

Study of Nasal Mucosal Flora in Acute and Chronic Sinusitis

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ABSTRACT

Introduction: Microbiological investigation and selection of antimicrobial agents are important for infection control and to avoid complications. It is also suggested to have continuous medical education for effective and early diagnosis, as well as providing appropriate care. The objective of the study is to analyze the microflora present in patients with acute and chronic sinusitis in the tertiary care teaching hospital.

Methods: A cross sectional hospital based study was conducted in order to determine the microorganism of paranasal sinuses in patients with acute and chronic sinusitis undergoing endoscopic sinus surgery. Swabs/ biopsy were taken from the infected sinus and were analyzed microbiologically within 4 hours of collection.

Results: The bacterial isolates including *Staphylococcus aureus* accounted for the maximum at 37.5% followed by *Klebsiella* spp., 7% and MRSA 2%. The fungal isolates including *Aspergillus flavus*, *Candida albicans* and *C. tropicalis* showed 8% and are considered as differential diagnosis in tropical regions. Further no cultural prints of anaerobes were found.

Conclusion: Based on this research work, it was identified that bacterial and fungal pathogens play vital role in causing infections in the sinuses for which empirical antibiotic therapy will be the choice for better management of the condition.

Keywords: Acute, Chronic, Sinusitis, Nasal Mucosa, Microbiology.

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INTRODUCTION

Most of the sinus infections are viral and few develop secondary bacterial infections. Rhinoviruses, influenza viruses and parainfluenza viruses are the most common causes of sinusitis.¹ The most common bacteria isolated from paediatric and adult patients with community acquired acute purulent sinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pyogenes*. *Staphylococcus aureus* and anaerobic bacteria (*Prevotella* and *Porphyromonas*, *Fusobacterium* and *Peptostreptococcus* spp.) are the main isolates in chronic sinusitis. *Pseudomonas aeruginosa* and other aerobic and facultative gram negative rods are commonly isolated from patients with nosocomial sinusitis, the immunocompromised host, those with HIV infection and in cystic fibrosis.^{1,2} Fungi and *Pseudomonas aeruginosa* are the most common isolates in neutropenic patients. The microbiology of sinusitis is influenced by the previous antimicrobial therapy, vaccinations and the presence

of normal flora capable of interfering with the growth of pathogens. Acute exacerbation of chronic sinusitis (AECS) represents a sudden worsening of the baseline chronic sinusitis with either worsening or appearance of new symptoms. Typically, the acute (not chronic) symptoms resolve completely between occurrences. An increase in antimicrobial resistance was also observed and has increased now a days.

Recommended treatments for most cases of sinusitis include rest and drinking enough water to thin mucus. Antibiotics are not recommended for most cases Decongestant nasal sprays containing oxymetazoline may provide relief.³ However, if symptoms do not resolve within 10 days, amoxicillin is to be used first then for treatment with amoxicillin/ clavulanate being indicated when symptoms do not improve. There is limited evidence to support short treatment with oral corticosteroids for chronic rhinosinusitis with nasal polyps.⁴

Surgery should only be considered for those people who do not benefit with medication. Chronic sinusitis is an infection of sinuses lasting for more than three months. Despite its prevalence the disease remains with poorly understood origin, pathogenesis and natural history. The etiology of chronic sinusitis continues to be the focus of much debate and research in the field of rhinology.^{5,6}

With initial use of antibiotics and agents that decrease mucosal edema now surgical methods are employed in whom medical treatment fails. Although diagnostic criteria for acute sinusitis are well established yet the definition of chronic sinusitis is controversial with respect to the importance of bacteria in the initiation and progression of disease. Chronic sinusitis has been considered to be chronic inflammatory condition rather than microbial infection. The role of bacteria in the pathogenesis of chronic sinusitis is currently being reassessed.^{6,7}

Fungal colonization of the nose and paranasal sinuses appears to be a common finding in both normal and diseased states. Fungal rhinosinusitis (FRS) is increasing in prevalence; it causes significant physical symptoms, negatively affects quality of life and it can substantially impair daily functioning.⁸

The use of endoscopies has made it possible to determine the microbiology of each sinus with a lower probability of contamination.^{6,9}

Persistence of infection causes mucosal changes such as loss of cilia, edema and polyp formation. We feel that lack of progress is largely due to paucity of knowledge in microbiology and histopathology of chronic sinus disease available to us. This was the impetus of our study to evaluate the microbiology of acute and chronic sinusitis in patients.

MATERIALS AND METHODS

This study is the cross sectional study conducted from June to August 2016. After getting approval from institutional ethical committee (IEC-No:30 dt. 15.04.2016) and informed consent from the participants, the details were collected.

In the present study, microbiological aspects were strictly followed according to the standard institutional operating protocols. The study was conducted in tertiary care teaching hospital, Tamilnadu. After explaining the study methodology to the participants/attenders, details were collected from 50 patients attended in ENT OP. Their socio-demographic status (age, sex and occupation), clinical complications, and laboratory parameters (microbiological)

were collected and entered in the proforma.

Inclusion criteria: Both sexes of all age groups; patients with acute and chronic inflammatory disease of sinuses; patients with chronic sinusitis with no response to initial treatment; patients with recurrent sinusitis; and patients not on antibiotics at least for 2 weeks were considered.

Exclusion criteria: The patients with malignancy of paranasal sinuses and patients on recent antibiotics were excluded from study.

Sample collection: The endoscope was sterilized in a glutaraldehyde solution for 10 min and washed prior to use. Endoscopic examination of the nasal cavity and the sinuses was done on all the patients. Endoscopic specimens were collected from nasal mucosa, which was not disinfected, and this culture was considered representative of the background nasal flora. Swab specimens for microbiological analysis aseptically transferred into a transport medium, and transported immediately to the laboratory.

Macroscopic observation: The specimens were examined macroscopically for

- a. Appearance (color) and
- b. Blood stain

Bacterial isolation: Within 1 hour of collecting the specimen, the specimens were cultured using Nutrient agar, Blood Agar and MacConkey Agar, and were incubated at 37°C for 24 hours. The cultures were examined for growth; the isolation and identification of bacterial isolates were carried out in accordance with Bergey's Manual of Determinative Bacteriology.^{10,11}

Fungal isolation: The specimens were inoculated in Saboroud's Dextrose agar and incubated at room temperature for 3 to 4 days. The cultures were examined for growth; the isolation and identification of fungal isolates by colony morphology and microscopy using lactophenol cotton blue staining.

RESULTS

In this study endoscopic nasal mucosal swabs were collected from 50 patients who attended the ENT OP and also from ten healthy controls.

Patients' Demography and Interventions

In this study, among the 50 patients included, 31 (62%) were males and 19 (38%) were females. (Figure 1) The distribution of cases in relation to age group is depicted in figure 2.

Figure 1: Sex wise distribution of the patients attended ENT OPD with the symptoms of acute and chronic sinusitis

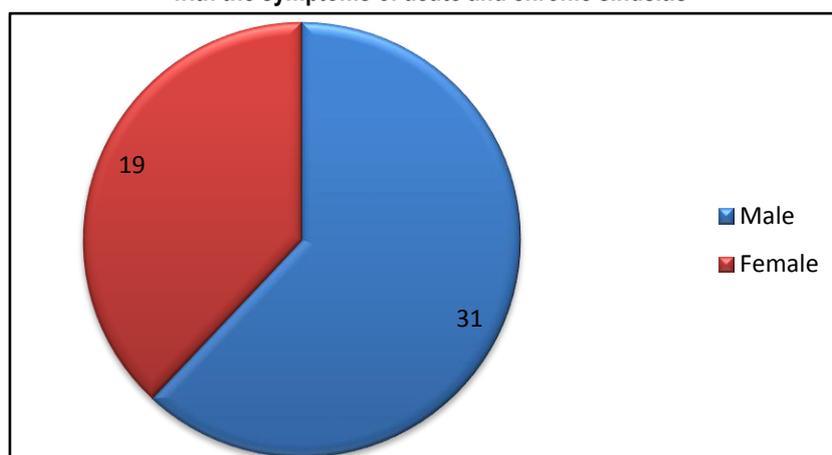


Figure 2: Age wise distribution of patients

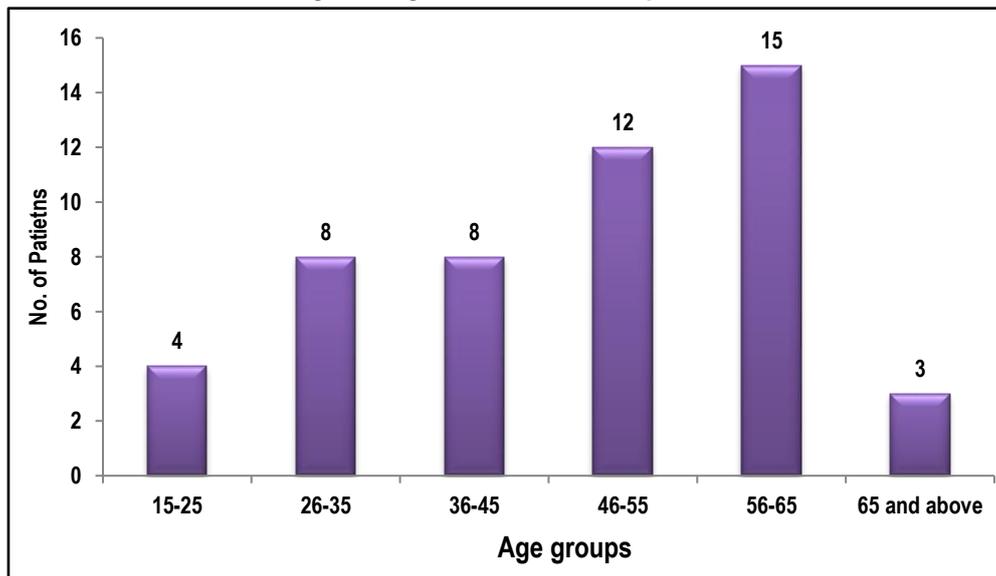


Table 1: Symptomology of the patients included in the study

Symptom	No. of patients	Percentage
Nasal discharge	27	54
Nasal obstruction	38	76
Headache	39	78
Disturbance of smell	11	22
Epistaxis	3	6
Sneezing	17	34
Sore throat	1	2
Hawking	1	2

Table 2: Microbiological analysis (Subjects – 50 and isolates – 60)

Microorganisms identified	No. of isolates verses specimens
Aerobic organisms (Both seen on smear and isolated)	31/ 50 (62)
Aerobic organism (Seen on smear but not isolated)	25/ 50 (50)
Anaerobic organisms	0/ 50 (0)
Fungus	4/ 50 (8)

[Figures in parenthesis denoted percentages]

Table 3: Aerobic microorganisms (bacteria) and its frequency (n=56)

Aerobic microorganisms (bacteria)	Frequency
<i>Staphylococcus aureus</i>	21 (37.5)
<i>Klebsiella</i> spp.	4 (7.1)
MRSA	1 (1.7)
<i>Streptococcus</i>	1 (1.7)
<i>Enterobacter</i> spp.	1 (1.7)
<i>Acinetobacter</i> spp.	1 (1.7)
<i>Pseudomonas</i> spp.	1 (1.7)
<i>Citrobacter</i> spp.	1 (1.7)
Gram positive bacilli	5 (8.9)
Gram negative bacilli	11 (19.6)
Gram positive cocci	8 (14.2)
Gram neg coccobacilli	1 (1.7)

[Figures in parenthesis denoted percentages]

Clinical symptoms of the patients

The clinical diagnosis of acute and chronic sinusitis was confirmed in all 50 patients. History of head ache was present in 39 (78%) of 50 patients. The nasal obstruction and nasal discharge was found among 38 (76%) and 27 (54%) respectively. Other clinical symptoms observed in this study subjects are given in table 1.

Microbiology results

Among 50 patients, bacterial and fungal pathogenic organisms were isolated from 31 samples. Out of 50 patients, 31 samples were positive for aerobic organisms, none were positive for

anaerobes, fungal growth was reported in 4 samples. (Table 2)

Further the study was extended to determine the type of microorganism by standard microscopic and biochemical characterization, thereby the maximum isolates was *Staphylococcus aureus* (21) followed by gram negative bacilli (11) and gram positive cocci (8). The detailed description of aerobic microorganisms and its frequency was interpreted in table 3.

The fungal isolation was *Aspergillus flavus* and *Candida* species. The detailed description of fungal isolates and its frequency were depicted in table 4 and Figure 3,4.

Table 4: Fungal isolation and its frequency (n=4)

Fungal isolates	Frequency
<i>Aspergillus flavus</i>	2 (50)
<i>Candida albicans</i>	1 (25)
<i>C. tropicalis</i>	1 (25)

[Figures in parenthesis denoted percentages]



Figure 3: KOH mount of the sinonasal polyp tissue showing slender septate fungal hyphae



Figure 4: LPCB mount of *Aspergillus flavus*

DISCUSSION AND CONCLUSION

The microbiology of infections of the acute and chronic sinus diseases can be anticipated according to the patient's age, clinical presentation, and immunocompetence. In acute sinus disease, viral upper respiratory infections frequently precede bacterial superinfection by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Staphylococci and respiratory anaerobes are common in chronic sinus infection, which may also be caused by exacerbations of infection with the bacterial species that cause acute disease. Enterobacteriaceae may be found in patients with nosocomial sinusitis who are predisposed to the development of sinusitis by prolonged nasogastric and nasotracheal intubation. Immunosuppressed patients have episodes of sinusitis caused by the usual agents associated with acute sinusitis in immunocompetent patients, and they may also become infected with a broad array of unusual agents, including mycobacterial species, fungi, and protozoa.¹²

In most of the studies, in order to determine the bacteriologic cause of acute sinusitis, a sample of sinus secretions must be obtained from one of the paranasal sinuses without contamination by normal respiratory or oral flora that colonize mucosal surfaces. When maxillary sinus aspiration is performed on children who have signs and symptoms of acute sinusitis, bacteria are recovered in high density from 70%. In patients with acute, subacute, or chronic sinusitis who are generally well except for

persistent respiratory symptoms, of nasal discharge or cough or both;¹³ the same technique also included in this study also.

In many studies, the biofilm of bacterial infections may account for many cases of antibiotic refractory chronic sinusitis⁴ and of 75% of patients undergoing surgery for chronic sinusitis.¹⁴ Biofilms are complex aggregates of extracellular matrix and interdependent microorganisms from multiple species, many of which may be difficult or impossible to isolate using standard clinical laboratory techniques. These bacteria have their antibiotic resistant increased up to 1000 times when compared to free living bacteria of same species. We attempted to follow a consistent and reproducible technique for isolating bacterial pathogens from the specimens. The non-isolation of bacterial pathogens may be due to technical factors, improper processing, non – infective/ allergic pathology or patients on past infections, now getting treated.

The microbiology of chronic sinusitis with polyposis considered as the major isolates of polymicrobial aerobic-anaerobic flora. Fungal colonization of the nose and paranasal sinuses is common in the normal and inflamed sinuses.¹⁵ The role of fungi in the pathogenesis of chronic sinusitis is controversial. Although fungi can be detected in the nose and paranasal sinuses of nearly all patients with chronic sinusitis and are present in almost all healthy controls, some studies suggest that there may be mechanisms by which fungi exert an effect on sinus mucosa in susceptible individuals only.¹⁶

Even though acute and chronic rhinosinusitis have similar symptoms, but severity is more among acute sinusitis. The signs and symptoms of cases were correlated with radiographic results. The most frequent microorganisms found in CRS patients were: *Staphylococcus aureus*, (37.5%); followed by *Klebsiella pneumoniae* (7.1%) etc. There was no growth of bacteria in 19 patients (38%). A short course of antibiotics was effective and patients improved well. The major strength of the study is rigid criteria adopted to select subjects and controls and good laboratory practice adopted to process the samples. Further, this study has certain limitations including

1. This study is made from single centre and limited to aerobic bacterial and fungal organisms.
2. The transversal studies tend to overestimate the long duration of infections and under estimate the short duration infection. Nevertheless this type of transversal analysis has fundamental importance for the knowledge.
3. The study has several important limitations which require discussion. Patient population at the study area may not be a representative of the community at large.

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