for both species being frequently isolated together from caries in children (34). The large scope of unique interactions observed in Candida–bacteria biofilms has created a need to better understand how these interactions occur and are maintained.

CANDIDA AND COAGGREGATION

Adhesion to various microbial cells, or coaggregation, is the initial step in intermicrobial interactions, making the adhesion between Candida and bacteria a key factor in colonization and pathogenesis (35). Deletion of C. albicans ALS3, which encodes a surface glycoprotein, results in the loss of C. albicans attachment to saliva-coated surfaces as the biofilm matures, promoting the importance of the ALS proteins in maintaining adhesion throughout the biofilm lifecycle (36). Coaggregation has been known for many years to be a vital component in cross-kingdom interactions. Utilizing a simple agglutination assay, Bagg and Silverwood demonstrated that C. albicans yeast cells coaggregated well with oral bacteria including Streptococcus sanguinis, Streptococcus salivarius, S. mutans, Streptococcus mitis, Fusobacterium nucleatum, and Actinomyces viscosus but not with Bacteroides melaninogenicus (Prevotella melaninogenica) (see Fig. 3). Their study provided evidence that bacterial surface lectins and yeast cell surface carbohydrates may interact to allow coagulation with specific bacteria species, such as Fusobacterium, but could not explain all adherence mechanisms, including those of oral streptococci (37). Jenkinson and colleagues followed up on the mechanisms of aggregation in regard to oral streptococci and C. albicans by starving yeast cells of glucose and testing their adherence with S. gordonii and S. sanguis. They determined that starvation promoted adherence of oral streptococci to C. albicans yeast and that since the conditions may have triggered expression of new surface molecules, the biofilm environment must also be taken into account when thinking about adhesion mechanisms (38).

Holmes and others further evaluated the interaction of C. albicans and S. gordonii, concluding that cell wall polysaccharides from S. gordonii participate in binding to C. albicans and that treatment with antipolysaccharide antibodies can abolish the adherence between the

FIGURE 2 Candida albicans strain DAY185 stained with a combination of calcofluor white (blue)/Syto9 (green) and imaged by confocal laser scanning microscopy (195).

FIGURE 3 Scanning electron micrographs of a polymicrobial biofilm formed on discs of hydroxyapatite. This shows the affinity of Streptococcus mutans to the Candida albicans hyphal elements as the streptococcal chains wrap around the hyphae. Small perforations are evident on the surfaces of the hydroxyapatite due to the highly acidic local microenvironment induced by the acidogenic bacterial species, S. mutans (196). Bar = 10 mm.

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organisms (39). Advances in in vitro biofilm growth have recently provided better insights into biofilm formation and its core components. Diaz and colleagues utilized a novel flow cell apparatus that incorporated mucosal tissue from the oral cavity and esophagus to better characterize the interaction between C. albicans and oral Streptococcus species. They saw that there was a cooperative interaction, as the presence of C. albicans increased the amount of Streptococcus oralis on oral mucosal tissue, and that invasion of C. albicans into the tissue increased when in coculture (40). Recently, Arzmi and others examined aggregation of polymicrobial biofilms using artificial saliva media and cocultured combinations of S. mutans and Actinomyces naeslundii with different strains of C. albicans. They discovered that the aggregation of bacteria and fungus was variable, depending on the specific strain of C. albicans present (41). These studies suggest that different surface receptors, each of which contributes to aggregation, may be expressed at different times during dual-species biofilm development, particularly in the oral cavity.

CANDIDA–BACTERIA INTERACTIONS IN THE HUMAN HOST

C. albicans often exists in a milieu of other microbial species. These relations are likely to affect how C. albicans interacts with the host. This section will discuss the various interspecies interactions that are dependent upon not only the environment of the host compartment, but also the resident microbial species in these compartments. While certain intermicrobial interactions can occur in multiple host compartments, we have grouped C. albicans with the historically recognized bacterial partner(s) in each compartment.

Interactions on the Skin and into Systemic Disease: Candida–Staphylococcus Species

Clinical Coisolation

Staphylococcus aureus, a Gram-positive bacterium, is a common colonizer of the skin and mucosa, such as the nasal cavity. Clearance of S. aureus from this particular body niche is dependent on a functioning Th17 axis of the immune system and neutrophil access (42). This requirement becomes problematic in immunocompromised patients, predominantly HIV-positive patients with low T-cell counts, in whom S. aureus may have an increased opportunity to interact with C. albicans. Such an interaction may be lethal to the host, with S. aureus frequently isolated from the blood of patients with candidemia, suggesting that patients with candidemia may have higher rates of bacteremia (9, 11). This co-isolation in bloodstream infections is present even in the neonatal population, where S. aureus and Candida species were coisolated in about 9% of polymicrobial bloodstream infections (12). Infective endocarditis, a major health concern due to the significant 16% in-hospital mortality, has occasionally been associated with polymicrobial infection with both staphylococcal and fungal species (43, 44).

Infectious Synergy

Multiple animal models have shown a significant increase in the virulence of both Candida and Staphylococcus species when coinfection exists. When mice were given intraperitoneal injections of C. albicans and S. aureus, mortality resulted within 2 days but was not seen when each organism was injected alone. In these mice, both fungus and bacteria were found together in the spleen, pancreas, and esophagus, indicating dissemination from the intraperitoneal site. S. aureus was always accompanied by C. albicans in these disseminated pockets of infection, suggesting a synergistic role between organisms (45–47). A neonatal colonization model has demonstrated that coinfection of C. albicans and S. epidermidis, another skin colonizer, caused delays in weight gain as the rat pups aged and an increase in morbidity at sublethal doses delivered subcutaneously. When young rat pups were given fluconazole prophylaxis prior to coinfection, there was a substantial increase in survival and weight gain, providing evidence of the importance of C. albicans in this infection process (48). A mouse model mimicking polymicrobial peritonitis, a complication that has increased with the usage of peritoneal dialysis methods, further supported previous studies that mono-infection of C. albicans or S. aureus are nonlethal, but dual-species infections raise mortality significantly. Larger bacterial and fungal burden was detected in kidneys and spleens of dual-infected mice compared to mono-infected mice, and increases in proinflammatory cytokines, such as IL-6 and G-CSF, were seen within 1 day in these organs. Increased neutrophil presence was also detected in the dual-species infection, and when mice were treated with nonsteroidal anti-inflammatory drugs (NSAIDs), all mice survived with lower bacterial and fungal burdens in kidneys and spleens. This protective effect was countered by application of PGE2 (prostaglandin E2), an oxylipin known to increase proinflammatory cytokines, and it suggests that polymicrobial infection control may involve modulating the host innate immune response (49).
With indwelling medical devices serving as excellent substrates to form biofilms, a polymicrobial infection model was developed using subcutaneous implanted titanium discs in mice and proximately injecting *S. aureus* and *C. albicans* nearby. These discs, coated with antimicrobial agents, were able to decrease some of the microbial burden on the implant but could not decrease the burden in nearby tissue. Such results exemplify how polymicrobial infections can be tenacious and difficult to treat, as well as the need for new and functional treatment methods (50). Taken together, these animal models have thoroughly demonstrated the potent power of *C. albicans–Staphylococcus* species infections in causing morbidity and mortality in multiple body niches.

In testing the impact of hyphae-formation in polymicrobial biofilm development, *S. aureus* adherence to mutants in the regulators of morphogenesis *CPH* and *EFG* was evaluated. It was found that *S. aureus* bound in high numbers to *C. albicans* hyphae produced by *cph1/cph1* and *efg1/efg1* single mutants. In contrast, when staphylococcal adherence to the *cph1/cph1*, *efg1/efg1* double mutant was evaluated, binding was nearly abolished. This is probably because of the tendency for *S. aureus* to preferentially bind hyphae since the double mutant produced a majority of yeast cells compared to the complete or partial hyphal production in the *cph1/cph1* and *efg1/efg1* single mutants, respectively. While this hyphae dependence on staphylococcal binding is very important, it should be noted that increased concentrations of serum strongly promote even nonspecific polymicrobial adherence within these biofilms. This alternative effector of adherence serves as a reminder that the proximal environment around the biofilm can play a potent role in its development (51, 52).

Harriot and Noverr elucidated part of this interaction by using different killing methods on *C. albicans* biofilms to see if this changed the polymicrobial biofilm dynamic with *S. aureus*. They saw that formalin- and

**FIGURE 4** We and others have previously reported the association of *Staphylococcus aureus* with *Candida albicans* hyphae during polymicrobial biofilm growth. High-resolution scanning electron microscopy confirmed these findings and demonstrated a three-dimensionally distributed pattern of *S. aureus* hyphal attachment. Not only can *S. aureus* be found bordering the basal layer of the hyphae-substratum interface, but bacterial cells are also seen attached to the upper portion of the hyphal surface. The precise architectural details and spatial arrangement cannot be fully appreciated like those in the cryo-SEM image of a *C. albicans–S. aureus* dual species biofilm on PVC catheter disks.
heat-killed, but not antifungal-treated (51), C. albicans hyphae were unable to sustain a thick and closely associated biofilm with S. aureus, suggesting that a fungal protein was the major player in this dual-species interaction (51). With yeast and hyphae being distinct forms in C. albicans, the protein composition as well as the secretome of both stages differs, opening the chance for new interactions that could explain the specificity of C. albicans hyphae and S. aureus association. Utilizing two-dimensional gel electrophoresis on protein extracts from dual-species biofilms, Peters and colleagues demonstrated that specific proteins for yeast and hyphae stages were upregulated when C. albicans was grown with S. aureus (31, 53). The yeast–S. aureus biofilm had the most changes in protein expression for both species; this may be because yeast-form C. albicans produces a unique quorum sensing molecule called farnesol, which causes a loss in S. aureus membrane integrity and decreased bacterial viability. Such a harsh environment may further explain the upregulation of S. aureus stress proteins in this interaction (31, 53).

**Candida Adhesins that Bind S. aureus**

The determination of fungal and bacterial adhesion mechanisms has been a central focus in the study of C. albicans and S. aureus interaction. These studies have concentrated on previously noted Candida surface adhesins, such as ALS (agglutinin-like sequence) proteins and Hwp1p (hyphal wall proteins), as well as hyphal transcription factors Ber1p and Tec1p (54). Initially, Harriot and Noverr took their cues from studies looking at S. epidermidis and C. albicans and decided to use mutants in multiple ALS proteins and HWP1. They found no significant effect of deleting any of these proteins when C. albicans and S. aureus were grown in dual-species biofilms with large concentrations of serum. However, these large concentrations of serum may have compensated for some of the mutations. The authors suspected that the Candida biofilm matrix was a key to polymicrobial development (51). This work was followed by Peters and colleagues, who used ALS mutants in serum-free media and noted a significant defect in S. aureus binding to C. albicans lacking Als3p (52) (see Fig. 6). These results suggest that environmental conditions can alter polymicrobial biofilm development and that there may be some nonspecific S. aureus–C. albicans interactions that involve sera components such as fibronectin and albumin. Beaussart and others used single force spectroscopy to examine the molecular interactions of S. epidermidis with C. albicans Als1/Als3 proteins, noticing that there was a reduced amount of binding in yeast cells compared to germ tube, the
previous lacking Als proteins. They performed single force spectroscopy with Als1/Als3 double knockouts as well as mannosyl-transferase mutants and concluded that Candida–staphylococci adherence required ALS proteins with correct o-mannosylations (55).

The importance of Als3p was verified in a recent study by Schlecht and colleagues using a dual-species oral infection model in an immunocompromised murine host, simulating the environment of a patient with oral candidiasis. In infected tongue tissues, S. aureus was shown to invade only with wild-type and Als3-complemented C. albicans strains. Such specificity was shown to impact systemic disease, with no S. aureus dissemination found in kidney tissues when coinfected on the tongue with the als3Δ mutant strain. Since the als3Δ mutant can still form hyphae and actively penetrate tissue, the authors concluded that this defect was attributed to specific S. aureus–C. albicans binding and that it prevented S. aureus from attaching with these invasive hyphae (32). However, other factors such as complicity of the host immune response or augmented microbial virulence may also come into play in this interaction. These results support one of the first studies examining polymicrobial intra-abdominal infections,

![Figure 6](image-url)
which was completed in 1983. Similar to the kidney dissemination witnessed by Schlecht and colleagues with dual infections of bacteria and fungus, Carlson observed that even at sublethal doses, *S. aureus* could be recovered from the bloodstream of mice coinfected with *C. albicans*. These findings were also shown during dual-species infection of *Serratia marcescens* and *Enterococcus faecalis* (46). The translation of the binding mechanism of *S. aureus* to Als3p protein in *C. albicans* from *in vitro* to *in vivo* is an important finding and suggests the need for biofilm treatments that address both organisms.

**Staphylococcus Adhesins that Bind C. albicans**

While the *C. albicans* binding protein in the *C. albicans*–*S. aureus* interaction has been elucidated, the *S. aureus* binding partner remains unknown, as does the impact of *S. aureus*–produced factors on the polymicrobial biofilm. Fehrmann and colleagues used biopanning to see which peptides produced by *S. aureus* would have strong binding to *C. albicans* biofilms in the presence of fibronectin and noted consistent binding with staphyloccocal coagulase and extracellular fibrinogen binding protein (Efb). Since these staphyloccocal components can block complement pathways used by the innate immune system, Fehrmann and others examined how these might complement pathways used by the innate immune system, which is the front line of defense. These findings were also shown during *in vitro* studies with *C. tropicalis* (58). Development of antimicrobial resistance by the biofilm organisms has traditionally been attributed to the complex matrix of the biofilm, rendering weak diffusion of the drug throughout the structure. Some in *vitro* studies have noted that *S. aureus* and *S. epidermidis* gain antimicrobial resistance in the presence of *C. albicans* (59, 60). Utilizing an *in vitro* catheter disk model system, Adam and colleagues demonstrated that some *S. epidermidis* strains released extracellular polymer slime that hindered fluconazole activity against *C. albicans* in a mixed-species biofilm. The authors also tested the effects of coculture with vancomycin treatment, a last resort drug for highly resistant staphyloccocal infections, and discovered that even in slime-negative *S. epidermidis* strains, coculture could protect against clinical levels of vancomycin (59). These findings show that *C. albicans* and *S. epidermidis* share mutual benefits to counteract the action of antimicrobials in polymicrobial biofilm development. Similarly, Al-Fattani and Douglas examined the biofilm matrices of *C. albicans* and *Candida tropicalis*, noting stark differences in their major constituents, with *C. albicans* matrix comprised of glucose and *C. tropicalis* matrix comprised of hexosamine. These differences made significant contributions to drug resistance in *Candida* biofilms, with *C. tropicalis* biofilms being almost completely resistant to both fungicidal and fungistatic agents (61).

Harriot and Noverr followed up on these observations by looking at *S. aureus–C. albicans* biofilms in the presence of antimicrobials and reported increased vancomycin resistance in *S. aureus* during coculture. They postulated that the resistance was conferred by secretion of extracellular matrix components of *C. albicans* into the biofilm. Through immunofluorescence microscopy, the authors showed that *S. aureus* could become coated in *C. albicans* matrix and that this could help block vancomycin from reaching its target of peptidoglycan (60). However, it should be noted that addition of *C. albicans* matrix alone did not restore vancomycin resistance in *S. aureus* to levels similar to that seen in polymicrobial biofilm culture, suggesting that several components mediate this interaction.

**Antimicrobial Resistance with Staphylococcus Species Induced by C. albicans**

*Staphylococcus* species are known for developing antimicrobial resistance in multiple clinical situations. The CDC’s most current report on invasive methicillin-resistant *S. aureus* (MRSA) infections has shown that health care–associated MRSA infections remain the deadliest in the United States even though overall rates of MRSA infection are decreasing in portions of the nation (58). Development of antimicrobial resistance by the biofilm organisms has traditionally been attributed to the complex matrix of the biofilm, rendering weak diffusion of the drug throughout the structure. Some in *vitro* studies have noted that *S. aureus* and *S. epidermidis* gain antimicrobial resistance in the presence of *C. albicans* (59, 60). Utilizing an *in vitro* catheter disk model system, Adam and colleagues demonstrated that some *S. epidermidis* strains released extracellular polymer slime that hindered fluconazole activity against *C. albicans* in a mixed-species biofilm. The authors also tested the effects of coculture with vancomycin treatment, a last resort drug for highly resistant staphyloccocal infections, and discovered that even in slime-negative *S. epidermidis* strains, coculture could protect against clinical levels of vancomycin (59). These findings show that *C. albicans* and *S. epidermidis* share mutual benefits to counteract the action of antimicrobials in polymicrobial biofilm development. Similarly, Al-Fattani and Douglas examined the biofilm matrices of *C. albicans* and *Candida tropicalis*, noting stark differences in their major constituents, with *C. albicans* matrix comprised of glucose and *C. tropicalis* matrix comprised of hexosamine. These differences made significant contributions to drug resistance in *Candida* biofilms, with *C. tropicalis* biofilms being almost completely resistant to both fungicidal and fungistatic agents (61).

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Importantly, *C. albicans* mutant Bcr1p was unable to adhere to plastic, formed a weak biofilm matrix, and was incapable of conferring vancomycin resistance when grown with *S. aureus*, supporting the concept that adhesion and biofilm matrix can be vital factors in antimicrobial resistance. However, dual-species biofilms grown with als3Δ and hwp1Δ *C. albicans* mutants were capable of conferring the vancomycin resistance as closely as to the wild-type strain. It is important to remember that Als proteins and Hwp1p are surface-expressed adhesins, some of which can be rendered unnecessary in the presence of large amounts of sera (utilized in these experiments) and that these mutants can still form biofilms (51). Such conditions with large concentrations of sera could possibly simulate growth on bloodstream catheters but would not be as applicable in deep tissue infections, further suggesting that staphylococcal factors may be important in this induced resistance.

**Interactions in the Lungs**

*Candida–P. aeruginosa*

*P. aeruginosa* is a Gram-negative bacterium that is ubiquitous in the environment but can become an opportunistic pathogen in the immunocompromised population. In the CDC’s 2013 report on antibiotic resistance, multidrug-resistant *P. aeruginosa* was listed as a serious threat, alongside pathogens such as MRSA and multidrug-resistant tuberculosis (62). Strikingly, *P. aeruginosa* engages in an antagonistic relationship when grown with *C. albicans* in *in vitro* biofilms, with a noticeable killing of hyphal filaments (30). However, these organisms have the opportunity to engage within an immunocompromised patient in multiple locations. Early studies utilized mouse burn models to examine if *P. aeruginosa* infection could influence or amplify a concurrent *C. albicans* infection. These studies demonstrated that a low-virulence *P. aeruginosa* strain could prime a mouse for lethal candidiasis; it should be noted that as *C. albicans* CFUs increased in these studies, *P. aeruginosa* CFUs decreased. The authors speculate that the large amount of elastase produced by this *P. aeruginosa* strain may have further damaged tissues and facilitated *C. albicans* dissemination from the skin into the kidneys, because purified elastase injected with *C. albicans* produced similar deaths (63). Yu and colleagues noted the similarities in structure of the *P. aeruginosa* pilus adhesin and the *C. albicans* fimbrial adhesin and investigated if the two structures could share a common epitope for immune recognition. Through agglutination assays, they detailed how monoclonal antibodies raised against bacteria could bind to fungus and vice versa (64). Since both pilus adhesin and fimbrial adhesin recognize glycosphingolipids on cell surfaces, a common antibody against each could be utilized in the prevention of *P. aeruginosa* or *C. albicans* biofilms through the action of neutralizing antibodies.

On the contrary, the fungicidal effects of *P. aeruginosa* on *C. albicans* in multiple *in vitro* coculture settings have been reported by several investigators (65–67). Hogan and Kolter provided a foundation in this work by showing that *P. aeruginosa* can attach to *C. albicans* hyphae and kill them. They also examined mutations in *P. aeruginosa* pili, structures responsible for adhesion to a variety of surfaces, and noted an overall decrease in attachment to hyphae with these pili mutants (30). This correlates with the data of Yu and others and suggests that pili are important to *Pseudomonas* in this relationship (64). Corroborating this antagonism, live/dead imaging revealed that *P. aeruginosa* bound to *C. albicans* hyphae and remained stationed on these particular hyphae as they caused cell lysis in the immediate area. Brand and colleagues further tested if direct contact was needed for *P. aeruginosa* to kill using spent media on fungal cultures and determined that secreted products in the media did have a modest inhibitory effect on *C. albicans* (68). Overall, most evidence suggests that direct interaction between *P. aeruginosa* and *C. albicans* is detrimental to *C. albicans*.

Like many bacteria, *P. aeruginosa* can use quorum sensing to signal between cells within biofilm structures; Gram-negative bacteria use homoserine lactones to accomplish these tasks. *P. aeruginosa* secretes N-3-oxo-C12 homoserine lactone (3OC12HSL) at large micromolar amounts in biofilms; when smaller amounts were introduced into *C. albicans* cultures, complete repression of filamentation was witnessed. This effect could also be seen when *C. albicans* was treated with similar 12-carbon backbone molecules, suggesting that *C. albicans* may be able to sense its environment and respond through detection of these small molecules (69). McAlester and colleagues followed these observations by examining multiple strains of *P. aeruginosa* and noticed that production of HSL varied between strains; when supernatant from these strains was placed on yeast-form *C. albicans*, only high HSL producers could inhibit the yeast-hyphae switch, while low HSL producers did not have any effects on fungal transition. Since *C. albicans* can also produce its own quorum sensing molecule, farnesol, the authors decided to see if this could alter growth or other aspects of *P. aeruginosa* and determined...
that micromolar concentrations of farnesol could stop swarming activity, an important method of dispersal for *P. aeruginosa* (70). At the molecular level concerning *P. aeruginosa*, Cugini and colleagues utilized qualitative PCR to show that while transcripts of genes in the *Pseudomonas* quinolone signal operon, another quorum sensing mechanism in *P. aeruginosa*, are decreased in the presence of farnesol, the modulating transcription factor for the operon was unaffected. Through electrophoretic mobility shift assays, the authors were able to show that farnesol interfered with the transcription factor and directed it toward an alternative site, ultimately reducing production of the bacterial virulence factor pyocyanin (71). Such findings strongly support the idea of an antagonistic relationship in the immunocompromised patient, with bacteria and fungus each producing their own molecules to silence the opponent.

In an early report, Kerr and colleagues demonstrated that pyocyanin produced by *P. aeruginosa* could be an antifungal agent, though not at the same levels as current antifungal treatments such as fluconazole (66). Since *P. aeruginosa* is known to produce phenazine compounds that can act as antimicrobial agents and because pyocyanin is a phenazine derivative through the shikimate pathway, Gibson and others examined if phenazines were important in these dual-species interactions. The authors documented a red pigment on *C. albicans* lawns inoculated with single points of *P. aeruginosa*; knocking out the *Pseudomonas* quinolone signal operon abolished this pigmentation, suggesting an association with pyocyanin. Through further genetic knockouts, the component responsible for the red pigment was determined to be a pyocyanin precursor, 5-methylphenazinium-1-carboxylate (5MPCA), and it appeared only in areas of fungal cell death, supporting the concept that pyocyanin components may act as antifungal agents (72). Further studies on 5MPCA by Morales and colleagues demonstrated that the phenazine compound was taken up by *C. albicans* cells and interacted with oxygen, generating reactive oxygen species (ROS) that could be detected by probes. When testing catalase-deficient *C. albicans* mutants to confirm the role of reactive oxygen species damage in this interaction, the authors saw that these *C. albicans* mutants, which could not handle oxidative stress, had increased death when treated with 5MPCA (73). All of these current studies show that quorum sensing molecules may play an important role in the cross-talks between *Candida* and *Pseudomonas* in coculture, and as such, purified molecules may be exploited in controlling biofilm-associated infections in the face of rapidly developing antimicrobial and antifungal resistance.

To further dissect the genetic effects of *P. aeruginosa* secreted factors on *C. albicans* biofilms, Holcombe and others performed a transcriptomic screen on the fungus and discovered that the expression of genes related to drug or toxin efflux increased, while biofilm and adhesion genes decreased (74). This inhibitory effect of *P. aeruginosa* on *Candida* biofilms has been shown for multiple *Candida* species. Biofilms of *C. tropicalis* and *Candida dubliniensis* grown with *P. aeruginosa* had decreased fungal viability and were thinner compared to mono-species fungal biofilms alone (75). Further studies by Bandara and colleagues on *P. aeruginosa* interactions with different *Candida* species have revealed that *P. aeruginosa* lipopolysaccharide (LPS) inhibited *Candida glabrata*, *Candida krusei*, and *C. dubliniensis* biofilm formation and maturation, suggesting that bacterial LPS can have an effect throughout biofilm development (76).

However, *Candida* species are not without their own defenses; Chen and colleagues pointed out that ethanol produced by the fungus can halt swarming behavior and promote biofilm development. Using a transposon screen, the authors determined that levels of cyclic-di-GMP, an important secondary messenger molecule, were decreased through WspR, a factor that stimulates exopolysaccharide production for biofilms (77). Lopez-Medina and others further examined *C. albicans* defense mechanisms against *P. aeruginosa* using a neutropenic murine mouse model and inoculated the gut with both pathogens. Their results demonstrated that *P. aeruginosa* virulence was attenuated in the presence of *C. albicans* and that when the transcriptome of *P. aeruginosa* was analyzed via RNAseq, genes encoding pyochelin and pyoverdine, siderophores used to sequester iron for bacteria, were highly downregulated. Utilizing spent media, the authors concluded that *C. albicans* secreted proteins that inhibited the pyochelin and pyoverdine produced by *P. aeruginosa* and that virulence of the bacteria could be rescued by the addition of iron in the murine model (78). While *P. aeruginosa* and *Candida* species maintain an antagonistic relationship, these studies elucidating defense mechanisms of each opportunistic pathogen might be applied to other polymicrobial biofilm situations and provide unique therapeutic targets.

**Candida–Burkholderia cenocepacia**

*Burkholderia cepacia* complex is comprised of 17 distinct species of Gram-negative bacteria that survive in a wide range of environments, including some anti-septic solutions, and can cause serious infection in immunocompromised patients (79). These bacteria possess
important virulence factors such as quorum sensing mechanisms, the ability to form biofilms on plastics and epithelial cells, and intrinsic resistance to several antibiotics (80). B. cenocepacia, one of the members of this complex, is responsible for causing an invasive respiratory disease called cepacia syndrome in individuals with cystic fibrosis, which can lead to death (81). The reports on interactions between B. cenocepacia and Candida are rare; however, they have the potential for interaction by overlap of niche in the cystic fibrosis lung environment. Boon and colleagues noted a molecule produced in most of the B. cepacia complex species, cis-2-dodecenioic acid (BDSF), was important to produce biofilms and extracellular polysaccharides in B. cenocepacia. In both coculture and with the addition of BDSF, C. albicans hyphal formation was stunted, and this was further supported by rescue of hyphal health through knocking out the genes controlling BDSF production (82). How this suppression of fungal growth is accomplished is currently unknown, as is any effect on C. albicans quorum sensing molecules on B. cepacia complex members. Such molecules may serve as a mechanism to establish dominance in the cystic fibrosis lung, and further analysis on the therapeutic potential of the purified BDSF molecule is needed.

Candida–Mycobacterium tuberculosis

While tuberculosis is not a major health concern in the Western world, throughout sub-Saharan Africa and portions of Asia, the disease is endemic. Patients with latent tuberculosis can serve as reservoirs and may progress to active disease once their immune system falters (83). With an increase in multidrug-resistant strains as well as patients immunocompromised from HIV infection, this provides a suitable environment for interaction of the acid-fast bacteria with Candida species. Examining a hospital in South India, Kali and colleagues noted that 40% of their surveyed population infected with M. tuberculosis also had C. albicans coinfection. The authors recovered several species of Candida, particularly in patients displaying pulmonary disease symptoms. This suggests that it may be clinically beneficial to screen patients with tuberculosis infection for fungal pathogens, especially female patients, who were determined to have a significantly higher chance of harboring dual infection (84). An earlier study in five hospitals across northern Kenya examined HIV-positive patients with positive tuberculosis tests, and of the 11 patients determined to harbor both virus and mycobacterium, 4 were coinfected with Candida species (85). Because there are such limited reports regarding the coisolation of Candida and M. tuberculosis in patients, little research has been conducted on the interaction between these pathogens of the immunocompromised.

Both M. tuberculosis and Candida infection have immunocompromise as a common point, though traditionally M. tuberculosis is associated with Th1 cytotoxic killing through interferon-γ, while Candida is associated with neutrophils, IL-17, and Th17 lineage. Genetic errors in IL-17A/F result in mucocutaneous candidiasis, while errors in interferon-γ genes lead to increased mycobacterial diseases, even from weak strains such as the tuberculosis vaccine strain, Mycobacterium bovis. A recent study noted that some pediatric patients have been infected with both pathogens, suggesting a common genetic mutation. Okada and others used genomic sequencing of seven pediatric patients over many ethnic backgrounds and found a common loss-of-function mutation in both copies of RAR-related orphan receptor C (RORC). This alteration led to a nonfunctioning RORγ, the vital transcription factor for the Th17 lineage, and the production of T-cells that could no longer produce IL-17A/F. Further examination of this genetic defect through analysis of the T-cell receptor repertoire demonstrated a decrease in rearrangement of 5′ portions of the V regions in the T-cell receptor, resulting in the absence of type 1 NK T-cells, which are important in recognizing the unique glycolipids of mycobacterium species (86). While no current literature has examined the phenotypic or genetic effects of mycobacterium and Candida coinfection on the respective organisms, it may prove important to further an understanding of different T-cell subsets, primarily invariant T-cells, of which little is currently known.

Interactions in the Oral Cavity

S. aureus and C. albicans

The discussion above of the interaction of S. aureus and C. albicans is also relevant to the oral cavity. S. aureus is often carried within the nares and on the skin of colonized hosts. Although S. aureus is generally thought to be a noncommensal, increasing numbers of culture and molecular-based studies have shown that this pathogenic species is more common on mucosal surfaces from healthy subjects than originally hypothesized (87–89). While this pathogen may not directly contribute to localized mucosal virulence in the oral cavity, the transient or persistent carriage of S. aureus on various mucosal surfaces can provide an infectious source for systemic disease, particularly when C. albicans is present (9, 32, 98–101).
**Candida–Streptococcus species**

The oral microbiome is comprised of many Streptococcus species, providing multiple locations for contact with Candida species, including dental appliances and the periodontal pocket. Some of the earliest work on cross-kingdom interactions reported on coaggregation between Candida and various oral bacteria species, including *S. mutans* and *S. salivarius* (37, 102). These studies suggested that binding of *C. albicans* with viridans streptococci (oral streptococcus that are α-hemolytic and optochin-resistant) was important for yeast colonization on the oral surfaces. Holmes and colleagues determined that *S. gordonii*, a streptococcus species that rapidly adheres to tooth surfaces, was able to bind to *C. albicans* through streptococcal surface proteins A and B (SspA and SspB) in addition to surface-associated proteins cshA and B. By expressing SspB on the surface of *E. faecalis*, the authors enabled Candida–Enterococcus binding, supporting their hypothesis (102). These original studies implied that a complex environment exists within the oral cavity, with salivary factors such as mucus interacting with Candida species and oral bacteria to provide multiple ligand–receptor interactions.

Recently, with a better understanding of the role of the oral microbiome in human health, studies have focused on determining how Candida–Streptococcus interactions occur in biofilms. Bamford and others showed that *S. gordonii* enhanced hyphal development and biofilm formation in *C. albicans* when in the presence of human saliva but that streptococcal contact with *C. albicans* through SspA and SspB was not the only factor for polymicrobial biofilm development. They investigated quorum sensing within *S. gordonii* through knockouts of the *luxS* system, which produces universal quorum sensing molecule autoinducer-2 (AI-2), and discovered that these mutants could no longer form dense biofilms with *C. albicans* (103). These results suggest that cross-talk between Candida and *S. gordonii* could be vital for promoting biofilm formation in the oral cavity and on oral appliances and that Candida species may be a bridge from a healthy microbiome to a pathogenic microbiome.

*Candida* adhesins ALS proteins have also been found to mediate interactions with streptococci during many steps in polymicrobial biofilm formation. Silverman and colleagues demonstrated that Als3 in *C. albicans* was required to form and sustain biofilms on a salivary pellicle (initial stage of attachment to tooth surfaces) when *S. gordonii* was present. To further support their conclusions, the authors used genetic alterations of *Saccharomyces cerevisiae*, yeast that cannot attach to *S. gordonii*, to express Als3p on the surface of *S. cerevisiae* and established yeast–bacteria adherence (36). However, several ALS proteins have similarities in structure, and it is not illogical for multiple ALS proteins to take part in these interactions. Hoyer and others reported that Als1 was also bound with *S. gordonii* during interkingdom interactions using NT-Als crystal structures to visualize the adhesion of Alsp1 to SspB on *S. gordonii* (104). Meanwhile, Dutton and colleagues have demonstrated that O-mannosylation of the *C. albicans* cell wall is required for hyphal to bind with *S. gordonii* (105). However, Hoyer and others noticed that even though the ALS protein of *C. albicans* bound open C-termini of respective ligands, in the case of *S. gordonii* SspB, the C-terminus is bound to peptidoglycans and obscured, suggesting that there must be some type of editing to free the SspB ligand (104).

*S. mutans*, the principle bacteria found in human caries, is another member of the viridans streptococci group that can interact directly with *C. albicans* and may exchange secretory products with the fungus. Jarosz and others examined the effects of spent media from *S. mutans* and a mutated strain of *S. mutans* that lacked the comC gene, encoding for both competence and a quorum sensing molecule, CSP. When these media were placed on *C. albicans*, only the wild-type *S. mutans*–derived media could inhibit hyphal formation; this was further supported by the use of synthetic CSP in a dose-dependent fashion also preventing hyphal formation and forcing reversion to yeast forms (106). Vilchez and colleagues continued exploration into components secreted by *S. mutans* and observed that one small molecule could inhibit the AI-2 quorum sensing. They pursued this further with nuclear magnetic resonance spectrometry to determine that the molecule was trans-2-decenoic acid and that when placed on *C. albicans*, it prevented hyphal formation but did not stunt growth overall. A possible mechanism of action for trans-2-decenoic acid is altering surface protein expression, because Hwp1 expression on *C. albicans* was terminated in the presence of the compound. This secreted molecule has a similar structure to molecules of the diffusible signal factor family, previously seen in *Burkholderia* species, and was also determined to be secreted from other cariogenic bacteria, such as *S. sanguinis* (107). These findings demonstrate that oral streptococci species and *C. albicans* can interact with each other but may limit each other, perhaps in the attempt to secure nutrients or other materials in the oral cavity.

Examination of young children with dental caries has shown that Candida species are found at a higher...
prevalence in caries-positive children than in caries-free children, with most children carrying *C. albicans* (108). Dental plaque collected from children with early childhood caries (where caries can be seen in those as young as six months) was found to have a positive association with *C. albicans* and *S. mutans*, whereas plaque from an older group of children with caries only had a positive association with *S. mutans* (109). These clinical findings promote the concept that *C. albicans* and *S. mutans* together may facilitate survival in the cariogenic environment; *in vitro* examination of these dual-species biofilms showed that even though extrapoly saccharide matrix (EPS) production was reduced in *S. mutans*, induction of quorum sensing systems in the bacteria increased production of mutacin, a broad-spectrum bacteriocin (110). Since the *S. mutans* quorum sensing system also controls competence, induction by *C. albicans* may enable the bacteria to acquire new genetic information quickly, which may alter cariogenicity and the landscape of the oral microbiome.

Previously, Gregoire and colleagues demonstrated that *S. mutans* preferred to bind to *C. albicans* cells that had glucans on their surfaces and that these reactions involved glucosyltransferases produced by *S. mutans*. Utilizing a micropipette technique to visualize individual adhesion events, the authors saw that beads coated with saliva adhered better to glucan-coated *C. albicans*. When *S. mutans* was added to the mixture, there was a significant increase in bacteria bound to saliva-coated structures with glucan-positive *C. albicans* (111). This study suggested that the glucosyltransferases from *S. mutans* can help to increase a glucan and fructan matrix on the tooth surface, promoting caries. While this appears to be contradicted by Sztajer and others, who reported a decrease of EPS production by *S. mutans*, it may be that in the earlier stages (as noted by Gregoire and others) a more intense matrix is produced, which decreases over time as *C. albicans* takes up more sucrose, and forces *S. mutans* to induce its quorum sensing machinery (110, 111). When these findings are taken together, it is obvious that the interaction between *Candida* and streptococci is more complex than just synergistic, but with their interaction positively associated with early childhood caries, it can be concluded that treatments for this disease must include a multikingdom approach to be successful.

**Candida—Porphyromonas gingivalis**

*Candida* species have been isolated from periodontal lesions; however, the association of *Candida* species with periodontal disease has not been solidified—only speculated. Periodontal disease affects the gingival tissue and tooth attachment/anchoring in the alveolar bone of the mouth. These diseases can range from mild gingival inflammation (gingivitis) to chronic gingival inflammation and alveolar bone loss (periodontitis) (112). It was shown that subgingival colonization of *C. albicans* was associated with the severity of chronic periodontitis and was the only yeast species to be present in all yeast-positive cases (113).

*P. gingivalis* is well known for its role in chronic periodontitis, with a variety of virulence factors such as LPS, gingipains, and fimbriae along with the ability to form biofilms (114). Unfortunately, interactions between *Candida* species and *P. gingivalis* have not been thoroughly assessed. Pretreatment of human gingival epithelial cells and human gingival fibroblasts with heat-killed *C. albicans* or mannanprotein-b-glucan complex (a major cell wall and biofilm matrix component of the fungus) derived from *C. albicans* enhanced *P. gingivalis* invasion of the cells. However, this enhancement was not through adhesion or upregulation of typical adhesive molecules, such as ICAM-1. These results suggest that *C. albicans* exacerbates periodontal disease by providing assistance to *P. gingivalis* invasion of epithelial cells, possibly by serving as a scaffold to allow the bacterium time to invade (115). In the presence of some evidence of symbiotic interactions between *Candida* and *P. gingivalis*, it is important to further explore the molecular mechanisms involved to determine better treatment methods for periodontal disease.

**Candida—Aggregatibacter actinomycetemcomitans**

Localized juvenile/aggressive periodontitis is an acute form of periodontal disease associated with anaerobic bacteria in the oral cavity, resulting in bone loss and periodontal ligament destruction around specific clusters of teeth. *A. actinomycetemcomitans* has been identified as a causative agent of localized juvenile/aggressive periodontitis (116). This organism produces a variety of virulence factors such as leukotoxin, cytotoxic, and peroxide toxins, Fc-binding factors, and proteases (117). It was shown that in dual-species biofilms, *A. actinomycetemcomitans* adhered to *C. albicans* and inhibited biofilm formation via the general quorum sensing molecule AI-2, synthesized by the luxS gene (118). This molecule can be used to communicate between both Gram-positive and Gram-negative bacteria, and Bachthar and colleagues demonstrated that synthetic AI-2 could also inhibit *C. albicans* biofilm formation, providing a possible treatment option by modulating the oral flora.
through quorum sensing. Periodontitis is linked with several systemic conditions, particularly diabetes mellitus, and patients with both diseases have been shown to harbor various Candida species, strikingly C. albicans (119). Determining how periodontal bacteria and Candida species interact within the gingival pocket will be vital to developing more effective oral health biomaterials.

Candida–Acinetobacter baumannii

A. baumannii, a Gram-negative bacterium, has emerged as one of the most troublesome pathogens for health care institutions globally. The ability of A. baumannii to adhere to and persist on surfaces in a biofilm, particularly on medical devices, has made the bacterium a concern in hospitals and war zones. A. baumannii is emerging as a pertinent opportunistic human pathogen and is part of the ESKAPE pathogens due to its development of multidrug resistance along with the biofilm-forming capability, earning it the term “superbug” (120). Given that C. albicans and A. baumannii are common etiological agents of nosocomial infections in the immunocompromised and that they overlap niches in the oral cavity, it is vital to understand any interactions between these organisms. It was demonstrated that outer membrane protein A (OmpA) of A. baumannii19606, a standard lab strain, was essential for bacterial attachment to C. albicans filaments through knockout and complement experiments (121). OmpA was also shown to be important for attachment and invasion of epithelial cells; since C. albicans binds to N-cadherins on epithelial cells through its protein Als3, it is possible that OmpA may bind to Als3 on C. albicans (122). This interaction between bacteria and fungus resulted in fungal death, visualized with fluorescent microscopy and only when A. baumannii was in direct contact with C. albicans filaments through a functional OmpA protein (121). These findings show antagonism between these two opportunists; determination of the mechanism of fungal killing by A. baumannii deserves further exploration to minimize serious infections in the immunocompromised population.

Interactions in the Gastrointestinal Tract

Candida–E. faecalis

The Gram-positive bacterium E. faecalis and C. albicans often cohabit the human large intestine and the oral cavity, with E. faecalis consistently isolated after failing endodontic (root canal) treatments (123, 124). Unfortunately, to date, the interactions between C. albicans and enterococci have not been thoroughly examined. Several studies showed that the relationship between E. faecalis and C. albicans in polymicrobial biofilms is antagonistic. In a Caenorhabditis elegans model of a coinfection with E. faecalis and C. albicans, E. faecalis inhibited C. albicans hyphal formation and protected C. elegans from being killed by C. albicans. Interestingly, the presence of C. albicans reduced E. faecalis cell death. These results correspond with findings in vitro, where E. faecalis inhibits Candida hyphal formation in a dual-species biofilm. The inhibition of Candida hyphal morphogenesis by E. faecalis was caused by a secreted heat-stable protein of approximately 10 kDa and was partially dependent on the Fsr quorum-sensing system, which regulates virulence in E. faecalis (125). Shekh and Roy identified an E. faecalis strain that produces an anti-Candida protein (APC), an anti-mycotic protein that was nonhemolytic and different from the one Cruz and colleagues discovered (126). Heat-killed E. faecalis prevented C. albicans from adhering to plastic substrates, and in a murine oral infection model, heat-killed E. faecalis protected against oral candidiasis, showing that direct cell contact between the two species plays an important role in cross-kingdom interactions (127). These findings indicate a role for quorum sensing molecules and other proteins produced by both C. albicans and E. faecalis in the interaction between these two organisms. These molecules may be promising in the quest to find new therapeutic strategies in the battle against antimicrobial drug resistance, and additional research may further elucidate the role of these molecules and their mechanism of action.

Candida–Escherichia coli

E. coli remains one of the most important organisms found in the gastrointestinal tract, and different virotypes of E. coli are responsible for causing a variety of infections, including diarrhea, respiratory tract infections, wound infections, and septicemia. E. coli and C. albicans are often found together in human tissues and body fluids (128). Polymicrobial intra-abdominal infections involving fungi result in higher mortality rates (up to 75%) compared to bacterial polymicrobial infections (up to 30%), and Candida species are the most commonly found fungi in these infections (129–132). In an experimental murine model of peritonitis, C. albicans showed synergism with E. coli. In this model, coinfection with both C. albicans and E. coli resulted in higher mortality in mice compared to a single-species infection (133). This Candida-associated mortality of experimental animals was augmented by E. coli and its LPS (134). Bandara and colleagues evaluated the effect of E. coli LPS on different Candida species biofilms in vitro and found that E. coli LPS caused a significant
reduction in the growth of C. tropicalis, Candida parapsilosis, C. krusei, and C. dubliniensis (135). In addition, E. coli secretory elements significantly impair Candida biofilm development, possibly by modulating hyphal-specific genes and their transcriptional regulation (136). Interestingly, when Candida–E. coli biofilms were treated with the fluoroquinolone antibiotic ofloxacin, the β-1, 3-glucan produced by C. albicans increased E. coli tolerance of ofloxacin (137).

These findings suggest that the interaction between Candida and E. coli is synergistic. Since they are commonly isolated from infection sites and seem to increase mortality and tolerance to antibacterial agents, they can cause serious problems in immunocompromised individuals. More research on the interaction between these two species is needed, and targeting β-1, 3-glucan production by C. albicans is currently used in the clinic through the echinocandin class of antifungals. These drugs may be useful in combination therapy with other antimicrobial agents, because there is still little fungal resistance to echinocandins (138).

**Candida–Salmonella species**

Salmonella species can survive intracellularly within epithelial cells, dendritic cells, and macrophages, causing chronic inflammation. The bacterium is well known for its ability to cause gastrointestinal pathology, ranging from asymptomatic carriage to gastroenteritis and typhoid fever (139). Since Candida appears as a commensal in the gastrointestinal tract, the overlap of niches provides an excellent point for interaction between species. In a C. elegans polymicrobial infection model, Salmonella enterica serovar Typhimurium inhibited C. albicans filamentation, stunting fungal virulence. Moreover, an in vitro coculture model showed that S. Typhimurium inhibits C. albicans viability and its ability to form a biofilm (140). The type III secretion systems are important virulence factors for Salmonella pathogenesis and are encoded by Salmonella pathogenicity island 1 (SPI-1) and SPI-2 on the bacterial chromosome (141). The type III secretion system allows the bacterium to inject effector proteins directly into the host cell (141, 142). There are more than 30 known SPI-1- and SPI-2-regulated effectors in Salmonella species that utilize these systems (143). One of these effectors, sopB, plays a critical role in interaction and competition with C. albicans by killing filaments. Deletion of sopB significantly increased the survival of C. albicans in vitro. The sopB effector translocates into filaments via SipB, killing C. albicans hyphae. In C. elegans, S. Typhimurium sopB decreased the viability of C. albicans filaments and repressed elongation of filaments, germ tubes, and biofilm formation during infection. Remarkably, researchers found that the sopB effector is associated with the transcriptional repression of CDC42 in C. albicans (which encodes a Rho-type GTPase related to viability) and suggested that the sopB effector of S. Typhimurium is important for competing against fungi (144).

Considering these findings, it is reasonable to conclude that interaction between S. Typhimurium and C. albicans is multifactorial and that the viability of C. albicans is associated with the S. Typhimurium sopB and sipB type III secretion system translocation machinery. However, only limited research has been done on the interaction of C. albicans with intestinal bacterial pathogens. The human intestinal tract has a remarkable microbial community, including Candida. Understanding the interactions between the diverse organisms within the complex milieu of the intestinal tract may expose important pathogenic and therapeutic insights.

**Candida–Helicobacter pylori**

H. pylori has been found to colonize the human gastrointestinal tract and is responsible for many conditions, ranging from gastritis and peptic ulcers to adenocarcinoma (145). In terms of association to Candida, Helicobacter has shown unique interactions with the fungus as illustrated by a handful of reports. H. pylori DNA was found within Candida yeasts isolated from cheek swabs of dyspeptic Iranian patients, a location that has an endemic H. pylori presence (146). This is an important finding in the cohabitation of these two pathogens, with reference to treatment, prevention, and the control of cross-transmission.

Analysis of the relationship between H. pylori and Candida using specimens obtained from patients with specific upper gastrointestinal tract disorders suggested a positive relationship of Helicobacter with Candida, which may help maintain the persistence of H. pylori in the oral cavity, possibly favoring reinoculation of the stomach with the bacterium and allowing the bacterium to exist in these endemic regions. Analysis of samples taken from patients with severe gastric ulcerations confirmed coinhabitation of Candida and Helicobacter in human disease conditions (147).

The intracellular existence of Helicobacter within Candida yeast cells was investigated by several groups in an attempt to explore the underlying mechanisms of this interaction. Fluorescent microscopy showed that labeled H. pylori survives as viable, fast-moving bodies inside the vacuoles of Candida yeast cells obtained from multiple niches (148–150). It has been suggested that the
yeast vacuoles serve as a niche that protects *H. pylori* against environmental stresses while nourishing the bacteria and providing it with sterols such as ergosterol (150). *H. pylori* seems to be vertically transmitted to the daughter cells of *C. albicans* and continues to express its own proteins to survive safely within the yeast cells (148, 149). The bacteria inside yeast cells produce peroxiredoxin and thiol peroxidase, substances which can counteract the respiratory bursts of most phagocytic immune cells. In addition, urease and VacA, two virulence factors of *H. pylori*, are produced when *H. pylori* is taken into *C. albicans* and are capable of modulating the host innate immune response to promote bacterial survival (150). These virulence mechanisms may thus play a crucial role in intracellular survival of *H. pylori* in both epithelial cells and yeast cells. Human gastric epithelial cells and human immune cells have been recognized as the only eukaryotic cells that host *H. pylori*. *H. pylori* might use this intracellular establishment within the yeast cells as a “Trojan horse” mechanism to invade and persist inside human epithelial and immune cells. This could be a crucial step in the colonization of the human gastrointestinal tract, as well as persistence in a variety of environmental conditions, being masked from the host immune defenses.

**Interactions in the Vulvovagina**

*Candida–Lactobacillus* species

*Lactobacillus* species are known for their probiotic effects and are widely known to negate the colonization of pathogens in the gastrointestinal tract and the female genitourinary tract (151). In the female genital tract, lactobacilli function as a barrier against infection with other pathogens by competing for adherence and producing several antimicrobial compounds, such as H2O2 and lactic acid. These compounds lower the vaginal pH to an inhospitable level for many microbial species (152–156). Lactobacilli isolated from the oral cavity were shown to inhibit the growth of *C. albicans* through the production of H2O2 (157). Lactobacilli vaginal isolates also inhibited the growth of *C. albicans*, but this effect was only partially attributed to the production of peroxides (158). It was shown that supernatants obtained from cultures of four *Lactobacillus* species (*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus reuteri*) impaired hyphal and biofilm formation of *Candida* (159). Taken together, these data show an inhibiting effect of lactobacilli on *Candida* growth, hyphal formation, and biofilm production.

Kohler and others showed that the growth of *Candida* was suppressed by lactic acid (produced by lactobacilli), and using fluorescent microscopy, they showed a loss in metabolic activity and cell viability. Moreover, when kept under coculture conditions, *C. albicans* showed an increase in expression of stress-related genes (e.g., SIS1, TPS3, HSP78, TPO3, SEO1), indicating that the cells were in hostile environments, most likely through the lower pH created by lactobacilli acid production (160). Interestingly, Wagner and Johnson showed that *Candida* activated the NF-κB pathway–associated genes IkBα and ELK1 upon infection of VK2/E6E7 vaginal epithelial cells. Lactobacilli suppressed this expression of NF-κB-related inflammatory genes and induced IL-1α and IL-1β expression via alternative signal transduction pathways, such as mitogen-activated protein kinase/AP-1. Activation of such alternative signaling mechanisms by lactobacilli may provide a mechanism to decrease the inflammatory damage caused by vulvovaginal candidiasis and demonstrates the importance of IL-1β to stimulate anti-*Candida* defenses in the innate immune system (161). *Lactobacillus crispatus*, a common species found in the female genital tract, inhibited growth and adhesion of *C. albicans* to HeLa cells. Toll-like receptors (TLR)-2 and -4 expressed by HeLa cells are modulated by both *L. crispatus* and *C. albicans*, and the production of IL-8 and human beta defensin (HBD)2/3 by both species is modulated via the TLR2/4 pathway. Given that *L. crispatus* inhibited *C. albicans* growth and adhesion to epithelial cells, this pathway could be a promising target in antifungal treatment (162).

Two other lactobacilli strains found in healthy vaginal microflora, *L. reuteri* RC-14 and *L. rhamnosus* GR-1, were found to inhibit *C. albicans* growth and attenuated virulence in vaginal cell cultures (163). In addition, Martinez and colleagues demonstrated that use of probiotic lactobacilli and antifungal drug combinations were more effective in controlling the *C. albicans* growth in vaginal candidiasis than antifungal agents alone (164). Chew and others expanded these findings, showing that inhibition of *C. glabrata* growth by *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains leads to cell death, an important observation due to an increase in non-*C. albicans* infections in some populations (165). Romani and colleagues described that some bacteria can support host-fungal symbiosis (166). Commensal lactobacilli and mammals together increase immune tolerance in response to *C. albicans* through the tryptophan catabolic pathway production of indoleamine 2,3-dioxygenase 1, which drives resources away from inflammatory T-cell lineages and promotes a T-regulatory
state. Given the commensal cohabitation by these microorganisms in humans and the frequency of vulvovaginal candidiasis among women worldwide, it is important to ascertain the inhibitory mechanisms of Lactobacillus on Candida so that its probiotic nature can be fine-tuned to work with the host and prevent recurring infections.

**DIAGNOSTICS FOR CANDIDA-ASSOCIATED POLYMICROBIAL INFECTIONS**

History and examination are the cornerstones of the diagnosis of infections, irrespective of their mono- or polymicrobial etiology. Evidence of contamination, presence of a foreign body, use of a prosthetic or iatrogenic instrument often can be ruled out by the clinical history that can be easily gathered from the patient. Further, the signs of inflammation and infection may be elucidated by a systematic clinical examination. In addition, the general assessment of the patient for hygiene and health is also important in the assessment of the degree of contamination and the level of the host’s immune competence, respectively. In case of the patient using prosthetic appliance therapy, the nature and quality of the prosthesis and its use should also be assessed and monitored.

Following the history and examination, various investigations including microbiological methods play a role in the diagnostic process. Conventional microbiological cultures using Sabourauds dextrose agar have been useful in isolating Candida from clinical samples. However, investigations using bacteria–fungi coculture both in blood cultures and animal models showed that usual microbiological techniques, including Gram stain and culture on solid media, were inadequate to detect fungemia when concomitant bacteremia was present (167). These investigators have suggested that the likely mechanisms for this fungal growth suppression included nutritional depletion and elaboration of a toxic substance by the bacteria. Consequently, the reported incidence of blood cultures with synchronous bacteremia and candidemia may underestimate the definite incidence. One possible alternative is to perform an additional and simultaneous culture using chrome-agar Candida medium that prevents bacterial growth and is an excellent growth platform for identification of most commonly encountered Candida species (168). In addition, the advances in molecular microbiology technology using PCR improved the rate of detection of Candida in blood cultures and has demonstrated Candida in the blood of patients with culture-negative results, confirming the lack of sensitivity of blood culture in disseminated candidiasis (169). Several other factors have been suggested to avert the synchronous isolation of Candida and bacteria in blood cultures. One of the reasons for difficulty in isolating Candida in blood culture is rapid bacterial proliferation that often inhibits the fungal proliferation (167). This phenomenon, if occurring in actual patient specimens, would lead to a reduction in numbers of blood cultures isolating Candida species. On the other hand, early commencement of antibacterial drugs immediately upon detection of fever in patients would lead to a reduced incidence of synchronous bacteremia and candidemia because of the presence of bactericidal antibiotics in the blood.

In the context of polymicrobial infections often related to biofilms, novel methods with the potential to identify and quantify each individual member of the polymicrobial community have the utmost importance. In particular, molecular biological methods have demonstrated the ability to precisely define the identity and the quantity of each species of a polymicrobial biofilm infection. For example, advanced techniques including metagenomic analyses are helpful to describe polymicrobial ecosystems (170). Flow cells are used to study microbial interactions in biofilms (171). Recently, microbiome-level analyses involving either conserved, phylogenetically informative genes such as bacterial 16S rRNA gene or whole shotgun metagenomic sequencing have provided promising results. Comprehensive, quantitative molecular diagnostic methods are the most efficient and effective ways to appropriately identify and characterize the complex bacterial and fungal components of such polymicrobial infections (172). Although various high-tech diagnostic methods have been suggested such as metagenomics analyses and flow cells, they have yet to reach clinical diagnostic laboratories in many parts of the world.

**MANAGING CANDIDA–BACTERIA POLYMICROBIAL INFECTIONS**

**Importance of Recognizing the Potential for Polymicrobial Infection**

In cases of bacterial or fungal infection, it is important to recognize that the infection may not be only bacterial or only fungal since standard diagnostic modalities (see above) for polymicrobial infections can often miss major players in the infectious milieu. Considering a polymicrobial infection in the differential diagnosis is important for three reasons: (i) designing appropriate chemotherapy, (ii) taking into account systemic bacterial infections due to Candida infections, and
(iii) recognizing the importance of infectious synergy on patient morbidity and mortality.

Designing appropriate chemotherapy
When Candida is missed during diagnosis, and a polymicrobial infection is treated as a bacterial infection, treatment and cure will not be obtained. A clinical assumption of a mono-species or mono-kingdom infection ignores the potential for a polymicrobial infection that necessarily impacts the disease management (i.e., antibiotic +/- antifungal administration). Therefore, patients often undergo an unnecessary infection cycle during which antibiotics are first used, followed by a fungal infection. Antibiotic therapy is halted and replaced by antifungal therapy, allowing the pathogenic bacterial species that was not eliminated by the abbreviated antibiotic regimen to become a fulminant infection again. Chasing this moving target of antimicrobially based infection resolution only increases the risk of patient morbidity and mortality as well as the development of antimicrobial-resistant strains. Therefore, once a Candida or fungal infection is found following an initial bacterial infection diagnosis and initiation of antibiotic therapy, the initial broad-spectrum or antibiotic sensitivity-directed antibiotic therapy should be continued to avoid the return and exacerbation of the original bacterial infection as well as the acquisition of multidrug-resistant bacteria such as vancomycin-resistant enterococci (173).

Since a contribution of Candida is often missed during diagnosis by standard culture, advanced techniques (e.g., PCR, IBIS, or differential/selective media) should be used, or if not present, can be considered during the differential diagnosis by noting particular risk factors for invasive candidiasis. Invasive candidiasis that can predispose patients to the potential for polymicrobial infection can be considered when patients demonstrate certain risk factors. Besides maintaining a good diet and avoiding social risk factors (obesity, smoking, alcohol/drug abuse), there are a number of other risk factors associated with developing invasive candidiasis. Some of these include broad-spectrum antibiotic usage, immunosuppression due to disease (e.g., Job’s disease, AIDS following uncontrolled HIV infection, etc.), administration of immunosuppressive agents (e.g., chemotherapy, steroids, or antirejection agents following organ transplant), some indwelling medical devices (e.g., endotracheal tubes, urinary catheters, intravenous catheters, and parenteral nutrition), diabetes and diabetic foot wounds, and extremes of age. These risk factors can result in failure of the host to keep Candida in check, an imbalance between commensal bacteria and fungi, or invasion of particular host niches by opportunistic bacterial and fungal pathogens (8).

Taking into account systemic bacterial infections due to Candida infections
Systemic bacterial infections and complications may result from localized invasive candidiasis or even superficial infections by this fungal pathogen (32, 101). Patient morbidity and mortality may increase due to systemic infections that result from the ability of Candida to deliver other microbial species into deep tissue layers or the circulatory system of the host. Therefore, systemic complications such as bacterial infections due to candidiasis should be considered and may require more empirical antibacterial coverage (174). As such, when selecting antibiotics it is important to consider the sensitivity, bioavailability, toxicity, degree of resistance formation, and stability (175). It has been demonstrated that Candida facilitated the invasion of bacteria into host tissues. The findings in the above studies demonstrate that superficial candidiasis may constitute a risk factor for disseminated bacterial disease, warranting awareness of therapeutic management of immunocompromised individuals. The identification of superficial candidiasis as a risk factor for disseminated bacterial disease has serious clinical implications. For instance, it is very important to improve awareness in terms of therapeutic management, particularly in immunocompromised individuals, who often experience recurrent episodes of superficial candidiasis affecting oral and vaginal mucosae, to avoid disseminated bacterial infections.

Impact of microbial infectious synergy on patient morbidity and mortality
Coinfection by C. albicans and microbial species may produce a synergistic infection due to the interaction of the host, Candida, and the bacterial species, thereby dramatically increasing the severity and unpredictability of the infection (9, 45, 174, 176). Therefore, coinfections should be taken extremely seriously. Characterizing the nature of the complex interaction between Candida and bacteria may be the first step in understanding the nature of their coexistence in the host. Unraveling the mechanisms that Candida and bacteria use in a competitive, polymicrobial environment would not only deepen our understanding of their coinhabitation but may also provide important insights into novel pathways that help the development of new antimicrobial drugs.
Control of Biofilm Formation

Since the interaction between Candida species and bacteria often occurs in a biofilm mode of growth, it is important to describe biofilm resolution strategies. Microbes can produce biofilms on synthetic materials or devitalized tissue that results in the failure of antimicrobial agents and the host immune response to resolve these infections. Management of biofilm-related polymicrobial infections, particularly those involving Candida, can be challenging. Removal of the infected device is generally needed to establish cure of Candida infections of medical devices. The mainstay for the resolution of biofilm-associated infections continues to be the removal of the nidus of infection (e.g., indwelling medical device, devitalized tissue, periodontal scaling, and/or debridement) (14, 177–188). There are a number of other biofilm resolution strategies: small molecules that interfere with either bacterial and fungal quorum sensing systems, signaling pathways that control biofilm formation and maintenance, or unique nutrient requirements by biofilm microorganisms to maintain localized pH and redox conditions. Also, macromolecular approaches to biofilm eradication have been proposed that include matrix-digesting enzymes and the development of biofilm-specific antibodies. Some examples that have been proposed include promoting programmed detachment, mechanical disruption, dispersal agents, DNases, nanoparticles (modified silver and gold), lyase, lactonase, alpha-amylases, lystostaphin (for staphylococcal strains), photodynamic therapy (see references 189 and 190 for a review of some of these strategies), and antibody-directed photacoustic killing (191).

Probiotics and prebiotics may have a role in preventing or even treating polymicrobial infections. Live microorganisms when administered in proper concentrations that have health benefits are known as probiotics. There are sets of particular bacterial species that are found in different body niches and are able to control Candida through their probiotic action (192). Probiotics are able to control various infections by exploiting the microbial interference as a mechanism for novel prophylactic or therapeutic management of polymicrobial diseases. Prebiotics, defined as an oligosaccharide indigestible by humans but able to be fermented by beneficial gut bacteria such as Lactobacillus and Bifidobacterium species, may also play a role. By combining a probiotic and a prebiotic (called a synbiotic [193]), microbial mucosal health can be dramatically improved. It is evident that bacteria such as Lactobacillus species have the potential to control yeast colonization in various body niches and therefore could be used in the management of Candida infections.

Phage therapy could also be used to control these polymicrobial infections. Phages are viruses that act against bacteria and that demonstrate a particular tropism for specific species. These phages infect and lyse bacterial populations by undergoing rounds of phage replication, thereby reducing bacterial populations. While bactericidal activity was once thought to primarily be a consequence of lytic events, it has been shown that phage particles encode depolymerases that exhibit enzymatic activity against bacterial matrices, including exopolymeric compounds. However, phage therapy has not entered phase III clinical trials or showed effective biofilm infection therapy in vivo against multiple microbial strains, even with decades of research attempts.

Polymicrobial vaccines may also play a role in the future control of polymicrobial infections. Now that we are aware that chronic and polymicrobial infections involve Candida and bacterial biofilms, investigators can develop appropriate vaccines that prevent these coinfections. When Candida or bacteria invade the host they can either be removed by the host innate immune response or get attached to the host extracellular matrix proteins to develop a localized biofilm community. The resulting proteome of the microorganisms becomes distinct once the population transforms into a biofilm phenotype and deviates remarkably from the characteristics of the planktonic proteome. According to Harro et al. (194), two components of the biofilm, i.e., bacterial cells within the biofilm and the biofilm matrix, although they vary between bacterial genera, species, and strains, could be considered as targets for vaccine development if common antigens are found. Moreover, to prevent polymicrobial infection, a vaccine composed of a multivalent cocktail of antigenic proteins from all microorganisms involved in disease pathology may have to be considered.

Once combined with conventional antibiotics to battle the microbial cells existing in the planktonic state, nonsurgical biofilm eradication may finally be realized. However, many of these antibiofilm strategies are in early in vitro, animal, or topical development and are not approved for use for invasive disease. Therefore, infections caused by Candida–bacteria interactions have significant complexity and are still in the infancy stages in vaccine development. Vaccine design for Candida–bacteria polymicrobial infections should consider the unique virulence attributes of the yeast and the bacteria in question to achieve success.
**SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS**

*Candida* can exist in a polymicrobial biofilm mode of growth attached to biotic and abiotic surfaces. Some microbes have evolved mutualistic or even synergistic relationships to facilitate cohabitation on epithelial surfaces and deep wounds, while others have developed competitive antagonistic approaches during cocolonization. Biofilm formation occurs within a complex milieu of host factors and other members of the human microbiota. Thus, bacteria can attenuate or enhance fungal invasion and virulence in *C. albicans* mucosal biofilms, not to mention the candidal effect of augmentation or abrogation of virulence on bacteria.

Complex *Candida*-bacteria interactions are not a rare occurrence; they do exist, interact, and coaggregate with common bacteria (both commensals and frank pathogens), and this interaction has great clinical significance. The effects of such interactions are relevant to the host, the microbe, and the environment. Elucidating the nature of these interactions at the proteomic, genetic, transcriptomic, metabolomics, and systems levels is imperative in the understanding, prevention, and management of polymicrobial infections. It is likely that interaction between host, *Candida*, and commensal microbiota dictates the types of host–fungus relationship, and the microbial dysbiosis may predispose to a variety of chronic fungal infections and diseases at local and distant sites. The elucidation of these complex interactions is important not only for a better understanding of the pathobiology of infections and microbial interactions but also for the identification of novel targets for future antimicrobial strategies as the age of antibiotics begins to wane.

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Candida-Bacteria Interactions


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