

SPECTRUM OF FUNGAL ISOLATES IN DIFFERENT CLINICAL SAMPLES

Dibya Prasana Mohanty¹, Susanta Kumar Sahu², Uday Hembram³

¹Assistant Professor, Department of Microbiology, SCB Medical College, Cuttack.

²Professor, Department of Microbiology, SLN Medical College, Koraput, Odisha.

³Tutor, Department of Microbiology, VSS Institute of Medical Sciences and Research, Burla.

ABSTRACT

BACKGROUND

Fungal infections are on the rise due to changing practices in medical care; increase in the immunocompromised states, and due to increase in the use of antibiotics and steroid therapy. Currently, the awareness levels among the medical personnel regarding fungal diseases are lacking. More and more cases of fungal infections are being reported from cases of AIDS, malignancy, and transplant recipients, patients in ICU or on steroid therapy. Indiscriminate use of antibiotic therapy is another reason for the growing incidence of fungal infections.

MATERIALS AND METHODS

A retrospective study was undertaken to know the burden of fungal infection in different clinical samples. A total number of 3146 clinical samples from different in-patient departments processed in the microbiology laboratory of a tertiary care teaching hospital were analysed. The samples were processed for microscopy by KOH mount, Gram stain, negative stain, Lactophenol cotton blue stain and culture on SDA with Chloramphenicol, BHI broth (for blood), Dalmau culture on CMA, Slide culture and relevant biochemical tests depending on the type of sample and were identified by standard algorithm.

RESULTS

It was seen that most of the isolates belonged to yeasts comprising *C. albicans* (45%) followed by *C. tropicalis* (33.6%). The samples from the genital tract, oropharyngeal swabs, blood, urine, CSF and faeces showed no isolation of filamentous fungi. Moulds were mainly isolated from respiratory samples, skin and its appendages, aural swabs and deep wounds. Dermatophytes formed the major isolates from skin and its appendages.

CONCLUSION

A more detailed study is needed to know the socio-epidemiological prevalence of these infections and their burden in the community and in hospitalised patients.

KEYWORDS

Fungi, Yeast, Filamentous Fungi, Candida.

HOW TO CITE THIS ARTICLE: Mohanty DP, Sahu SK, Hembram U. Spectrum of fungal isolates in different clinical samples. *J. Evolution Med. Dent. Sci.* 2017;6(69):4906-4911, DOI: 10.14260/Jemds/2017/1064

BACKGROUND

The fungi belong to a group of microorganisms with characteristics different from other disease-causing organisms like bacteria and parasites. Among the 1.5 million fungal species described, only about 300 species are associated with human disease and only about a 100 of them are capable of causing infection in normal individuals.^{1,2} With changing practices in medical care, fungal infections have been on the rise. Availability of improved diagnostic methods also helps us to conclusively identify fungal infections, which were earlier not possible, and were attributed to other organisms. Fungi are implicated in various allergy, infectious diseases and toxic manifestations. The pattern of fungal diseases varies according to various geophysical and climatic condition of a particular area.

Financial or Other, Competing Interest: None.

Submission 24-07-2017, Peer Review 18-08-2017,

Acceptance 23-08-2017, Published 28-08-2017.

Corresponding Author:

Dr. Susanta Kumar Sahu,

Professor, Department of Microbiology,

SLN Medical College,

Koraput-764020, Odisha.

E-mail: drsusantavss@gmail.com

DOI: 10.14260/jemds/2017/1064



Although superficial fungal infections are very common in human population they are usually easy to diagnose, and are curable. It is the rather uncommon invasive infections which are not only life threatening, but also difficult to diagnose.³

Fungal infections of human beings originate mostly from exogenous sources and are acquired mainly by inhalation and traumatic implantation. Some infections particularly of *Candida* are mostly derived from endogenous sources. Last few decades have witnessed an alarming rise in the incidence of fungal infections particularly in cases of AIDS, malignancy, transplant recipients, patients in ICU, and patients on steroid therapy. Indiscriminate use of antibiotics has led to the suppression of the local microflora, which has helped in the growing incidence of fungal infections.^{4,5,6} Besides this, fungi pose a difficult situation from the clinicians' point of view because of non-availability of proper laboratory facilities, limited number of antifungals available, their side effects and the long duration of treatment needed to eradicate the infection. As most of these fungi are ubiquitous and free-living in the environment it is very difficult on the part of the laboratory to prove the cause effect relationship between the fungus and the disease they are attributed to. Although fungal infections contribute substantially to human morbidity and mortality, their burden on human health is not widely scientifically studied. We also lack in providing efficient

diagnostic facilities in most clinical laboratories and lag behind in research in this field.

Keeping this in view a retrospective analysis of data regarding the distribution of different types of fungi in various clinical samples processed in the mycology laboratory was undertaken in a tertiary care teaching hospital in Odisha.

MATERIALS AND METHODS

Type of Study

Retrospective, Observational.

Clinical specimens processed in the Microbiology Department for mycological tests during the period from January 2015 to June 2016 were analysed. No record or history of taking antifungal treatment by any patient was observed. The samples included respiratory tract samples, blood, urine, corneal scrapings, specimens from skin and its appendages (Nail and Hair), oropharyngeal swabs, faeces, aural swabs, pus & wound swabs, high vaginal swabs, cerebrospinal fluid (CSF) etc. Respiratory samples included sputum, bronchoalveolar lavage fluid (BAL), pleural fluid and endotracheal aspirates.

Samples were examined for microscopy and culture. Microscopic examination included 10% KOH mount, Gram stain, wet mount & Negative staining by Nigrosin depending upon the type of sample. The blood samples were cultured in Brain Heart Infusion (BHI) broth and incubated for 4 weeks. Subcultures from blood were made periodically onto Sabouraud Dextrose Agar (SDA) and incubated at room temperature for 2 weeks before they were discarded as negative. Centrifuged CSF deposits were cultured in SDA. The other samples were inoculated in two sets of SDA with chloramphenicol, one set incubated at 22-25°C and the other set at 37°C for 6 weeks before discarding them as negative.

Yeast like colonies isolated were stained with Gram stain to know the shape, budding, presence of pseudohyphae, capsule etc. Yeast like colonies from CSF samples & wound swabs and respiratory samples were also examined by negative staining to rule out *Cryptococcus*. The other yeast colonies were subjected to a panel of tests, namely morphological tests, by Germ tube test, Cut streak (Dalmau) culture, sugar fermentation, sugar assimilation test, urease test, surface growth etc. Standard algorithm was used for identification of these organisms. Dalmau culture plate was done by inoculating the yeast on Corn meal agar (CMA) by cut streak, and incubating the plate at 20°C for 4 weeks after covering the inoculated area by a sterile glass coverslip. This helps to see the ability of the yeasts to produce mycelium or pseudo-mycelium and enhances Chlamydo-spore production in *C. albicans*. It also helps production of Arthrospores by *Trichosporon*. The plate is observed periodically under an inverted microscope.^{7,8} *Rhodotorula* was identified by its typical salmon pink coloured colonies and absence of mycelium in Dalmau preparation.

Filamentous fungi were identified by gross morphology of the colony on the SDA medium and microscopic appearance of the teased-out growth in Lactophenol cotton blue mount and by slide culture method.^{7,8}

After the processing was over the clinical specimens and culture were discarded in discard jars containing chemical disinfectants (Cresol 5% usually); these were later on taken

by personnel from hospital biomedical waste management department for further treatment.

RESULTS

The results of the study are depicted in Table 1. We analysed 3146 clinical specimens collected during the period from January 2015 to June 2016. The overall proportion of fungal isolates is 15.1% comprising predominantly (12.9%) of yeasts, while filamentous fungi contributed a minor portion (2.2%). It was observed that maximum percentage of fungal isolates was obtained from aural swabs (46.4%), vaginal discharges (35.4%), corneal scrapings (32.1%), respiratory samples (28.5%), skin and its appendages (27.1%) and faeces samples (23.9%). Urine and blood which constituted more than half the samples showed isolation rates of 13.5% and 13.3% respectively. All of these isolates belonged to yeasts only. Least proportion of fungal isolation was seen in wound swabs & pus, CSF, and oropharyngeal swabs where the respective isolation rates were 5%, 9.1% and 10.6% respectively.

Filamentous fungi were the major isolates from corneal scrapings, aural swabs and skin scrapings with a proportion of 25%, 25% and 19.6% respectively, in comparison to yeast isolates where the comparative values were 7.1%, 21.4% and 7.5%. Yeasts were the only isolates from urine, blood vaginal discharge, oropharyngeal swabs and faeces, and formed a major portion from respiratory samples, wound swabs & pus. CSF showed isolation of 2 numbers of *Cryptococcus neoformans* from 22 samples.

The distribution of different yeasts and moulds isolated from the clinical samples are summarised in the Tables 2 & 3. *Candida* is the major isolate among the yeasts, with a minority contributed by others like *Trichosporon*, *Geotrichum*, *Rhodotorula* and *Blastoschizomyces* spp. Among the *Candida* spp., *C. albicans* contributed to 45%, whereas non-*albicans Candida* (NAC) were isolated in the remaining 55%. Most of the NAC belonged to *C. tropicalis*, *C. parapsilosis* and *C. krusei*. Among the filamentous fungi isolated, *Aspergillus* is the major isolate. Most of the isolates from corneal scrapings (25%) belonged to environmental moulds, namely *Fusarium* (2), *Aspergillus* (2), *Penicillium* (1), *Curvularia* (1). A rare isolate *Blastoschizomyces* (1) was also isolated from corneal scrapings.

Dermatophytes, namely members of the genus *Trichophyton*, *Epidermophyton* and *Microsporum* were the major isolates from skin and its appendages as shown in Table 4, whereas only 8 out of the total 29 isolates from skin and its appendages were *Candida* spp. [*Candida albicans* (3) and NAC (5)].

Clinical Sample	No. of Samples	Isolate		
		Yeasts	Mycelial Fungi	Total %
Urine	1224	163 (13.3)	0	13.3
Wound swabs & Pus	685	28 (4.1)	6 (0.9)	5.0
Blood	453	61 (13.5)	0	13.5
Respiratory samples	375	86 (22.9)	21 (5.6)	28.5
Skin scrapings	107	8 (7.5)	21 (19.6) *	27.1
Oropharyngeal scrapings	85	9 (10.6)	0	10.6
Vaginal Discharge	65	23 (35.4)	0	35.4

Aural swabs	56	12 (21.4)	14 (25)	46.4
Faeces	46	11 (23.9)	0	23.9
Corneal scrapings	28	2 (7.1)	7 (25)	32.1
CSF	22	2 (9.1) †	0	9.1
Total	3146	405 (12.9)	69 (2.2)	15.1

Table 1. Distribution of Yeast and Mycelial Fungi among Different Clinical Samples

† All the 2 isolates are Cryptococcus neoformans

C. albicans	182 (45.0)
C. tropicalis	136 (33.6)
C. parapsilosis	47 (11.6)
C. krusei	20 (4.9)
C. glabrata	4 (1.0)
other Candida spp.	2 (0.5)
Trichosporon	5 (1.2)
Geotrichum	3 (0.7)
Rhodotorula	2 (0.5)
B. capitatus	1 (0.2)
Cryptococcus	3 (0.7)
Total	405

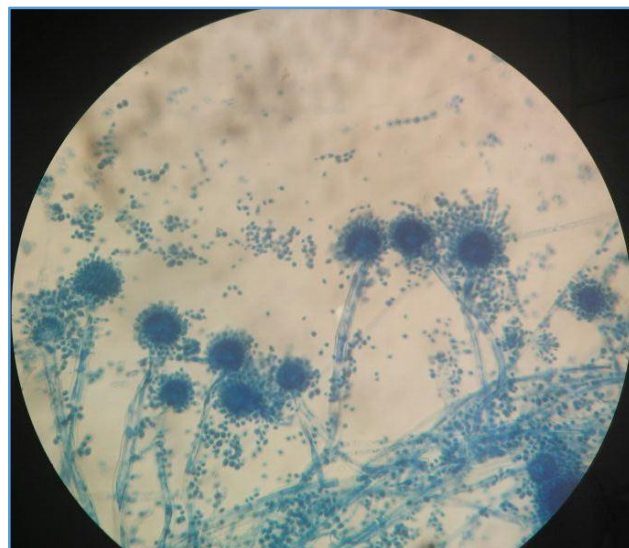
Table 2. Distribution of the Yeasts

A. fumigatus	15 (31.3)
A. flavus	9 (18.8)
A. niger	8 (16.7)
Penicillium	5 (10.4)
Rhizopus	3 (6.3)
Mucor	2 (4.2)
Curvularia	2 (4.2)
Fusarium	2 (4.2)
Exophiala	1 (2.1)
Others	1 (2.1)
Total	48

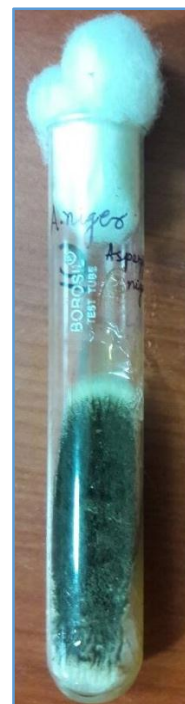
Table 3. Distribution of the Filamentous Fungi (Excluding the Dermatophytes)

Name of the Isolate	Skin (82)	Hair (11)	Nail (14)
Trichophyton rubrum	4	1	2
Trichophyton mentagrophyte	4	0	1
Other Trichophyton spp.	1	1	1
Microsporum spp.	2	1	0
Epidermophyton spp.	2	0	1
Malassezia furfur	1	0	0
Candida albicans	2	0	1
Non-albicans Candida	2	0	2
	20	3	6

Table 4. Distribution of the Fungal Isolates from Skin & Appendages



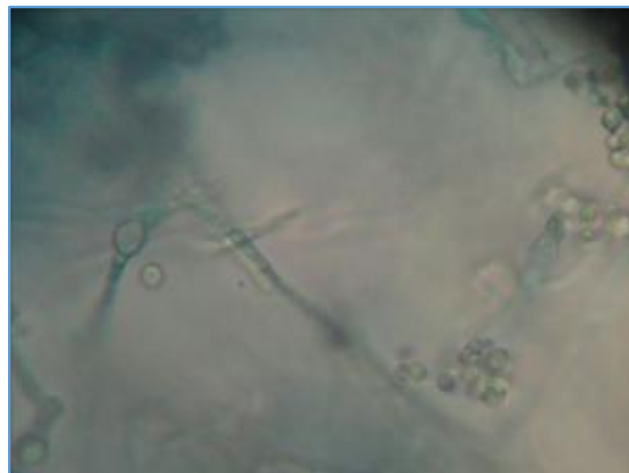
A. flavus-LPCB mount



A. niger-on SDA



A. flavus on SDA



C. albicans-Chlamydo-spore- in Dalmau Preparation



Candida -Pseudohyphae-Gram stain



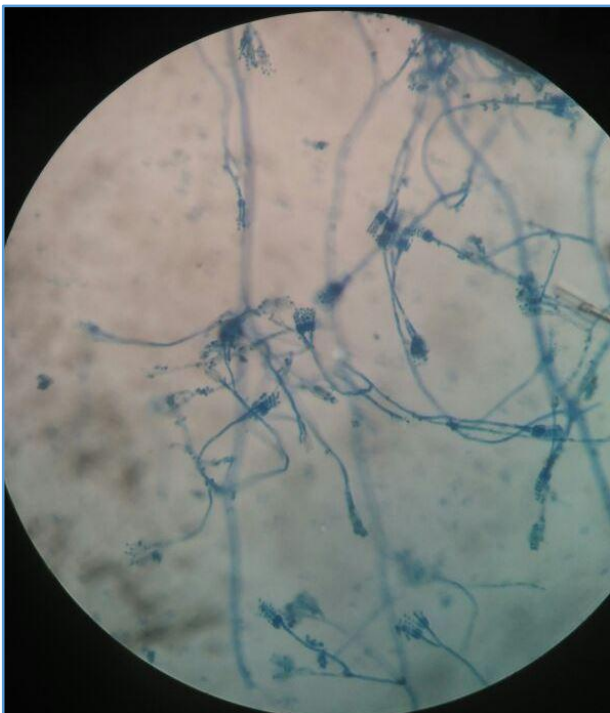
Rhizopus -on SDA



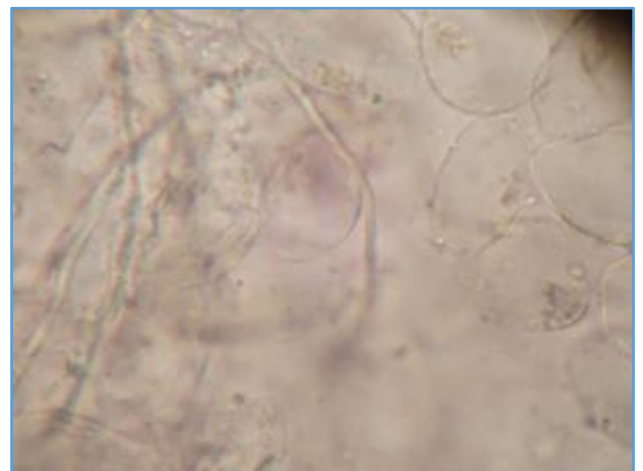
Penicillium spp.



Rhizopus-LPCB mount



Penicillium -LPCB Mount



Skin Scrapings-KOH mount

DISCUSSION

With increasing incidence of immunocompromised conditions due to AIDS, diabetes, malignancy, invasive medical procedures, transplantation, extended ICU stay, and increased life expectancy rates, the agents of mycotic infections yet to be known, and mostly environmental contaminants, have emerged as one of the important causes of infection. The use of indiscriminate use of antibiotics and steroids has further increased the diseases caused by fungi.

In the present study, we noted that the incidence of fungal isolates from clinical specimens was 15.1%, consisting predominantly of yeasts (12.9%), and in part, by filamentous fungi (2.2%), mainly *Aspergillus*. Similar finding is also described by other studies.^{3,4}

From the genital tract, oropharynx and faeces samples, *Candida* is the sole isolate as it is a member of the microbial flora of these regions. In these areas also, *C. albicans* is the major isolate followed by *C. tropicalis*. Similar observations were also made by Kaur et al.⁴

In our study, it was seen that *C. albicans* was the most common isolate from total samples followed by *C. tropicalis* among the yeasts which is supported by the findings of others.^{2,4,5} Globally, the incidence of *C. albicans* is in a declining trend and incidence of the non-*albicans* *Candida* are on the rise.⁶ In our study also we found that the NAC comprised 209 number of *Candida* whereas number of *C. albicans* was only 183.

Nosocomial candidaemia is one of the common morbid conditions in ICU and in neonates, it is more fatal. *C. albicans* is the most common cause of nosocomial blood stream infections worldwide. This is also evident from our study. In our study, it is seen that yeasts contributed 13.5% cases of blood stream infection. This might be due to the fact that the blood cultures tested in our department were mostly obtained from neonatal ICU cases, who were immunologically immature.⁹⁻¹¹

Among the urine cultures, 13.3% showed growth of Yeasts. *C. albicans* is the most common isolate among them followed by *C. tropicalis*, *C. parapsilosis* and *C. krusei* which agrees with the findings of other researchers.^{10,12}

In respiratory samples which comprised of sputum, BAL fluid, endotracheal aspirate and pleural fluid, the major isolate was yeasts belonging mostly to *Candida* spp. But a significant proportion are also contributed by filamentous fungi like *Aspergillus*, *Penicillium* and *Rhizopus* spp. It was observed that all the isolates from endotracheal aspirates are *Candida*. This finding corroborates with the observation of Kaur R et al and Dorko E et al.^{4,13}

The pus samples collected from superficial wounds, like burn wounds and bed sores, showed mainly the growth of *Candida*, whereas the sample collected from deep wounds or sinuses mostly showed filamentous fungi belonging to the dematiaceous group. These results are in agreement with the findings of Dowd SE et al.¹³ Samples from unopened abscess cavities and surgical sites showed no growth of fungi.¹⁴

From skin and its appendages dermatophytes were the major isolates consisting of *Trichophyton rubrum*, *T. mentagrophyte*, *Microsporum* and *Epidermophyton* spp. followed by the *Candida*. For obvious reasons of keratinophilic nature of the dermatophyte they predominate in keratinised tissues.¹⁵ Corneal scrapings showed

filamentous fungi (25%) belonging to *Aspergillus*, *Penicillium*, *Fusarium* spp. as major isolates, as also described by Thomas PA. Yeasts were only isolated in 7% of the corneal specimens.¹⁶

This study is only a preliminary observation to know the burden of the fungal infections in the hospital. More detailed multicentric prospective studies taking into account the demographic data, risk factors like immune status, history of endocrinopathy, malignancy, steroid and antibiotic therapy, occupation and socioeconomic condition of the patient should be undertaken to have a first-hand data of these diseases. These studies supported by more detailed laboratory investigations including serological tests and histopathological studies, molecular methods and antifungal sensitivity testing are needed for a clearer picture of the burden of these infections and to plan out remedial measures.

CONCLUSION

Though fungi were associated with both allergic and infectious diseases, it can be concluded from our study that a significant proportion of infections are contributed by fungus. *Candida* spp., particularly *C. albicans* was still the predominant fungal isolate in these cases in almost all samples except skin and its appendages and corneal scrapings where dermatophytic moulds and *Aspergillus* spp. were predominant.

Although the incidence of fungal infections is increasing with advances in the patient management techniques, the awareness among the medical fraternity in general regarding fungal pathogens is lagging behind. To counter this there is a need to carry out surveillance programmes to have an idea of the socio-epidemiological behaviour and the actual burden of these infections. The clinicians should consider including fungal pathogens in the differential diagnosis of many infectious and allergic diseases. Availability of improved diagnostic methods widely will make this more feasible and logical. More research to identify newer and safer antifungal agents and to develop vaccines against these also will go a long way in our fight against microbial infections.

REFERENCES

- [1] Richardson MD, Wamock DW. Fungal infections: diagnosis and management. 3rd edn. Massachusetts: Blackwell Publishing Ltd., 2003; p. 4.
- [2] Types of Fungal diseases. Atlanta: Center for Disease Control and Prevention. 2017. <https://www.cdc.gov/fungal/diseases/index.html#one>
- [3] Weinberger M, Sacks T, Sulkes J, et al. Increasing fungal isolation from clinical specimens: experience in a university hospital over a decade. *J Hosp Infect* 1997;35(3):185-95.
- [4] Kaur R, Dhakad MS, Goyal R, et al. Spectrum of opportunistic fungal infection in HIV/AIDS patients in tertiary care hospital in India. *Can J Infect Dis Med Microbiol* Article ID 2373424, 2016;2016:1-7.
- [5] Brown GD, Denning DW, Gow NAR, et al. Hidden killers: human fungal infections. *Sci Transl Med* 2012;4(165):165rv 13. (http://texasgrassfedbeef.com/sites/default/files/pdf/human_fungal_infections_killers_by_brown_scitransl_med_2012.pdf)

- [6] Guinea J. Global trends in the distribution of *Candida* species causing candidemia. *Clin Microbiol Infect* 2014;20(Suppl 6):5-10.
- [7] Milne LJR. Fungi. In: Collee JG, Frase AG, Marmion BP, eds. *Mackie & McCartney practical medical microbiology*. 14th edn. Edinburgh: Churchill Livingstone 1996:695-717.
- [8] Fothergill AW. Medically significant fungi. In: Mahon CR, Lehman DC, Manuselis G. eds. *Text book of diagnostic microbiology*. 5th edn. Missouri: Saunders/Elsevier Publishers 2015:589-622.
- [9] Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24, 179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004;39(3):309-17.
- [10] Pahwa N, Kumar R, Nirkhivale S, et al. Species distribution and drug susceptibility of *Candida* in clinical isolates from a tertiary care centre at Indore. *Indian J Med Microbiol* 2014;32(1):44-8.
- [11] Giri S, Kindo AJ. A review of *Candida* species causing blood stream infection. *Indian J Med Microbiol* 2012;30(3):270-8.
- [12] Sukumaran J, Sundaram JM, Sivan RR. Changing trend in the clinical distribution of *Candida* species in a tertiary care hospital. *J NTR Univ Health Sci* 2012;1(4):222-6.
- [13] Dorko E, Pilipcinec E, Tkasikova L. Fungal diseases of the respiratory tract. *Folia Microbiol* 2002;47(3):302-4.
- [14] Dowd SE, Hanson JD, Rees E, et al. Survey of fungi and yeast in polymicrobial infections in chronic wounds. *J Wound Care* 2010;20(1):40-7.
- [15] Sharma M, Sharma R. Profile of Dermatophytic and other fungal infections in Jaipur. *Ind J Microbiol* 2012;52(2):270-4.
- [16] Thomas PA. Fungal infections of the cornea. *Eye (Lond)* 2003;17(8):852-62.