A review of human pathogenic fungi prevalence, resistance and detection methods

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Abstract. Against the background of overuse of antibiotics, the problem of their resistance is growing. Pathogenic fungi can cause dermatologic, histologic, and systemic diseases in humans. This paper reviews the status of drug resistance in human pathogenic fungi and related research progress. It describes the common pathogenic fungal species and their pathogenic mechanisms. Common pathogenic fungi include *Cryptococcus, Candida, and Aspergillus.* Based on the common antifungal drugs, the main mechanisms of fungal resistance, and the results of resistance epidemiology survey, the status of research and development of novel antifungal drugs such as azoles and polyenes, as well as fungal detection methods such as second-generation sequencing and PCR are integrated. In contrast, the outlook of the existing Clinical Fungus Detection Methods and the status of fungal resistance research is presented.

Keywords: Pathogenic fungi, Drug resistance, Epidemic diseases.

1. Introduction of pathogenic fungi

1.1. Common pathogenic fungi

Of the approximately 5 million species of fungi known to exist in humans, fewer than 10,000 have the ability to infect humans and cause disease. The World Health Organization classifies pathogenic fungi into three categories according to priority: highest priority, high priority, and medium priority, with *Cryptococcus neoformans, Candida otorhinolae, Aspergillus fumigatus*, and *Candida albicans* belonging to the highest priority category. High-priority fungal pathogens include *Candida smooth, Histoplasma spp, Podoconiosis spp, Trichoderma spp, Fusarium spp, Candida tropicalis, and Candida near-smooth.* Medium-priority fungal pathogens include *Serratia marcescens, Sporothrix multilocularis, Coccidioides spp, Pseudomyces krusei, Cryptococcus gattii, Cyanobacterium marneffei, Pneumocystis japonicus, and Coccidioides parapsilosis.*

Approximately 1.7 billion people worldwide suffer from fungal infections of the skin surface, with *Aspergillus*, *Pneumocystis*, *Candida*, and C*ryptococcus* contributing to 90% of all deaths, and a team of Prof. David Denning of the University of Manchester published an article in The Lancet Infectious Diseases in 2024 based on patient data from more than 80 countries stating that There are approximately 6.55 million acute fungal-associated cases worldwide each year, resulting in 3.75 million deaths.[1] In fact, since the fungus is present in a wide variety of people, and more undetected and undiscovered cases are not counted among them, these infection numbers are only going to get higher. The Centers for Disease Control and Prevention has stated that the cost of treating fungal diseases is equivalent to almost 1.1% of the U.S. GDP. With at least hundreds of billions of dollars spent globally, the importance of its treatment cannot be overstated.

1.2. The *Candida*

The genus Candida, for example, contains more than 150 heterogeneous species (Calderone, 2002), but human candidiasis is associated with only a small concentration of Candida. Approximately 65% of *Candida* species are known to be unable to grow at temperatures of 37°C, which prevents these species from being successful pathogens or true human commensals, with more than 80% of *Candida* isolates obtained via humans being *Candida albicans* (NAC). *Candida albicans* can grow abnormally

in the presence of abnormal ph equivalents leading to candidiasis with a mortality rate of up to 60%.[2] Clinically, the treatment of *Candida albicans* infection is one of the sources of risk for diseases such as hematologic malignancies, solid cancers, preterm births, cardiac disease, trauma, neurological disorders, gastrointestinal disorders, organ transplants, pulmonary diseases, vascular diseases, HIV, hereditary disorders/congenital malformations, renal disorders, diabetes mellitus, liver disorders and pancreatic disorders, and according to the relevant reports, Candida in U.S. hospitals is a significant threat with the highest prevalence of associated diseases.

In addition, oral hygiene, systems including dietary nutrient intake, and living conditions play a key role in the prevalence of *Candida albicans* and NAC infections in different national populations. A study of data from 263 patients in a university hospital in Rio Grande do Sul in 2013-2014 showed that more than 60% of Candida isolates in gynecological clinical cases were identified as *Candida albicans*; whereas NAC was present in 8.6% of symptomatic patients and 14.3% of asymptomatic patients. A case of *Candida* bloodstream infections in a population of Barcelona, Spain, in 2003-2004 study showed that of the 340 patients monitored, 44% died within 30 days and 22% died within 7 days.[3] The Health Sciences Research Center, Faculty of Health Sciences, Sari Mazandaran University of Medical Sciences, Sari Mazandaran, Iran, based on 12 studies spanning 2000-2013, concluded that the prevalence of *Candida albicans*-induced denture stomatitis was relatively high, at 28.9%, among the cases of patients with 2271 infections in Iran.[4] Another study conducted on patients with invasive candidiasis in Southwest China reported *Candida albicans* as the most frequently isolated Candida species, and NAC was also frequently isolated. Numerous epidemiologic surveillance reports have shown that *Candida albicans* isolated from patients with invasive candidiasis remain the most common species despite a marked increase in cases of NAC infection.

Other *Candida* also have a significant role in human disease, with *Candida albicans* accounting for only 68% of Candida isolates obtained outside of the blood of cancer patients, as well as 12% Candida tropicalis, 10% Candida sparingly, and a large number of *Candida glabrata* in patients with oral candidiasis. Isolation of blood samples from drug-addicted patients yielded *Pseudomonas parapsilosis*, also a potentially curative isolate of Candida, in addition to *Pseudomonas aeruginosa*, which is one of the most frequently isolated fungi from pathogens and the second most common *Candida* isolated from the usually sterile body parts of hospitalized patients. Also, geographic disparities can make the percentage of patients infected with different *Candida* vary.

1.3. The *Aspergillus*

Invasive Aspergillosis, caused by *Aspergillus*, is one of the most clinically serious invasive fungal infections, occurring most often in patients with malignant tumors, AIDS, solid organ transplantation (SOT), or potentially immunocompromised populations receiving immunosuppressive therapy, with a mortality rate that can be as high as 50%. At least 300-400 known species of *Aspergillus* exist, with the five major groups of *Aspergillus* fumigatus, *Aspergillus flavus, Aspergillus niger, Aspergillus terreus and Aspergillus nidulans* receiving the most attention, and the most common species causing invasive disease are *Aspergillus flavus*, *Aspergillus niger,* and *Aspergillus terreus*.[5-6]

Invasive Aspergillosis was the most common mold infection among approximately 5,500 patients who underwent HSCT, and although 0.7% of HSCT recipients had mold infections, *Aspergillus* infections were the most common, followed by *Fusarium, Trichoderma,* and *Sedospora* infections. Among organ transplant recipients, the highest incidence of cardiac disease was found in heart transplant recipients (4.8%), followed by lung transplant recipients (4.1%), and a significant decrease in liver transplant recipients (0.8%) and kidney transplant recipients (0.3%). The Transplantation-Related Infection Surveillance Network (TRANSNET) study, which evaluated hematopoietic stem cell transplant and organ transplant recipients from 23 countries in the U.S., showed a 12-month cumulative incidence of invasive treponemal disease of 1.6% in all hematopoietic stem cell transplant recipients compared with 0.63% in SOT recipients. The 12-week all-cause mortality rate was 57.5% for HSCT recipients and 34.4% for organ transplant recipients.

As a common prevalent pathogenic fungus, *Aspergillus* is extremely abundant in the air, with the average day's inhalation containing hundreds of *Aspergillus* spores, and conidia are more readily dispersed into the environment during the saprophytic cycle, creating a favorable environment for transmission. Among these, *Aspergillus* fumigatus is more prevalent, and its genome contains genes encoding glycosyl hydrolases and extracellular proteases, the products of which support the ability to grow successfully by degrading polysaccharides from plant cells and obtaining nitrogen through the degradation of protein substrates, and *Aspergillus* fumigatus not only grows on a wide variety of substrates but also tolerates the stresses exerted by prolonged freezing or dehydration. This is one of the main reasons why *Aspergillus* is a major invasive fungal disease.

1.4. Other pathogenic fungi

Novel *Cryptococcus* is also one of the popular clinical research objects. *Cryptococcus* is extremely adaptable to the environment, and its pathogenicity is mainly through the respiratory tract into the lungs to cause chronic meningitis. Its polysaccharide pods allow for environmental adaptations such as desiccation, its heat shock proteins help it adapt to human body temperature, and its regulated iron uptake system helps it target the infiltration of hemoglobin and hemoglobin. The global incidence of cryptococcal meningoencephalitis is estimated to be more than 220,000 cases per year, resulting in approximately 180,000 deaths annually. *Clostridium neoformans* is also a long-lasting, latent type of pathogenic fungus that is widespread in soil and poultry feces and can establish symptomatic pulmonary infections leading to pneumonia, acute respiratory distress syndrome, and subsequent extrapulmonary spread. Another group of fungi can cause a wide range of human skin diseases, such as *Trichoderma* and *Marsupialia*, and tend to infect body hair, skin, and nails through direct contact with the body.[7]

2. Fungal Drug Resistance

Invasive fungal diseases caused by pathogenic fungi have long been recognized as a threat that cannot be ignored. And with the existing medical technology means, the most direct and effective treatment is to use the existing antibiotics to resist the invasion of pathogenic fungi, with the popularity and advancement of antibiotic treatment, traditional antibiotics are becoming less effective in dealing with pathogenic fungi, the World Health Organization once declared that “fungi causing common infections are becoming increasingly resistant to therapeutic drugs, and are increasingly likely to develop into more invasive infectious diseases in the general population to develop more aggressive infectious diseases.” Since the discovery of penicillin in 1928, there has been an increasing reliance on antibiotics, which has led to an increase in the resistance of pathogenic fungi to antibiotics through natural selection. Coupled with the lack of new and effective antibiotics and the emergence of more species of fungi that cause invasive diseases, the phenomenon of fungal drug resistance has become a major focus of modern medicine.

CHIF-NET published a 9-year epidemiologic and antifungal drug susceptibility monitoring study of *Candida tropicalis* in invasive candidiasis, which showed that the azole susceptibility rate of *Candida tropicalis* showed a decreasing trend in China over the past 9 years, and the fluconazole resistance rate increased from 5.7% to 31.8%. The resistance rate of *Candida tropicalis* to fluconazole was 29.55%, to voriconazole was 26.24%, itraconazole and posaconazole NWT were 21.99% and 76.60% respectively, and amphotericin B NWT was 0.24%. And the study found the emergence of a certain percentage of echinocandin-like resistant strains. Another study of clinical *Candida* drug resistance surveillance in 37 hospitals in East China carried out in 2018-2022 showed that 44.51% of *Candida albicans* was the highest percentage of the composition ratio of the tested fungi among 3026 more *Candida* strains.[8] It is clear that a number of medical surveillance studies and subjects have shown a growing trend of resistance of the dominant pathogenic fungi to existing antifungal drugs.

Classification of fungal drug resistance is similar to immune function, mainly including primary resistance, secondary resistance and clinical resistance, usually based on the source of drug resistance

generated by the fungus itself. Primary, also known as intrinsic resistance, refers to the resistance that exists naturally in the pathogenic fungus, secondary resistance, also known as acquired resistance, refers to the resistance that arises from changes in the genetic shape and genotype of the fungus itself, and clinical resistance refers to the resistance of the fungus itself that is very low, but arises from the physiological environment of the host body in the course of clinical treatment.

Azoles are a commonly used antifungal drug that has been discovered in recent times, including imidazoles and triazoles. Imidazoles include ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), miconazole ($C_{18}H_{14}Cl_4N_2O$), econazole ($C_{18}H_{15}Cl_3N_2O$), clotrimazole ($C_{22}H_{17}ClN_2$) and so on, which are more toxic when taken orally, and have the ability to inhibit the fungal cytochrome P-450-dependent 14-alpha-demethylase, which makes the accumulation of 14-alpha-methylsterols. resulting in the pharmacological effect of cell membrane ergosterol cannot be synthesized. It is used as a topical agent for the treatment of superficial fungal infections and Candida infections of the skin and mucous membranes. Triazoles, including itraconazole ($C35H_{38}Cl_2N_8O_4$), fluconazole ($C_{13}H_{12}F_2N_6O$), etc., can be used as therapeutic drugs for deep fungal infections. Similar to imidazoles, it interferes with the synthesis of ergosterol in fungal cells mainly by inhibiting 14α-sterol demethylation mediated by cytochrome P450 (CYP450) in fungi. Compared with other antifungal drugs, many azoles have exceptional oral bioavailability and can be used for oral and intravenous administration. However, they only inhibit the growth of *Candida* and *Cryptococcus*, among others, and do not kill the fungal body at all.[9]

In the case of azole antifungal drugs, the key to achieving the effect is by targeting the cytochrome p450-dependent enzyme lanosterol 14-alpha-demethylase, which inhibits the biosynthesis of ergosterol, a key component of the fungal cell membrane, causing the fungus to die. The most prevalent mechanism of azole resistance at this stage is mainly through mutations or overexpression of genes among altered ERG11/cyp51A/cyp51B. Mutations include *Pseudomallei* and *Aspergillus-resistant* strains, which are usually susceptible to amino acid substitutions in the region close to the heme-binding site of the enzyme. Additionally, in a 2018 study from the University of Toronto on the mechanism of resistance generation in a *Pseudohyphomyces* yeast, mutations in the transcription factor Pdr1 in *Candida glabrata* led to the upregulation of the drug efflux pumps, which enhanced resistance to azoles. Not only that, the fungus can also enhance drug efflux and reduce drug concentration in the cell by upregulating the expression of multidrug transporter proteins.

A drug sensitivity test on azole resistance analysis by the Department of Laboratory Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, in 2013 showed that *Candida smoothii*, *Candida quinquefasciatus* and *Candida tropicalis* were sequentially ranked at 27.2%, 24.3% and 21.9% resistance rates, respectively, and *Candida tropicalis*, *Candida smoothii* and *Candida quinquefasciatus* were sequentially ranked at 21.9%, 15% and 8.1% resistance, respectively.

A new antifungal drug that has entered the market in the last two decades of the new century is echinocandin, which causes disruption of cell wall integrity and osmotic pressure imbalance, ultimately killing the fungal body. Currently available on the market are caspofungin ($C_{52}H_{88}N_{10}O_{15}$), micafungin ($C_{56}H_{71}N_9O_{23}S$), and anidulafungin ($C_{58}H_{73}N_7O_{17}$). Echinocandins kill fungi by non-competitively inhibiting the synthesis of β-1, 3-glucans in the cell wall of the fungus and disrupting the normal structure of the fungus in its cell wall.[10]

Echinocandins are drugs that do not exist in mammals by destroying the fungal cell wall, and their main advantage centers on their favorable safety profile. These drugs have been clinically proven to be effective in the treatment of invasive candidiasis but are not effective against *Cryptococcus*, for example. Echinocandin resistance arises mainly through amino acid substitutions within the highly conserved region of the Fks subunit of glucan synthase. The level of resistance depends on the mutation and expression levels of these genes. Also, complex cellular circuitry coordinated in response to cell wall stressors can confer echinocandin tolerance and drug resistance. A survey at a hospital in the United States showed that the overall resistance rate to echinocandins increased from 4.9% to 12.3% in the last decade. Novel drugs currently emerging from clinical studies are 1, 3-β- d

-glucan synthase inhibitors, which support oral and intravenous administration and do not have a high level of resistance for the time being.

Polyenes are an old and common antifungal drug, introduced into clinical therapy in the 1950s, which kill fungi by binding to ergosterol on the fungal cytoplasmic membrane and forming a drug-lipid complex, thereby altering cell membrane permeability. Recently, it has also been shown that polyene antifungal drugs such as amphotericin can achieve direct uptake of fungal intracellular ergosterol by forming aggregates outside the cell membrane. In the face of this drug, the pathogen can reduce the binding capacity of the drug by overexpressing the enzyme target protein encoded by the ERG11 gene that binds to the drug. This alteration usually occurs near the critical site of binding to the drug, resulting in drug failure. The most commonly used polyene amphotericin B has demonstrated good efficacy against most clinically pathogenic fungi, including many species of *Pseudomonas, Aspergillus*, and *Cryptococcus*, but is not preferred in clinical practice due to the poor oral utilization of amphotericin and host dependence on the drug dose.

3. Clinical Pathogen Testing

The widespread existence of pathogenic fungi not only depends on the survival environment of the fungi themselves and drug resistance and other evolutionary results but also depends on the survival of fungi found by people using specific detection means. Fungi, as eukaryotic organisms, have intact nuclei, nuclear membranes, cell membranes, and cell walls, and infections have no specific clinical manifestations, which makes them highly susceptible to misdiagnosis such as omission in the clinic, and thus the development and application of fungal detection technologies are crucial.

The microdilution method was detailed in a study on antifungal drug mechanisms at the University of Toronto, Canada 2018 to perform drug sensitivity testing. A certain concentration of fungi was inoculated in media containing different concentrations of antifungal drugs, and their sensitivity to the drugs was assessed by observing the growth of the fungi. After 24 to 48 hours of incubation, the growth of the fungus in each petri dish was observed. By comparing the presence or absence of significant growth, the minimum inhibitory concentration (MIC), which is the lowest concentration of drug required to inhibit the growth of the fungus, can be determined.[11]

A 2010 review by MenGen provides a detailed analysis of *Aspergillus* tests covering the major common fungal tests. Using the Nested PCR method, specific primers are designed to target the internal transcribed spacer (ITS) region of the fungus for the first PCR amplification. These initial amplification products are used as templates for a second PCR amplification. Nested PCR enhances the specificity and sensitivity of the target DNA by using different primers in the second amplification. Target fungi can be detected with great sensitivity at DNA amounts as low as 10-100 copies. In addition, nested PCR is faster compared to traditional culture methods, producing results within hours and eliminating the need to culture the fungus, thus reducing the risk of cross-contamination.

A novel DNA microarray technique extracts the genomic DNA of fungi from a sample and then designs oligonucleotide probes that target specific fungi. These probes are immobilized on nylon membranes or microtiter plates. The DNA in the sample is amplified by PCR and then hybridized with the probes. By detecting the fluorescent signal after hybridization, it is possible to determine whether the sample contains the target fungus. The researchers used this technique to detect 64 clinically important fungi in 32 genera. The entire process, from isolation of colonies to detection of arrays, took only 24 hours, and the limit of detection was as low as 10 pg. This technique not only increases the speed of detection but also significantly improves sensitivity and specificity.[12]

In addition, an assay utilizing random amplification of polymorphic DNA is also of interest. After performing PCR on DNA from fungal samples, the size and number of amplification products are analyzed by gel electrophoresis to observe the polymorphism of the fragments. These polymorphic fragments can be used to construct DNA fingerprints to help identify fungal species. It can quickly identify target fungi in mixed samples.

Not only that, Next-Generation Sequencing (NGS) has also shown great strength in the detection of specific fungi. In clinical applications, NGS has been used to identify pathogens that cause keratitis. In a study from Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology 2021, NGS was performed on corneal samples combined with mass spectrometry by analyzing mass spectrometry data of proteins and metabolites in fungal samples. Nectria haematococca, the causative agent of corneal fungal infections, was successfully detected. Through simple steps such as DNA extraction, library construction, sequencing, and data analysis, followed by comparison with microbial genome databases, NGS can rapidly classify and identify the fungal species in the samples.[13] The high sensitivity and specificity of this technology make it an important tool for the diagnosis of complex fungal infections, providing medical professionals and researchers with more comprehensive pathogen information.

4. Conclusion

In summary, the article reviews the mechanisms of drug resistance in pathogenic fungi, suggesting that different fungi may enhance resistance to antifungal drugs in a number of ways. Current epidemiologic investigations of drug resistance in pathogenic fungi have shown that certain high-priority fungi such as *Cryptococcus* neoformans and *Candida albicans* are becoming more resistant in the clinic, and that the mainstream antifungal drugs such as azoles and polyenes have already produced extensive resistance in a variety of common strains of fungi, such as *Cryptococcus neoformans* and *Candida albicans*, and that most of the blocking pathways are still stuck in the biosynthesis pathway of ergosterol and that the clinical resistance rate is increasing and threatening the health of patients. Future research should focus on finding new drugs and their mechanisms of action to deal with the increasing complexity of drug-resistant fungal infections. There is an urgent need to find a drug that is less stimulating to the evolution of fungi and can meet the requirements of resistance to fungi. With the maturation of next-generation DNA sequencing and protein structure prediction technologies, we expect to see more advanced fungal detection tools to nip pathogenic fungi in the bud.

References

[1] Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis. 2024 Jul; 24 (7): e428 - e438. doi: 10.1016/S1473-3099(23)00692-8. Epub 2024 Jan 12. PMID: 38224705.

[2] Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012 Mar; 36 (2): 288 - 305. doi: 10.1111/j.1574 - 6976.2011. 00278. x. Epub 2011 Jun 6. PMID: 21569057.

[3] Dadar M, Tiwari R, Karthik K, Chakraborty S, Shahali Y, Dhama K. Candida albicans - Biology, molecular characterization, pathogenicity, and advances in diagnosis and control - An update. Microb Pathog. 2018 Apr; 117: 128 - 138. doi: 10.1016/j.micpath.2018.02.028. Epub 2018 Feb 16. PMID: 29454824.

[4] Moosazadeh M, Akbari M, Tabrizi R, Ghorbani A, Golkari A, Banakar M, Sekhavati E, Kavari SH, Bagheri Lankarani L. Denture Stomatitis and Candida albicans in Iranian Population: A Systematic Review and Meta-Analysis. J Dent (Shiraz). 2016 Sep; 17 (3 Suppl): 283 - 292. PMID: 27840842; PMCID: PMC5103476.

[5] Nji QN, Babalola OO, Mwanza M. Soil Aspergillus Species, Pathogenicity and Control Perspectives. J Fungi (Basel). 2023 Jul 20; 9 (7): 766. doi: 10.3390/jof9070766. PMID: 37504754; PMCID: PMC10381279.

[6] Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latgé JP, Steinbach WJ. Aspergillus fumigatus and related species. Cold Spring Harb Perspect Med. 2014 Nov 6; 5 (2): a019786. doi: 10.1101/cshperspect. a019786. PMID: 25377144; PMCID: PMC4315914.

[7] May RC, Stone NR, Wiesner DL, Bicanic T, Nielsen K. cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol. 2016 Feb; 14 (2): 106 - 17. doi: 10.1038/nrmicro.2015.6. Epub 2015 Dec 21. PMID: 26685750; PMCID: PMC5019959.

[8] Wang Y, Fan X, Wang H, Kudinha T, Mei YN, Ni F, Pan YH, Gao LM, Xu H, Kong HS, Yang Q, Wang WP, Xi HY, Luo YP, Ye LY, Xiao M; China Hospital Invasive Fungal Surveillance Net (CHIF-NET) Study Group. Continual Decline in Azole Susceptibility Rates in Candida tropicalis Over a 9-Year Period in China. Front Microbiol. 2021 Jul 9; 12: 702839. doi: 10.3389/fmicb.2021.702839. PMID: 34305872; PMCID: PMC8299486.

[9] Revie NM, Iyer KR, Robbins N, Cowen LE. Antifungal drug resistance: evolution, mechanisms and impact. Curr Opin Microbiol. 2018 Oct; 45: 70 - 76. doi: 10.1016/j.mib.2018.02.005. Epub 2018 Mar 13. PMID: 29547801; PMCID: PMC6135714.

[10] Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal Drug Resistance: Molecular Mechanisms in Candida albicans and beyond. Chem Rev. 2021 Mar 24; 121 (6): 3390 - 3411. doi: 10.1021/acs.chemrev.0c00199. Epub 2020 May 22. PMID: 32441527; PMCID: PMC8519031.

[11] Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis. 2017 Dec; 17 (12): e383-e392. doi: 10.1016/S1473 - 3099 (17) 30316 - X. Epub 2017 Jul 31. PMID: 28774698.

[12] Abdin MZ, Ahmad MM, Javed S. Advances in molecular detection of Aspergillus: an update. Arch Microbiol. 2010 Jun; 192 (6): 409 - 25. doi: 10.1007/s00203-010-0563-y. Epub 2010 Apr 1. PMID: 20358179.

[13] Wang W, Luo B, Yuan X, Xu L, Gao J, Liu S, Den C, Tan Y, Su G, Chen Z, Zhang H, Zheng Z, Li G, Zhou X. Diagnosis of fungal keratitis caused by Nectria haematodocha through next-generation sequencing: review of literature and report of three cases. Int J Infect Dis. 2023 Jun; 131: 53 - 56. doi: 10.1016/j.ijid.2022.12.032. Epub 2023 Jan 6. PMID: 36621753.