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Isolation, Identification and Determination of Antifungal Sensitivity of Fungi Isolated from A Sample of Patients with Rhinosinusitis in Baghdad City

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Abstract

Background Rhinosinusitis is inflammation of the sinuses, most commonly caused by a viral or bacterial infection or by allergic (or non-allergic; non-allergic could be due to infection). Fungal infections of the sinuses are uncommon and usually occur in individuals who are immunocompromised. However, recently, the occurrence of fungal sinusitis has increased in the immunocompetent population.

Objective Isolation and identification of fungi in Rhinosinusitis with antifungal sensitivity..

Methods The study was carried out on 112 patients who were suffering from rhinosinusitis, and then identification was done for the isolated fungi associated with rhinosinusitis 1-Specimens (Swabs (from nose and sinuses) and Water nasal washing) collection. 2- Direct microscopic exam. 3-Fungal isolation and diagnosis. 4-Anti-Fungal sensitivity test. 5- Patients (112 patient) were diagnosed by specialist Doctors according to the clinical symptoms and CT scan findings and biopsy. attending Al-Yarmouk Teaching hospital, and Baghdad Medical City. From February to June 2015.

Results The results showed that 38 (33.9%) patients were suffering from fungal infection in this study. The most common fungi isolated were *Aspergillus spp*. 18 (47.3%), followed by *Rhizopus spp*. 7 (18.4%), *Trichophyton spp*. 5 (13.1%), *Penicillium spp*. 3 (7.8%), yeast *Candida spp*. 2 (5.2%), *Alternaria spp*. 1 (2.6%), *Cryptococcus neoformans* 1 (2.6%) and *Blastomyces dermatitidis* 1 (2.6%). The antifungal of the most common three fungi isolated, which were (*Aspergillus spp*., *Rhizopus spp*., and *Trichophyton spp*.,) was determined for Amphotericin B, Griseofulvin and Ketoconazole. The result showed that tested fungi were sensitive to Amphotericin B and resistant to Griseofulvin and Ketoconazole.

Conclusion Fungal rhinosinusitis was moderate infection. Aspergillus spp., Rhizopus spp. and Trichophyton spp. were the most commonly fungus iolated from rhinosinusitis. They were sensitive to Amphotericin B and resistant to grisofulvin and ketoconazole.

Keywords Fungal sinusitis, rhinosinusitis, sinusitis, fungal infection in the nose.

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List of abbreviation: CT = Computerized tomography, KOH = Potassium hydroxide, SDA = Sabouraud's Dextrose Agar

Introduction

The sinuses are hollow cavities in the bones around the nose. Sinusitis is one of the most common medical conditions. About 10 to 15 million people each year were suffering from sinusitis [(1)]. Fungal infections of the sinuses are uncommon and usually occur in individuals who are immunocompromised. However, recently, the occurrence of fungal sinusitis has increased in

the immunocompetent population [2]. Many of the causative fungi are opportunists and are not usually pathogenic unless they enter a compromised host. Opportunistic fungal infection is particularly likely to occur in patients with diabetes mellitus, acquired immune deficiency syndrome, tuberculosis, lymphoma, leukemia, or burns. Candidiasis, aspergillosis, mucormycosis, mycetoma, nocardiosis, and cryptococcosis are typical opportunistic infection [1,3].

There are four types of fungal sinus infections: chronic invasive fungal sinusitis, fungal ball, acute invasive fungal sinusitis and allergic fungal sinusitis. Symptoms of chronic invasive fungal sinusitis and acute invasive fungal sinusitis include nasal obstruction, sinus and facial pain and fever. They have tendency to for local invasion. Other symptoms can include runny nose, headache, excessive phlegm buildup and loss of appetite. Fungal ball infections always need to be treated by medication and surgical debridement [4]. The diagnosis is based on clinical suspicion, computerized tomography (CT) scan findings and biopsy [5].

Aspergillosis is the group of diseases, which caused by *Aspergillus*. The most common subtype among paranasal sinus infections associated with aspergillosis is *Aspergillus fumigatus* [1,6,7]. *Aspergillus* is a very a common indoor mold that can be found on every place [7,8]. Mucormycosis is an aggressive, opportunistic infection caused by fungi in the class of Zycomycetes. The genera most commonly responsible for mucormycosis usually are Mucor or Rhizopus [9,10].

The study aim was for isolation, identification and determination of the antifungal activity of fungi isolated from a sample of Iraqi patients with rhinosinusitis.

Methods

Culture Media

In this study Sabouraud's Dextrose Agar (SDA) (Himedia/ India) was used as fungal medium. This medium (with antibiotic procaine penicillin and streptomycin) was used for culture purposes [11].

Antifungal drugs

1) Amfoterisin B (50 mg) from Bristol-Myers Squibb - Turkey.
2) Griseofulvin (500 mg) from Shanghai Pharmaceutical CO., LTD - China.
3) Fluconazole capsules USP (150 mg) from Microlaslimited - India.

Patients

Patients characters

This study was carried out on 112 patients who are attended Al-Yarmouk Teaching Hospital and Baghdad Medical City who suffered from sinusitis were diagnosed by specialized doctors according to the clinical symptoms. Swabs were taken from patients from nose, sinuses. The other sample taken from the other group of patients was nasal wash in ear, nose and throat department.

Specimens collection

1- Swabs: Sterile, clean swabs were used for sample collection, swabs was covered, to prevent contamination of air pollution and retention of the sample without contamination until cultured within a few hours if necessary. Concerning nasal wash, Cotton swabs were wetted with 0.85% normal saline then used to wipe the nose several times, and then the swabs conducted for direct smear and culture purposes. The fungal infections were detected in laboratory when the samples were grown on (SDA) at 37 °C for 72 hr [12].

2- Water nasal washing: Sinus wash was put in sterile containers and then was centrifuged. The sedimentation was examined microscopically; 1 to 2 drops were taken from the sediment for culturing on (SDA) medium with antibiotics.

Direct microscopic examination

Samples have been examined microscopically by taking rubbing the swabs on clean slides then stained with lactophenol cotton blue then

cover slip was put on and examined [13]. Nasal wash was underwent direct examination, by taking a drop of the wash with lactophenol cotton blue the examined microscopically after putting cover slip.

Fungal isolation and diagnosis

The diagnosis of fungi was depend on the colony morphology and color on petri dishes from both sides, in addition to the shape of sporangium if found, hyphae and spores according to the following references [14,15].

Antifungal sensitivity test

In this study, 5 mg of anti-fungal (Amphotericin B, Griseofulvin and fluconazole) were dissolved in 5 ml D. W., then added as 100 µL, 200 µL, 300 µL respectively [16].

Results

Patients

This study was carried out on 112 patients who were suffering from sinusitis, the ages of patients ranged between (3-54) years with mean (28.5) years. In this study, the results revealed that 38 (33.9%) patients were with fungal growth. The fungal infection was recorded among females as 22 (57.8%) cases, which were higher than males 16 (42.1%). Identification of fungal infections in this study was based on direct microscopically examination KOH mount, lacto phenol blue smears, which were showed the fungal elements were in 9 (18.7%) of the total cases (this 9 samples give positive result when cultured on BDA medium) while fungal growth was positive in 48% of total cases. Concerning sex distribution it has been found that 61 (54.4%) positive females' cases and 51 (45.5%) positive cases belong to males.

Characteristics of patients with fungal sinusitis

Patients samples included 102 nasal swab, and 10 samples of nasal wash (Table 1). The ages of patients in this study ranged between 32-48 years (figure 1), the highest rate of fungal infection was between 36-40 years, followed by age group 41-45 years, the average age of patients with fungal infection (40.4). Fungal isolates were recorded among females 22 (57.8%) cases, which is higher than males 16 (42.1%) (figure 2).

The diagnosed isolates were distributed in these patients as follows:

1) Eighteen patients, (47.3%) were of the genus *Aspergillus spp*.

2) Seven patients, (18.4%) were isolated belonging to the genus *Rhizopus spp*.

3) Five patients, (13.1%) were belonging to the genus *Trichophyton spp*.

4) Three of the patients, (7.8%) were belonging to the genus *Penicillium spp*.

5) Two patients, (5.2%) were *Candida spp*.

6) One patient, (2.6%) was *Alternaria spp*.

7) One patient, (2.6%) was *Cryptococcus neoformans*.

8) One patient, (2.6%) was *Blastomyces dermatitidis*.

As for the genus *Aspergillus spp*. (Table 2). The highest proportion recorded in *A. fumigatus* (50%) followed by *A. niger* (27.7%).

Sensitivity testing of isolated fungi to anti-fungal agent

The sensitivity of the most frequently isolated fungi (three fungi: *Aspergillus fumigatus*, *Rhizopus spp.* and *Trichophyton spp.*) was tested for the following anti-fungi drug (Amphotericin B, Griseofulvin and Ketoconazole) by all concentrations 100 µL, 200 µL, 300 µL for each antifungal drug and compared them with control (control: fungus cultured on media without anti-fungal). *A. fumigatus, Rhizopus spp.* and *Trichophyton spp.* were resistance to Griseofulvin and Ketoconazole in three concentration (100 µL, 200 µL, 300 µL) and sensitive to Amphotericin B (figures 3, 4, 5 and 6).

Table 1. Positive sample and percentage

Parameter	Nasal swap	Water nasal washing	Grand total
The total number of samples	102	10	112
Positive samples	34	4	38
Percentage %	33.3	40	33.9

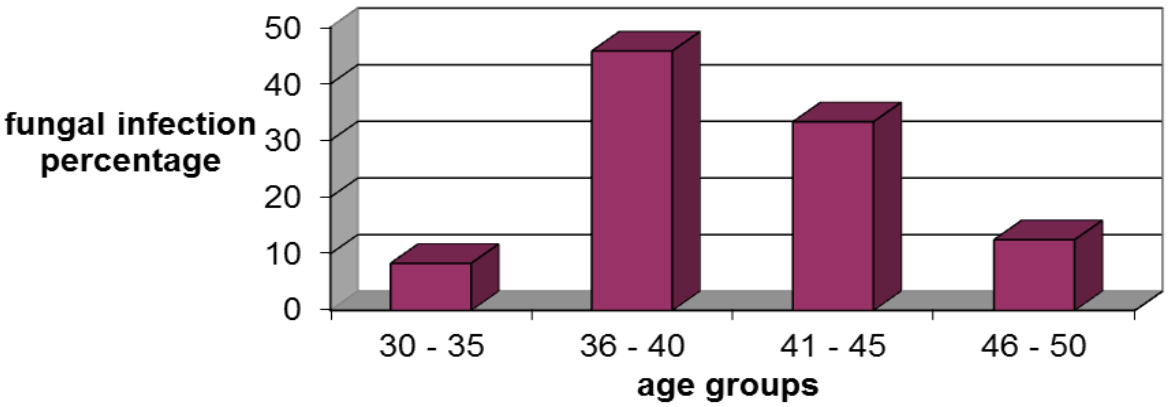


Figure 1. Percentage of age group of patients with fungal infection

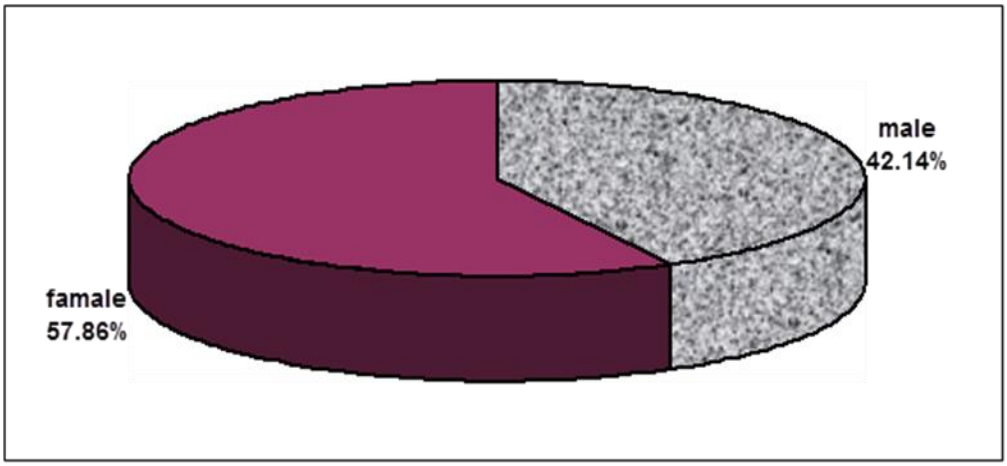
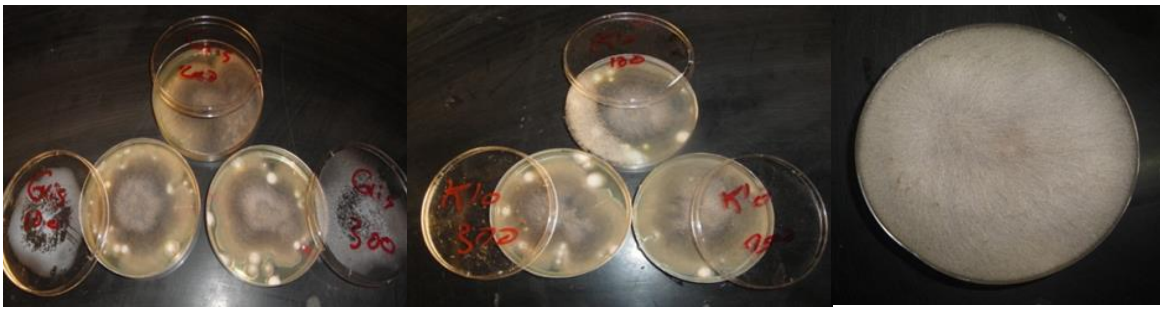


Figure 2. Percentage of sex patients with fungal infection

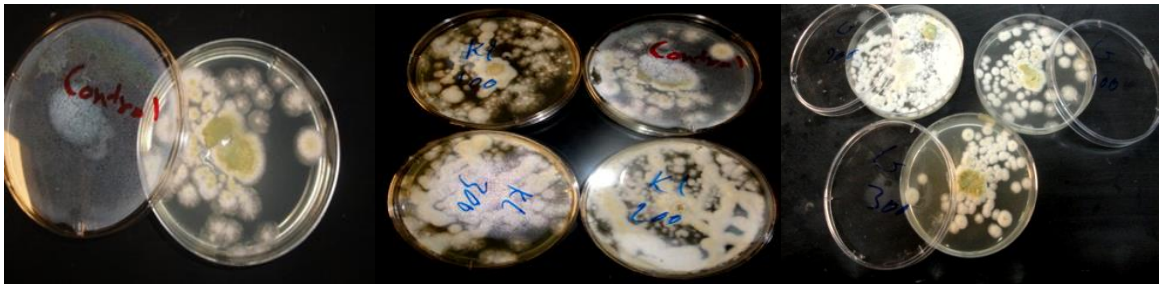
Table 2. The percentage of *Aspergillus spp*. was isolated in this study

Aspergillus spp.	Number of isolates	Percentage
A. fumigatus	9	50%
A. niger	5	27.8%
A. flavus	4	22.2%
Grand total	18	100%



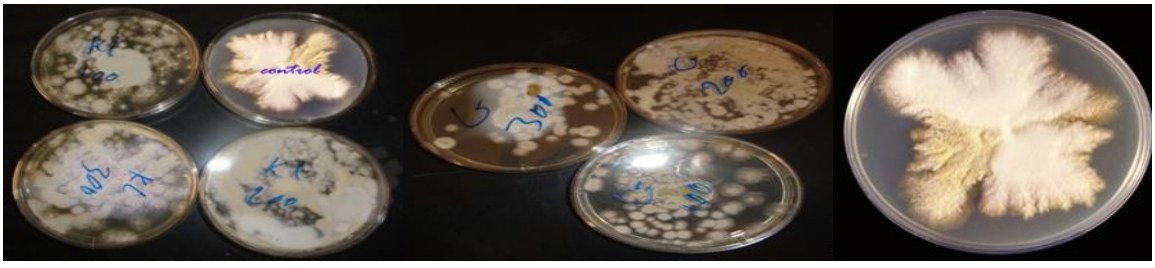
Griseofulvin | ketoconazole | Control

Figure 3. The resistant of *Rhizopus spp*. to Griseofulvin and Ketoconazole (100 µL, 200 µL, 300 µL)



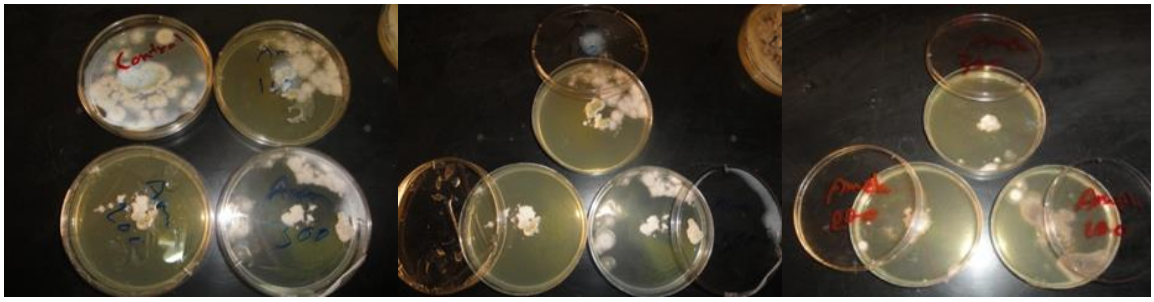
Griseofulvin | ketoconazole | Control

Figure 4. The resistant of *Aspergillus fumigatus* to Griseofulvin and Ketoconazole (100 µL, 200 µL, 300 µL)



Griseofulvin | ketoconazole | Control

Figure 5. The resistant of *Trichophyton spp*. to Griseofulvin and Ketoconazole (100 µL, 200 µL, 300 µL)



Trichophyton | *Rhizopus* | *A. fumigates*

Figure 6. The sensitivity of *A. fumigates*, *Rhizopus spp*. and *Trichophyton spp*. to Amphotericin B (100 µL, 200 µL, 300 µL)

Discussion

In this study, the ages of patients ranged between (3-54) years with mean (28.5) years. The wide variation in ages indicates clearly the different natural of inflammation of the rhinosinuses and possible infect all ages [17,18]. The isolated fungi were few (33.9%) compared with similar study in Tikirt/Iraq 60% in (2011) [19]; the results in a study in Turkey showed 63.67% [20] and in USA was 44% [21].

Fungal organisms are ubiquitous, and our exposure to these organisms occurs on a daily basis. A common location for these organisms to enter the human body is through the sinonasal cavity. Luckily, our immune system helps to prevent infection by these organisms. In those who do develop infection, a benign, noninvasive process usually occurs. However, in some patients, invasive disease does occur. As invasive fungal infections can lead to serious morbidity and mortality; it is important for the clinician to be able to recognize the difference between noninvasive and invasive fungal disease [22].

The study focuses on isolating the fungi from patients in sinus, because most cases of sinus infection are caused by bacteria. As such these types of sinus infection can be effectively treated with antibiotics. Yet fungal sinus infections may not respond to the types of antibiotics most commonly used for sinus infections [23].

Fungal infections are more common in a compromised host or people who already have some chronic disease, which make them all the more susceptible to infections. Many of the causative fungi are opportunists and are not usually pathogenic unless they enter a compromised host [1,2].

Results above were showed the fungal rhinosinusitis in male more than in female perhaps due to the vulnerability of women compared to men or to the short time of the study. Allergic fungal rhinosinusitis was more common in male patients and in patients aged 20-30 years [24]. This study showed *Aspergillus spp.* (47.3%), formed higher isolation of fungi, this result agrees with many studies such as [8,25], the most common subtype among paranasal sinus infections associated with aspergillosis is *Aspergillus fumigatus*. The researcher in Jordan (2001) indicated that *Pencillium spp. and Aspergillus spp*. was the first causative agent and most frequently in chronic sinusitis. While the results from in Tikrit *Pencillium spp*. give high frequently in sinusitis [19].

The antifungal conventional Amphotericin B (Fungizone) remains the standard therapy for many invasive or life-threatening mycoses, because Amphotericin B acts by binding to ergosterol in the cell membrane integrity and results in osmotic instability [26].

The conclusions of this study were: (1) Fungal rhinosinusitis was moderate infection. (2) The most fungal isolated was *Aspergillus spp*., *Rhizopus spp*., *Trichophyton spp*. (3) The anti-fungal Amfoterisin B was stronger agent

against *Aspergillus spp.*, *Rhizopus spp.*, *Trichophyton spp*.

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Conflict of interest

None.

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