**INTRODUCTION**

Pathogenic fungi cause not only superficial infections but also life-threatening invasive infections, particularly in immunocompromised individuals [1]. According to estimates, 1.7 billion people experience superficial fungal infections, and 300 million people experience serious fungal infections annually worldwide [1,2]. Approximately 1.5 million people die from invasive fungal infections every year [1,3]. Fungal infections have recently gained worldwide attention because of the notable spikes in their incidence rates observed over the past several years; these spikes are probably a result of surges in the use of clinically invasive procedures, such as central venous catheters and shunts, the overuse of broad-spectrum antibiotics, the increase in prolonged hospital and intensive care unit stays, the prevalence of human immunodeficiency virus infections and other immunocompromised conditions, and a shift toward older populations [3-5]. In addition, the coronavirus disease 2019 pandemic, caused by severe acute respiratory syndrome coronavirus 2, has further exacerbated clinical situations [6]. Coinfection of *Aspergillus, Mucorales*, and *Candida* species, including *Candida auris*, for example, together with severe acute respiratory syndrome coronavirus 2, has been found to result in higher morbidity and mortality rates than observed with single infections with any of these pathogens alone [6].
Candida species are the most common fungal pathogens causing mucosal candidiasis, deep organ infections, and bloodstream infections [7]. Despite receiving treatment with antifungal drugs, more than 40% of patients with invasive Candida infections die [1,8,9]. Candida albicans is the most frequently isolated Candida species in clinical settings, and thus has received substantial research attention [3,10]. Over the past two decades, however, epidemiological surveillance has indicated a shift toward the isolation of non-albicans Candida species, probably because of an increase in the use of antifungal drugs in clinical practices [9,11]. Specially, non-albicans Candida species of the Candida haemulonii complex and the closely related species Candida auris, which in some studies has been classified in the Candida haemulonii complex [12,13], have garnered substantial attention among both clinical and basic research communities. Multidrug resistance is a notably common characteristic of the Candida haemulonii species complex.

Most pathogenic Candida species (except for Candida glabrata) phylogenetically belong to the CTG clade; these species translate the CTG codon to serine rather than the canonical leucine [14]. The CTG clade is composed of the Metschnikowiaceae and Debaryomycetaceae clades [15]. Candida albicans, Candida tropicalis, and Candida parapsilosis belong to the Debaryomycetaceae clade, whereas Candida auris and the Candida haemulonii species complex are classified into the Metschnikowiaceae clade. Species in the Candida haemulonii complex are particularly concerning in hospital settings, owing to their rapidly increased emergence worldwide, and their intrinsic resistance to existing antifungal drugs [16,17]. Here, we review the past two decades of research progress in the epidemiology, biology, virulence, and drug resistance of the Candida haemulonii species complex.

**EPIDEMIOLOGY**

C. haemulonii was first isolated from seawater in the Atlantic Ocean and the intestines of Haemulon sciurus fish in 1962 [18]. The first clinical isolate of C. haemulonii was recovered from the blood of a patient with renal failure in 1984 [19]. In 2007, the first outbreak of C. haemulonii fungemia was reported in a neonatal intensive care unit in Kuwait, where seven isolates were identified from the blood of four neonates [20]. Fungemia caused by C. haemulonii was later reported in hospitalized patients in China and Korea in 2009 [21-23]. Other species of the Candida haemulonii complex, including Candida haemulonii sensu stricto, Candida duobushaemulonii, Candida pseudohaemulonii, Candida haemulonii var. vulnera, and Candida vulturna have been sporadically identified from patients in hospital settings (Fig 1) [16,24-27].

C. duobushaemulonii was first isolated from a patient with a foot ulcer in 1990 [28]. A retrospective study

![FIGURE 1](image_url) | Representative species of the Candida CTG clade. A. Dates of the first reports of isolates of species in the Candida haemulonii complex. B. Maximum-likelihood phylogenetic tree of C. haemulonii species complex and closely related species, constructed on the basis of ITS sequences and 1,000 bootstrap replicates. All sequences were acquired from the NCBI GenBank database. General time reversible (GTR) and gamma distribution with invariant sites (G+I) models were used. Red lines indicate species of the Candida haemulonii complex.
Candida haemulonii Species Complex: Emerging Fungal Pathogens of the Metschnikowiaceae Clade

BIOLOGY

Similarly to the major human fungal pathogen C. albicans, species of the C. haemulonii complex can undergo morphological transitions and form biofilms, which are important processes contributing to fungal pathogenesis [38-41]. Clinical isolates of C. haemulonii produce smooth, round colonies under standard laboratory culture conditions, and form colonies with light to dark viola
ten coloration on CHROMagar, a chromogenic medium used to isolate and differentiate certain clinically relevant Candida species [42]. C. haemulonii phenotypic transitions have recently been characterized [40]. C. haemulonii displays different phenotypes (white, pink, or filamentous) in response to specific growth conditions. The transition between the white and pink phenotypes appears to be the primary switching system in C. haemulonii. Clinical isolates of C. haemulonii often form both white and pink colonies at 25°C on yeast peptone dextrose agar plates containing the red dye phloxine B. The cells from pink colonies are larger than those from white colonies. Similarly to the white-opaque switch of C. albicans, the white-pink switch of C. haemulonii appears to be heritable and reversible. Moreover, C. haemulonii pink cells can form wrinkled colonies containing elongated filaments at 25°C on yeast peptone glycerol agar plates. This C. haemulonii filamentous phenotype is relatively stable and therefore may be regulated through genetic or epigenetic mechanisms. These distinct C. haemulonii phenotypes also differ in gene expression profiles, metabolic profiles, production of secreted aspartyl proteases (Saps), and virulence [40].

Beyond morphological transitions, the ability to form biofilms is another important virulence factor for pathogenic Candida species [8,41]. A fungal biofilm is a coordinated and functional community of fungal cells encased in an extracellular matrix. Fungal biofilms have greater drug resistance than free-floating fungal cells. Like C. albicans, species of the C. haemulonii complex can form biofilms on indwelling medical devices [8], thus significantly increasing morbidity and mortality among hospitalized patients [43,44]. For example, C. haemulonii has been shown to cause serious bloodstream infections originating from biofilms formed on the surfaces of indwelling intravascular catheters [41]. Proteins and carbohydrates are the main components of the extracellular matrix of biofilms formed by clinical isolates of the C. haemulonii species complex [41]. Interestingly, species of the C. haemulonii complex can form biofilms of different biomasses on several types of catheter surfaces, including vascular (polystyrene), urinary (siliconized latex), nasoenteric (polyurethane), and nasogastric (polyvinyl chloride) catheters [41]. Although the structure and function of biofilms formed by the C. haemulonii species complex have been described, the underlying mechanisms of biofilm formation in these species remain unclear [23,41,45,46]. In general, the abilities to undergo phenotypic transitions and to form biofilms are important in the environmental adaptation and virulence of the C. haemulonii species complex. Future exploration of the regulatory mechanisms involved in these processes will provide new insights into the development of novel strategies for the prevention and treatment of fungal infections.

VIRULENCE

Saps, phospholipases, and esterases are important virulence factors that facilitate the colonization and survival of pathogenic Candida species in their hosts [47]. The genomes of the C. haemulonii species complex genomes contain multiple genes encoding Sap-like conserved domains, and the production of Saps by both clinical and environmental C. haemulonii species complex isolates has been reported [33,48-51]. In fact, polyclonal antibodies specific to C. albicans Sap1, Sap2, and Sap3 recognize C. haemulonii Sap-like proteins [49]. Other hydrolytic enzymes, including serine proteases, phospholipases, and esterases, have
also been found in species of the *C. haemulonii* complex [33,48,50,52,53].

Compared with other pathogenic *Candida* species, in species of the *C. haemulonii* complex, the dose-dependent virulence has been reported to be relatively low in an immunocompetent murine model of disseminated infection [54]. In this model, all mice inoculated with *C. haemulonii* survived; however, half the mice died when the inoculum of *C. haemulonii* cells increased by 1 to 2 log. *C. haemulonii* yeast cells were recovered from various organs of infected mice on days 5 and 10 post-infection, regardless of the inoculum size [54]. Another study has indicated that *C. haemulonii* is completely nonvirulent in an immunosuppressed mouse model of disseminated infection: no viable yeast cells were recovered from the kidneys of infected mice 12 days post-infection [55]. In a *Galleria mellonella* infection model, diminished fungal burdens and prolonged host survival have been observed for species of the *C. haemulonii* complex compared with *C. auris* [56]. Indeed, in both the mouse and *G. mellonella* infection models, host survival rates after infection with species of the *C. haemulonii* complex have been found to be higher than those after infection with *C. auris* or *C. albicans* [56]. Future virulence studies will be important to shed new light on the mechanisms of pathogenesis of the *C. haemulonii* species complex compared with other *Candida* species.

**DRUG RESISTANCE**

Species of the *C. haemulonii* complex are often resistant to multiple antifungal drugs, and this resistance is a common reason for treatment failure [17]. Most clinical isolates of the *C. haemulonii* complex exhibit limited susceptibility to the triazoles and to amphotericin B, with elevated minimum inhibitory concentrations [20,22,26,30,42,57,58]. Several clinical isolates of the *C. haemulonii* complex also exhibit resistance to the echinocandins and to flucytosine [16,26,27,31,33,34,59,60].

Mutations in the lanosterol 14α-demethylase-encoding gene *ERG11* and upregulation of the efflux pump-encoding genes, such as *CDR1*, are associated with azole resistance in the *C. haemulonii* species complex [28,61,62]. Interestingly, efflux pump inhibitors have been shown to reverse the observed azole resistance caused by these mutations [62]. The mechanisms of echinocandins and amphotericin B resistance in the *C. haemulonii* species complex remain to be investigated. Given the common multidrug resistance characteristics of *C. haemulonii* complex species, the associated mechanisms are likely to be complex, and substantial research efforts should be focused on this area in the future.

**PERSPECTIVES**

Infections with historically “rare” fungal pathogens, such as *C. auris* and the *C. haemulonii* species complex, have been increasingly observed in clinical settings over the past two decades. Given the difficulties in accurately identifying these species through conventional phenotypic methods or standard biochemical methods, the prevalence of infections caused by these species is likely to be underestimated. The more widespread use of molecular identification techniques, such as metagenomic next-generation sequencing and matrix-assisted laser desorption/ionization-time of flight mass spectrometry in clinical settings is expected to greatly improve the accuracy of identification and the characterization of these fungal species.

The emergence of new fungal pathogens and the increased reports of multidrug resistant *C. auris* and species of the *C. haemulonii* complex from different countries may have several potential explanations. First, the widespread use of antifungal drugs in clinical settings and of fungicides in the environment (for example, in agriculture and wood preservation) may promote the evolution and emergence of drug resistant fungal species. Second, the increase in immunocompromised and older populations, combined with the use of clinical antifungal drug treatment regimens, provides an additional avenue for the evolution of antifungal drug resistance. Third, ecological factors, including climate change, combined with exposure to fungicides in the environment, provide yet another avenue for new emerging multidrug resistant fungal pathogens to infect human hosts.

Although the global prevalence of the *C. haemulonii* species complex remains relatively low with respect to that of other fungal pathogens, nosocomial outbreaks have been reported with increasing frequency in many countries. To limit infections caused by the *C. haemulonii* species complex, future studies should focus on understanding the epidemiology, pathogenesis, and drug resistance of these important emerging fungal pathogens. In particular, the transmission of the *C. haemulonii* species complex is a notable area for future research, because how transmission to the host occurs is not clearly understood. Given the increase in reported cases and the multidrug resistance of the associated isolates, the *C. haemulonii* species complex should be considered a serious public health threat worldwide.

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**CONFLICTS OF INTEREST**

Clarissa J. Nobile is a cofounder of BioSynesis, Inc., a company developing diagnostics and therapeutics for biofilm infections. All other authors have no competing interests to disclose.
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REFERENCES

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