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ORIGINAL ARTICLE

Antibiotic Sensitivity of Fungi Isolated from Patients with Sinusitis

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ABSTRACT

Objectives: The present study aimed at identifying the fungi that accompany sinusitis patients and the possibility of controlling them using antifungal agents.

Methods: Seventy eight swabs were taken from patients with sinusitis to the Diwaniyah Teaching Hospital for the period from November 2012 to April 2013 for the purpose of isolating and diagnosing the fungi associated with sinusitis and testing their susceptibility to the antifungal agents In a disc method and minimum inhibitory concentration.

Results: The fungus *Aspergillus* sp was the first fungus in terms of the number of isolates and the percentage of its frequency in sinus patients, as the isolates was 14 isolates, representing 31.8%, *penicillium* spp in the second ,the percentage was 18.18% from 8 isolates of the total number of fungi, the fungus *Cladosporium* sp and *B.dermatitidis* came third in terms of the number of isolates and the percentage of the emergence where they appeared 11.36% form five isolates of the total isolates, while *Ttichophyton* sp and *Alternaria* sp came fourth and were followed by *Ulocladium* sp, *Rhizopus* sp, *Mucor* sp, *Trouulpsis* sp, *Bipolaris* sp, *Rhodotorula* sp, *Pacilomyces* sp and *Geotrichum* sp.

Antibiotic sensitivity test by disc method showed a significant difference in inhibiting of growth of *Penicillium* sp by the antifungal ketoconazole with an inhibitory capacity of 36 mm. The antifungal Econazole 30 mm and nystatin 28 mm. *Aspergillus* sp, Econazole and Nystatin were the most effective in inhibiting of its growth. The same applies to the rest of the isolated fungi. Econazole and Nystatin are superior to the rest of the antifungal agents.

Conclusion: The sinuses are a suitable place for the growth of many opportunistic fungus for nutrients, heat and darkness suitable for the growth of these fungus, especially *Aspergillus* sp and *penicillium* sp. Ketoconazole is effective in inhibiting the growth of these fungi and clotrimazole is second grade.

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INTRODUCTION

Sinus is an air chamber filled with air inside the bones of the skull connected to the cavity of the nose with narrow openings lining a membrane called the mucous membrane produces mucus that acts as antimicrobial defense against bacteria, viruses and fungi. These secretions contain proteins and fats that perform the function of defense of the airways and sinuses function Arrange and warm the air inside the nose and change its temperature to suit the temperature of the body before the air reaches the lungs by the mucous membrane that lining of its surface and reduce the weight of the head and absorption of shocks and the simple and medium to the head and face and the composition of sound tone ¹.

Sinusitis is a common disease that needs to be accurately described for antibiotics to be cured. National health studies have reported that about 17% of all states in the United States have been diagnosed with this disease. In Canada, it is 5% of adults and in Europe, 1-5% of adults with acute sinusitis are diagnosed ². Sinusitis is defined as inflammation of the mucous layer of the sinus lining, since the lining of the nasal mucosa is connected to the mucous lining of the sinuses, so the nasal discharge leads to sinusitis and when abscess can be moved to other areas of the eye and brain. Microorganisms play an important role in sinusitis. The infection is not initial but occurs after secondary infection of the upper respiratory tract ³. In most cases, the colds caused by the virus and complete with the bacterial infection, doctors cannot distinguish between viral and bacterial infection through examination During the duration of the symptoms, approximately 80% of the upper respiratory tract infections cause nasal and sinus infections and more than 100,000 cases of nasal and sinusitis occur in the United States alone ⁴. The fungal pathogens of humans are big problem when they reach to the Sinuses as they can penetrate the inner lining in many cases of diseases, although they are few compared with other microorganism, but it represents a real danger for people who are immunocompromised and the diagnosis of these infections is difficult and needs to effort Highly skilled and experienced, and the fungal infections accounting for 90% of patients with sinusitis in nasal drainage and about 10% in patients undergoing nasal and sinus surgeries⁵. In Europe, the proportion of chronic sinusitis patients undergoing surgery About 75% ⁶.

Most of the cases of allergic fungal sinusitis in the United States, especially the South and Southwest, linked with melanin-producing fungi such as *Bipolaris* sp, *Alternaria* sp, and *Curvularia* sp, and may be heterogeneous due to differences in geographic distribution and the use of different diagnostic techniques. The indiscriminate use of antibiotics and Frequent uses create suitable environmental conditions for the growth of bacteria and resistance to antibiotics. For example, *Aspergillus* does not usually cause an invasion of the central nervous system, but its resistance to antibiotics caused a large number of deaths as a result of its invasion of the nervous system, as well as some

species of fungus *Pacilomyces* sp resistant for many of drugs and antifungal agents ⁷.

People suffering from immunodeficiency disorder are exposed to dangerous levels of infection with fungal threats to their life because they are missing cellular defense mechanism, especially the species belonging to the genus *Aspergillus* as the rate of infection is estimated at 6%, while the mortality rate is 55 - 92% after organ transplant and order Mucorales caused about 10% of the fungal infections of patients suffering from diseases of the blood and usually the advanced infection caused the increase in mortality rates ⁸.

Aim of study: due to the importance of the subject and the lack of studies on fungus causing sinusitis, this study aimed of investigating of fungi associated with sinusitis and the possibility of treatment using antifungal agents.

MATERIALS AND METHODS

Collection of samples: A total of 97 clinical patients were recruited from Diwanayah Educational Hospital in the Division of Ear, Nose and Throat in the city of Diwanayah. Those infected patients were within the age groups of 11 to 76 years and for both sexes for the period between 1-5-2012 and 1-5-2013 with taking into account that patient do not use the antibiotics. A form has been prepared for each patient, in which some important informations are proven, such as age, sex, nature of work, housing, and chronic diseases. The conference was done with the help of the specialist doctor. After the clinical examination, the samples were collected using cotton swabs. Inside the nose of the patient under the supervision of the doctor, then transferred to the laboratory of Microbiology for the purpose of culture and diagnosis and the media of Sabouraud Dextrose Agar and blood agar have been used in growing and isolate of fungi after its incubated in temperature of 37 ° C for a period of 4 to 21 days ⁹.

Isolation: The fungal isolates were isolated by direct isolation and using the medium of Sabouraud Dextrose Agar by solving it in the water, sterilized and cold to 45 centigrade. The antibiotic chloramphenicol was added 0.25 mg / ml to prevent the growth of bacteria and then spread in the petri dishes and left to cool down and harden. The temperature of 37 degrees Celsius for 4 - 21 days has been repeated the same steps above but using of blood agar ¹⁰. After the emergence of the growths were purified by re culture it on the media that mentioned to get pure colonies.

Diagnosis: The developing colonies were diagnosed by the phenotypic characteristics of the colonies, which included color, odor, growth speed, height, tissue and pigment production, as well as microscopic characteristics such as the shape of hyphae, spores and fruit body of fungi using 15% potassium hydroxide solution and blue cotton dye ¹¹.

Pharmacological sensitivity test for antifungal agents: Six types of antifungal agents have been used to treat the isolates that isolated for the purpose of pharmacological sensitivity test. These are Econazole,

Clotrimazole, Nystatin, Ketoconazole Amphotericine B and Itraconazole and have been followed the method described in ¹² as follows:

Preparation of fungal suspension: The fungal strain was obtained by taking the fungal growth from a colony surface at the age of 5 to 7 days by means of a ring conveyor placed in test tubes containing 5% of the sterilized physiological solution Mix thoroughly using the carburetor and Prepared a concentration of 10^5 spore per ml of fungal suspension, and the number was determined using the red blood cell count ¹³.

Disc infusion method: prepared standard discs of the antibiotics mentioned above used then follow the steps below :

Prepare media of Emons and Sabouraud agar that incubate for 24 hours at a temperature of 37 degrees to ensure that there is no contamination, put 0.2 ml of fungal suspension containing 10^5 spores per ml using a micro pipette and spread on the surface of the medium prepared in dishes previously using glass rod L letter . Put each disc and according to the concentration in a dish with sterile forceps and then press the disc carefully to fix it and then incubated dishes at 37 C ° for 24 to 48 hours then inhibitory area was measured by the ruler ¹⁴.

Measuring the minimum inhibitory concentration: A series of concentrations were prepared according to the method described in ⁴ and the concentrations are (1000, 500, 250 µg / ml, 0.24 µg / ml), which were distributed in test tubes and given serial numbers, 5 µl of the fungal suspension of each fungus Test tubes containing different concentrations of fungal antibiotics and then incubated all tubes in the temperature of 35 to 37 C ° for 24 to 48 hours then the results were recorded and the concentration was considered to prevent the growth of fungi is the minimum inhibitory concentration ¹⁵.

RESULTS

Percentage of appearance of fungal isolates

Table 1 shows that the fungus *Aspergillus* spp is the first fungus in terms of the number of isolates and the percentage of its frequency in sinus patients. The number of isolates was 14 isolates, which constituted 31.8%. The reason for this increase because it is opportunistic saprophytic fungus in which characterized by rapid spread present In water, air, soil, and decomposing materials, and it is capable of produce many branches of hyphae, which are composed of small reproductive units in large quantities transmitted through the air, which facilitates their access to the lungs and sinuses through inhalation, which is the main source of infection. this fungus named a sprinkler and it is form Large numbers of spores and spread in the weather. This fungus is known for its strong resistance to antibiotics, drought, lack of food and difficult conditions. It can produce toxins, especially aflatoxins, which cause fibrosis, liver damage, and cause chronic or severe diseases. The penetration of tissue is facilitate by the deficiency of immunity, especially in people with chronic diseases or who are taking immunosuppressive drugs ¹⁶.

Penicillium spp was the second highest in terms of number of isolates, and the percentage of the number of isolates was 18.18% from The total number of fungi. This fungus is as follows *Aspergillus* spp in terms of resistance in the high conditions of hunger and drought and its high potential to reproduce and production of conidia and can grow in all environments and uses food materials, as a source of carbon and nitrogen, and this explains his presence on paper books and wood exists and clothing and others.

It appears from the same table above for both *Cladosporium* sp and *B.dermatidis* were the third in terms of the number of isolates and the percentage of the emergence 11.36% of five isolates appeared of the total isolates, the fungus *B.dermatidis* is dimorphic fungi and It is found at 25 °C as a filamentous form or as a yeast body at 37 °C. This fungus attacks the skin and lungs and can reach the nasal sinuses by inhalation. This is common in people who breed dogs indoors and can be transmitted by cows, bats and chickens and cats ¹⁷.

Cladosporium sp is known as a widespread opportunistic fungus that causes asthma and allergies when inhaling of its spores in the air and is one of the causes of sinus diseases ¹¹.

The fungus *Trichophyton* sp and *Alternaria* sp came in fourth place because of the first fungus is from dermatophytes fungi that may reach through the nose to the sinuses. The second is an opportunistic fungus that can attack the sinuses in immunocompromised patients, especially people with asthma, diabetes and other diseases, this is the case with other isolated fungi: *Ulocladium* sp, *Rhizopus* sp, *Mucor* sp, *Trouloopsis* sp, *Bipolaris* sp, *Rhodotorula* sp, *Pacilomyces* sp and *Geotrichum* sp as they are opportunistic yeast and molds growing in the sinuses with moisture, food, and temperature to cause Health problems in this area ¹⁸.

Table 1: Number and percentage of the appearance of fungal isolates of patients with sinusitis.

Fungal species	No of isolates	%
<i>Aspergillus</i> spp	14	31.8
<i>Penicillium</i> sp	8	18.18
<i>Blastomyces dermatidis</i>	5	11.36
<i>Cladosporium</i> sp	5	11.36
<i>Alternaria</i> sp	2	4.54
<i>Trichophyton</i> sp	2	4.54
<i>Trouloopsis</i> sp	1	2.27
<i>Mucor</i> sp	1	2.27
<i>Rhizopus</i> sp	1	2.27
<i>Ulocladium</i> sp	1	2.27
<i>Geotrichum</i> sp	1	2.27
<i>Bipolaris</i> sp	1	2.27
Total	44	100%

Antifungal sensitivity of fungi with discs infusion method

Table 2 shows a clear contrast in the effect of antifungal agents on isolated fungi of sinusitis patients. The ketoconazole showed a significant effect in inhibiting the growth of *Penicillium* sp and an inhibitory capacity of it is 36 mm, which is known to affect the cell

membrane of the innate cell and inhibit enzymes during the process of forming the cell membrane, the second antifungal was Econazole 30 mm and nystatin 28 mm due to their interaction with the cell membrane structure of the fungi and the creation of gaps in the membrane, making it completely permeable to the different materials, thus losing its ability to be semipermeable causing cell death.

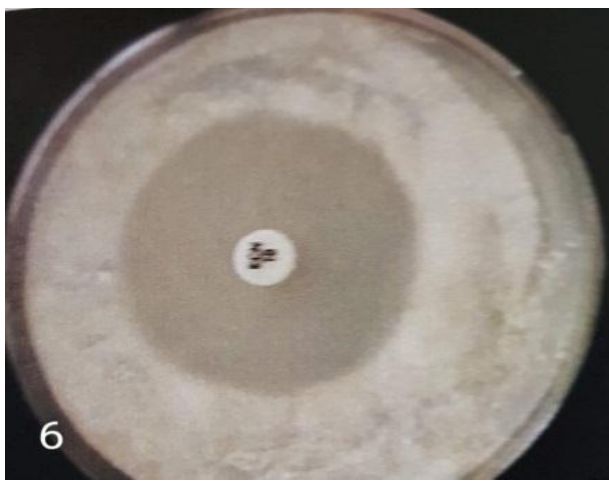
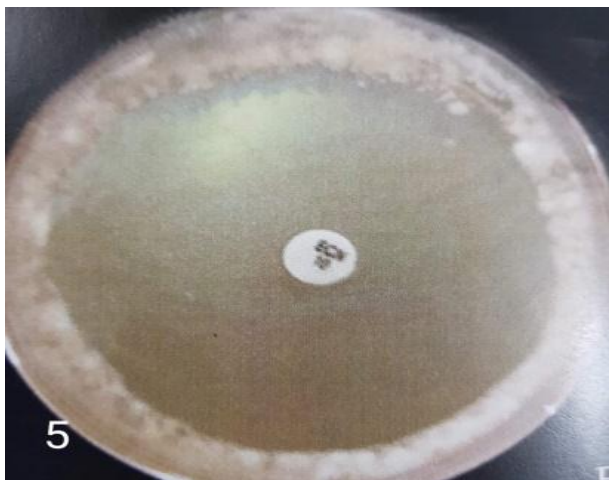
For *Aspergillus* sp, the two antifungals econazole and nystatin were the most efficient in inhibiting its growth due to their efficiency due to the above mentioned reasons, and the same applies to the rest of the isolated fungi, as the superiority of the econazole and nystatin on the rest of the antifungal agents.

Table 2: Sensitivity of fungi isolated from sinus patients towards antifungal agents in terms of inhibition zones measured in millimeters.

Fungi	Antifungal agents					
	Itraconazole*	Ketoconazole	Econazole	Clotrimazole	Nystatin	Amph.B
<i>Aspergillus</i> spp	16	16	33	10	32	10
<i>Penicillium</i> sp	12	18	20	20	15	10
<i>Blastomyces dermatidis</i>	16	25	30	12	20	8
<i>Cladosporium</i> sp	13	22	40	0	25	12
<i>Alternaria</i> sp	15	20	35	10	33	26
<i>Trichophyton</i> sp	26	26	39	21	26	18
<i>Trouloopsis</i> sp	21	28	27	25	30	17
<i>Mucor</i> sp	14	16	31	9	26	22
<i>Rhizopus</i> sp	12	11	20	7	26	20
<i>Ulocladium</i> sp	14	15	31	9	27	22
<i>Geotrichum</i> sp	18	23	19	12	22	18
<i>Bipolaris</i> sp	25	32	44	23	27	21

* Results were compared to Standard Tables (NCCLS)





Figures (1-6): Areas of inhibition of fungicides towards a number of isolated fungi.

- 1-Itraconazole * *Aspergillus* sp 2- Itraconazole * *Rhizopus* sp
 3- ketoconazole * *Aspergillus* sp 4- Itraconazole * *Peicillium* sp
 5- Ketoconazole * *Bipolaris* sp 6- Econazole vs *Penicillium* sp

The minimum inhibitory concentration

Table 3 shows the minimum inhibitory concentrations of antifungals agents against isolated fungi in this study. It is clear that the antifungal Econazole was the most effective in this field, and the minimum inhibitory concentrations ranged between 1.1-1.2 $\mu\text{g/ml}$ followed by Nystatin with minimum inhibitory concentrations ranged (10 - 4) $\mu\text{g/ml}$, while all isolates exhibited a clear resistance to Clotrimazole. The effect of the Clotrimazole and Nystatin is by attacking the plasma membrane of the fungal cell during the process of building it, both antifungal agents prevent synthesis of ergosterol, which is essential in the membrane, makes it deformed and contains holes causing the death of the cell¹⁹. The cause of antifungal resistance to antimicrobial Nystatin may be due to possessing appropriate resistance mechanisms Such as a solid cellular wall or its formation of specialized enzymes by destroying this antifungal agent²⁰.

Table 3: Minimum inhibitory concentration (MIC) for antifungals agents used against isolated fungi.

Fungi	Minimum inhibitory concentration ($\mu\text{g/ml}$)		
	Econazole	Nystatin	Clotrimazole
<i>Aspergillus niger</i>	*1.1	125	5.5
<i>Aspergillus</i> spp	1.1	125	8
<i>Penicillium</i> sp	1.2	100	7
<i>Blastomyces dermatitidis</i>	1.1	100	8
<i>Cladosporium</i> sp	1.2	100	7
<i>Alternaria</i> sp	1.1	100	10
<i>Trichophyton</i> sp	1.2	125	4
<i>Troulopsis</i> sp	1.1	100	8
<i>Mucor</i> sp	1.2	125	8
<i>Rhizopus</i> sp	1.1	100	8.5
<i>Ulocladium</i> sp	1.2	125	6
<i>Geotrichum</i> sp	1.1	100	7
<i>Bipolaris</i> sp	1.1	100	9

*Results were compared to Standard Tables (NCCLS)

Discussion

Aspergillus sp is characterized by its high ability to survive and spread. It can grow in drought conditions and lack of food resources, it is resistant to antifungal agents, and it can produce multiplication units in large numbers, which can spread these small units of reproduction and are airborne and fall to an appropriate environment. Fast-growing fungal mycelium, This applies to fungus *Penicillium* sp, which is known for its antibiotic production and thin hyphae. These two fungi have been recorded for many of them, including respiratory diseases, especially sinuses²¹.

Opportunistic fungi such as *Fusarium*, *Rhizopus* and *Cladosporium* are saprobe fungi of the original, but may become parasitic when appropriate conditions are available. When they have a suitable host, they can cause various diseases such as dermatitis, nose and eye diseases, respiratory tract, lungs and urinary tract. For many of these fungi because they contain moisture, food, temperature and pH suitable for growth and reproduction, and the conditions of disease caused by the simple, which can be eliminated by treatment to complex cases that Needs health care and surgical intervention that may cause death²².

The effect of econazole is caused by its effect on the cell membrane, especially on the compound called Ergosterole, which causes malfunction in the construction of the cell membrane and becomes perforated and permeable. There are reports indicating its effect at the level of RNA and building protein and may affect the metabolism of fat in the cell²³.

The clotrimazole inhibits pathogenic fungi by inhibiting the cytochrome P450 oxidase in the cell membrane, which causes problems in membrane permeability and thus the death of the fungus cell²⁴.

As in the rest of the fungus belonging to the polynalic group, nystatin is associated with egesterol, the main

component of the cytoplasmic membrane in breakfast, and, when available at appropriate concentrations, causes holes in the membrane, leading to extracellular potassium leakage and thus cell death²⁵.

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