

## The Fungi Flora of Healthy Nasal Mucosa in Kerman, Iran

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### Abstract

#### Introduction:

Environmental fungi, molds and yeasts, can infest the nasal cavity through inhaled air. There is some evidence that they could be the main cause of Chronic Rhinosinusitis (CRS) but little is known about the normal fungal flora in the human nose.

The objective of this study was to assess the normal fungal flora of the nasal mucus in adults in Kerman.

#### Materials and Methods:

We conducted a cross sectional study. Nasal swabs were used to sample the nasal cavity of 100 adults, 46 men and 54 women between 17 and 60 years old, currently living in Kerman, Iran.

#### Results:

Among 100 healthy people, one or more types of fungi were detected in 31 (31%) persons; *Candida* in 12 persons, *Aspergillus* in 8 persons, *Streptomyces* in 8 persons, and *Penicillium*, *Nocardia* and *Mucor* in a few persons. In only 4 persons, more than one type of fungi was detected. There was no significant relation between age, sex, education or smoking with the presence of fungi.

#### Conclusion:

Fungi have been considered one of the causative agents of CRS and differences in climatic conditions can influence the fungi flora.

#### Keywords:

Fungi, Flora, Nasal

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### **Introduction**

The inflammation of paranasal sinuses, often known as rhinosinusitis, is one of the most common and very costly diseases. In the United States, over 14% of the population, i.e. every seventh person is inflicted with an acute or chronic type of the disease (1). Even though most of its cases are acute, rhinosinusitis is one of the most common chronic diseases in the world (2). The annual direct cost of the disease in the United States is \$4.3 billion, and in 2001, one out of each five antibiotic prescription was for the treatment of rhinosinusitis (2).

The cost of leave from work due to this disease is also high. In 1992, in the U.S., there were 73 million leave from work days due to rhinosinusitis (1).

Rhinosinusitis can be classified based on clinical presentation, the type of the affected sinus cavity, the responsible organisms (viral, bacterial and fungal) etc. (3).

Various predisposing and causative factors have been identified in chronic rhinosinusitis, including local factors, systemic factors, immune-deficiency-related factors, environmental factors, and infectious factors such as bacteria and fungi (3).

The causative agent of chronic rhinosinusitis can be an allergy (e.g. to fungi) or an inflammatory reaction to the presence of bacteria and/or fungi. Fungi have recently summoned a lot of attention as the causative agents of this condition. Different types of fungi have adjusted to various climatic conditions, and their spores can hence be found everywhere in the world.

Differences in climatic and weather conditions (the AMSL height, sunlight, humidity, type of the soil, and the presence or absence of fresh, salty, current, still, ground, and or surface water, etc.) can influence the fungal flora of a region directly, or indirectly by influencing the flora and fauna of the region. For instance, *Histoplasma* is common in such areas as Missouri and Mississippi, in the U.S.,

where there is an abundance of bat and bird droppings. Nonetheless, rhinosporidiosis is usually found in Asian and African regions with contaminated water (4). Yet *Paracoccidioidomycosis* is found in the hot and humid regions of Latin America (5), and the *Aspergillus* found in chronic pulmonary conditions and in paranasal sinuses is especially common in Sudan and Saudi Arabia (5).

Fungi have certain microscopic forms, and their spores, through the inhaled air or contaminated water and food, make their way into the human body, particularly via the oral and nasal cavities. Were it not for the immune system in the mucosal secretions and the blood-rich mucus of the nasal cavity, the warm and damp cavity would have provided an ideal medium for the growth and reproduction of fungi.

However, before conclusively identifying fungi as the major causative agent of all or some (for instance allergic) types of rhinosinusitis, we should have a good knowledge of the fungal flora of the nasal cavity of a healthy individual. No doubt, to diagnose any pathologic condition, we should first know and define the "normal" state as the first step.

Therefore, to systematically examine the role of fungi in chronic rhinosinusitis, we should first identify the normal fungal flora of a healthy individual, an undertaking which has been performed inadequately, and which, because of the various differences in this flora caused by climatic differences, should be repeated in different parts of the world.

The aim of this study was to identify the fungal flora of the nasal mucus of healthy individuals in Kerman, Iran. To the best of our knowledge, this is the first study of this type conducted in the country.

### **Materials and Methods**

This was a cross-sectional study. A group of 100 patients (54 females, 46 males) visiting the clinic of Shafa Hospital, Kerman, were entered into the study after

the primary selection based on case histories and self-report questionnaires.

The selected individuals ranged between 17 and 60 in age, and had been residing in Kerman for at least one month prior to the commencement of the study. For each individual, a questionnaire was completed by one of the administrators, in which, in addition to personal information of the subject (e.g. name, age and address), information was recorded on the symptoms, habits, medications and medical conditions of the subject.

Those who had reported chief complaints of upper respiratory tracts or of active acute rhinosinusitis were eliminated from the study. Those eliminated also included subjects who had nasal obstruction, pathological nasal discharge, previous allergic episodes, any nasal medication in the last month leading to the study, hospitalization and surgery in the last month leading to the study, histories of rheumatological conditions, local or systemic use of antibiotics in the last month leading to the study, histories of autoimmune conditions, malignancies, radiotherapy, chemotherapy, immunosuppressive agents, and cleft palate.

Those deemed qualified for the study at the case history stage underwent physical examinations. Thorough oral and nasal, and if necessary endoscopic, examinations were performed by otorhinolaryngology residents or specialists. The physical examination of the nose was meant to ensure that the nasal mucus was healthy and that there were no signs of disease, such as pathological nasal discharge, inflammation of the nasal mucus membranes, nasal polyps, internal/external nasal anomalies, pathological discoloration of the nasal mucus, or abnormal dryness of the nasal mucus. The physical examination of the mouth was to ensure that there were no signs of infection or oropharyngeal inflammation, posterior pharyngeal discharge or cleft palate.

Sampling was performed on qualified subjects with sterile swabs only from the mucosal secretions. In order to prevent the swab from being contaminated while passing through the nasal vestibule, a sterile disposable plastic tube was inserted in this part of the nose, and the swab was passed through this tube and was robbed against the anterior nasal mucus and that of the inferior nasal concha. The moistened swab was then placed in a test tube containing saline solution; the tube was sealed, kept in a cool and dry condition and was transferred to a mycology lab as soon as possible. Sampling was performed bilaterally on every subject.

At the lab, three separate slides were made for each sample, one slide for direct examination, and two for staining. The former was diluted with a 10% potash solution, and the latter two were stained with Gram and Giemsa. The slides were examined with an Olympus CX21 microscope.

The samples taken to the lab were also cultured. Three media were employed for this purpose: Sabouraud dextrose agar, Mueller-Hinton agar, and fluid Thioglycollate. The tube containing Thioglycollate, used for anaerobic cultures, was placed in an anaerobic glass container. The culture plates were placed in an incubator, and were examined after the required time (two weeks maximum). The results were recorded on the questionnaires relating to each subject and were used for statistical analyses.

### **Results**

This cross-sectional study, which included 100 healthy individuals from Kerman, Iran, took six months. These individuals had no nasopharyngeal conditions and were, in this regard, in perfect health. The samples were examined microscopically, and through aerobic, anaerobic and fungal cultures. The demographics of the subjects are presented in table 1.

**Table 1:** Demographic characteristics of study participants

		NO
<b>Gender</b>	Female	54
	Male	46
<b>Education</b>	Illiterate and primary school	8
	Guidance school	14
	Diploma	53
	University	25
<b>Smoking histories</b>	Positive	11
	Negative	89
<b>Total</b>		100

Only 11 subjects, all of whom were male, had smoking histories.

The smear and culture results are presented in Table 2, respectively. The smear and culture had negative results in 19 subjects (19%). Bacterial culture was reported only for 50 subjects. The fungal smear was positive in 31 cases. While 19 subjects had only one type of fungus in the smear and the culture, in four cases, two types of fungi had grown in the culture. Eight subjects had both fungal and bacterial growths in their cultures.

The total number of those who had fungal growths in their culture was 31 (31%). Table 2 gives the detailed statistics of different types of fungi as follows: 12 subjects had Yeasts, 12 had Saprophytes (eight cases of *Aspergillus*, three cases of *Penicillium*, and one case of *Mucor*), and 11 had Actinomycetes (three cases of *Nocardia* and eight cases of *Streptomyces*). In four cases, there were two types of fungi in the smear, two of which were a mixture of Yeasts and Saprophytes, one of which was a mixture of Yeasts and Actinomycetes, and one of which was a mixture of Saprophytes and Actinomycetes.

**Table 2:** Smear and culture test results in the study participants

Test results	NO
Negative smear and culture	19
Positive smear and culture	81
Only bacteria	50
With fungi	31
Mixture of fungi and bacteria	8
One type of fungi	19
Two type of fungi	4
Yeasts	12
Saprophytes	12
Actinomycetes fungi	11

Out of the 46 male subjects, 16 (34.8%) were positive for fungi. This figure was 15 (27.8%) for women. The difference in the growth rate of the fungi was not significant between men and women ( $P=0.69$ ).

The highest fungal growth rate belonged to the 40-49 year-old group, and the lowest to the 50+ year-olds (at 0%). The difference between age groups was not significant.

The highest fungal growth rate was found among those with a high school diploma (39.2%), while this figure was the lowest in the less literate or illiterate group (16.67%). This difference was not statistically significant in different educational groups ( $P=0.37$ ).

Smokers had a lower fungal growth rate (18%) compared with nonsmokers (32.6%). However, this difference was not significant ( $P=0.57$ ).

### Discussion

Invasive fungal infections of the nose caused by *Aspergillus* were identified in the late 1800s (6). This type of fungal infection is almost exclusively seen in individuals who were

immunocompromised regardless of the cause.

Non-invasive fungal infections of the nose were identified by Safirstein in the late 1970s, who described them as allergic fungal sinusitis, and were brought to attention by Katzenstein in the early 1980s (7,8), and the role of fungi was then recognized. In fact, *Aspergillus* was, at that time, known as one of the causative agents of chronic non-invasive rhinosinusitis.

With the advancement of laboratory equipment and the development of modern culture techniques in the early 1990s, Manning, studying allergic *Aspergillus* sinusitis, then still a newly discovered and little-known condition, realized that out of 22 patients who showed symptoms of allergic *Aspergillus* sinusitis, only one person had a positive *Aspergillus* culture, and that other fungi such as *Bipolaris*, *Curvularia* and *Alternaria* played a greater and more important role in causing the condition. In other words, he realized that allergic *Aspergillus* sinusitis was only one type of allergic fungal sinusitis (9).

Over the last three decades, the diagnosis of fungal rhinosinusitis has been rising, and researchers have become increasingly aware of the significance of fungi in the pathogenesis of rhinosinusitis (10-12), and since the early 21<sup>st</sup> Century, fungi have been considered one of the likeliest causative agents of chronic rhinosinusitis, particularly the polypoid type (10,13).

About 1980, non-invasive fungal sinusitis attracted attention. Along the increasing diagnosis of fungal infections of the nose and sinuses in the following years, it was gradually hypothesized that apart from the known allergic fungal rhinosinusitis, fungi were likely to play a major role in the pathogenesis of chronic rhinosinusitis in general. The hypothesis is now the subject of much debate and research, and if proven, it can certainly have profound influences on the treatment of chronic rhinosinusitis, as the current antibiotics have no effect on fungal strains.

There is still a major question, nevertheless, which has not been fully answered: excluding pathological conditions, what fungi and how frequently exist in the mucus of healthy individuals? And can rhinosinusitis have been mistakenly attributed to fungi in certain cases.

As for the normal flora of the nose, there have been few studies, and little information is available. Certain studies have confirmed the existence of fungi in the healthy mucal secretions of adults (14,15) and even of infants (16).

In a similar research to ours, in Saudi Arabia in 2002, 100 healthy individuals aged 17-60 years old were studied. In the nasal cultures of 21 subjects (21%), there were different types of *Aspergillus*, yet no cases of *Candida* were observed (17).

In 2004, in Helsinki, Finland, a study was conducted on twenty healthy individuals, and in no cases of nasal cultures, were there any fungi (18).

In 2007, the normal fungal flora of 135 healthy residents of Barcelona, aged 18-35 years old, was studied. There were fungal growths in 41.5% of the healthy individuals, where *Cladosporium* had the highest rate at 23.6%. Other types of fungi were *Penicillium*, *Aspergillus* and *Alternaria*. As in our case, in these studies also there was no significant difference between sexes and among different age groups (18).

In our study, the growth rate of different types of fungi was 31%, and the fungi included various yeasts, Saprophytes (*Aspergillus*, *Penicillium*, and *Mucor*) and Actinomycetes (*Streptomyces* and *Nocardia*).

Since weather conditions, geographical and other climatic factors influence the flora and fauna of an area, and naturally the different types of the fungal flora there, it can be expected that nasal samplings in different geographical regions will show different fungal growths.

For instance, the fungal culture results of the research in Saudi Arabia and ours, both of which in the dry Middle Eastern region, are more similar to each other than to those in Barcelona, where a greater fungal growth is shown and where one type of fungus, *Cladosporium*, was observed which was never seen in our studies. In Helsinki there were also no fungal growths, which might be due to different climates, geographical locations or sampling methods (19).

### **Conclusion**

Despite their thorough efforts, the authors of this paper were unable to find any studies or researches about the normal fungal flora of the nose in healthy individuals in Iran. In a research conducted in the otorhinolaryngology ward of Rasool-e-Akram Medical Center, Tehran, on patients (some with high and some low IgE) with nasal polyps, and where sampling had been performed during the surgery, the highest positive fungal growth belonged to *Aspergillus* and *Candida*. *Penicillium*, *Alternaria* and *Curvularia* were in the following ranks. However, it should be noted that the objective of that research was the diagnosis of patients with allergic fungal sinusitis, and that having a nasal polyp was the criterion for being selected for the study (20).

Admittedly, our study did have some limitations; for instance, sampling was performed the easy way, by selecting people who visited our clinic. While in this study sufficient examinations were conducted to ensure that the nasal mucus was healthy, a larger sampling population would have been more ideal. However, this was impossible for the authors at that time and in view of the limited resources and facilities available.

Further studies of this type in other parts of the country or of the world can, through the identification of normal fungal flora of the nose, help understand the normal and pathological states of the nasal mucus, and ultimately help better cure rhinosinusitis, and at the same time help identify the climatic effects on the nasal flora.

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