



## Original Article

### Endoscopically guided sinus cultures in recalcitrant sinusitis

**Shawky M. Elmorsy,<sup>1</sup> Shirien Amin Rakha,<sup>2</sup> Noha Tharwat Abou El-khier<sup>3</sup>**

<sup>1</sup>Otorhinolaryngology Department, <sup>2</sup>Clinical Pathology Department, <sup>3</sup>Microbiology Department, Faculty of Medicine, Mansoura University, Egypt

Correspondence to: Shawky M. Elmorsy, Email: shawky\_morsy2003@yahoo.com

**Introduction:** *Despite maximal medical and surgical therapies, subsets of patients continue to have persistent inflammation of paranasal sinuses, treatment of this group of patients, often referred to as recalcitrant chronic sinusitis, is quite challenging and is a subject of considerable debate in the medical literature.*

**Patients and Methods:** *Sinus cultures were obtained in 48 patients with recalcitrant sinusitis after ESS. Between January 2012 and January 2014, Antibiotics were stopped 7 days before cultures.*

**Results:** *Pseudomonas aeruginosa was isolated in 5(10.4%) patients, Staphylococcus aureus was isolated in 12(25%) patients and Aspergillus was isolated in 3(6.2%) patients. 28 (58.3%) patients showed no bacterial or fungal growth on culture.*

**Conclusion:** *Intracellular bacteria should be taken into consideration when designing novel treatment strategies to lessen the chance of reinfection.*

*More research is needed to know contributing factors in chronicity and resistance.*

**Keywords:** *Recalcitrant sinusitis, Biofilms, middle meatus culture, Local treatment.*

## INTRODUCTION

Chronic rhinosinusitis (CRS) is a common, though poorly understood, group of diseases that affects upwards of 16% of the US population. Unlike acute rhinosinusitis (ARS), which can be clearly linked to pathogenic bacteria, the causes of CRS are not as straightforward. Recent evidence has increased our knowledge concerning the inflammatory etiology of CRS as it relates to factors such as environmental allergies, asthma, fungal infection, and aspirin sensitivity. In addition to these factors it is quite likely that bacterial infection plays a role in CRS, whether as a causative agent or an exacerbating factor. It is therefore important to understand how these systemic factors influence the sinonasal environment and the bacteria that grow there. Conversely, We must appreciate

how the bacterial flora affects the sinonasal environment.<sup>(1)</sup>

However despite maximal medical and surgical therapies, subsets of patients continue to have persistent inflammation of paranasal sinuses, treatment of this group of patients, often referred to as recalcitrant chronic sinusitis, is quite challenging and is a subject of considerable debate in the medical literature.

The pathophysiology of recalcitrant CRS is poorly understood, but seems to be multi-factorial. Over the years, most CRS research has focused on the sinus mucosa. The immune response in this layer has been studied extensively, including the role of cellular infiltrates and local inflammatory markers. However, an

overall understanding of the complex interplay of the diverse mucosal factors remains elusive.<sup>(2)</sup>

A new hypothesis reviewed by Harvey et al.<sup>(3)</sup> focuses on biofilm. Biofilm continually presents antigen, resulting in chronic inflammation of the mucosa. It might also act as an

unsurpassable barrier for innate host defense mechanisms as well as preventing antibiotics from reaching the causative micro-organisms. These characteristics of biofilm

could potentially explain important clinical features of recalcitrant CRS.

The care of patients failing the typical medical and surgical management for chronic rhinosinusitis who need further evaluation to rule out various mimics of sinus disease and other disorders that may require specific treatment plans. There is increasing interest in the underlying bone of the paranasal sinuses as an important player in recalcitrant CRS. This concept is based not only on the close anatomical relation between the mucosa and the underlying sinus bone, but also on radiological changes seen in patients with CRS.<sup>(2)</sup>

*Pseudomonas* exacerbation of chronic rhinosinusitis following functional endoscopic sinus surgery (FESS) is a common condition seen in over 30% of endoscopically obtained sinonasal cultures.<sup>(4)</sup>

These infections are frequently relapsing and often refractory to oral antibiotics. Colonization with *Pseudomonas aeruginosa* and subsequent biofilm formation have been demonstrated to occur in the lower airways and within the sinonasal mucosa.<sup>(5)</sup>

In patients with allergic rhinitis an association has been found with higher carriage rates of *Staphylococcus aureus*. The significance of this is unclear but may be related to bacterial superantigen production leading to TH2-mediated inflammation. Endoscopic sinus surgery does not appear to change the bacterial flora, though it may decrease the presence of bacterial biofilms in sinus cavities.<sup>(5)</sup>

*S. aureus* biofilms play a dominant role in negatively affecting outcomes of ESS with persisting postoperative symptoms, ongoing mucosal inflammation, and infections.<sup>(6)</sup>

This study gives further evidence supporting a role of intracellular *S aureus* in CRS. In all cases intracellular infection was associated with surface biofilm, suggesting a potential relationship between the two. Further work is required to delineate the true mechanisms of intracellular persistence and also the role that it plays in the recalcitrant nature of CRS.<sup>(7)</sup>

A review of recent literature shows a high incidence of positive cultures for *Staphylococcus aureus* from the sinuses of patients with chronic rhinosinusitis, both before and after surgery, but has not produced convincing evidence that *Staphylococcus aureus* has a significant role in the pathogenesis or clinical course of the disease.<sup>(8)</sup>

*S. aureus* persists in the sinonasal cavity despite ESS. The postoperative culture of sinonasal *S. aureus* in patients previously biofilm-positive but culture-negative may reflect the dynamic ability of *S. aureus* to adapt to the surgically-altered microenvironment with subsequent biofilm dispersal and release of planktonic clones.<sup>(9)</sup> Clinical and microbiological relapse of disease following ESS is significantly associated with intracellular *S. aureus*. Evidence suggests that this disease association is independent to surface biofilm status. Intracellular bacteria should be taken into consideration when designing novel treatment strategies to lessen the chance of reinfection.<sup>(10)</sup>

## PATIENTS AND METHODS

Sinus cultures were obtained from 48 patients with recalcitrant sinusitis after ESS. Between January 2012 and January 2014. Antibiotics were stopped 7 days before cultures.

Middle meatus secretions were collected from one side only, by means of a sterilized 2mm suction tip coupled to the Specimen Trap model 076-0490 (Sherwood Medical, St. Louis, EUA) collection container. . All types of cultures including aerobic, anaerobic and fungal cultures were included.

Samples were referred to the laboratory no later than 1 hour after harvesting, in Stuart (Starplex Scientific, Ontario, Canada) transportation means for the rearing of aerobic microorganisms; and in thyoglycolate broth for anaerobic rearing. In the laboratory, the bacterioscopic exam was carried out using the Gram stain.

For aerobic culture, the material was spread in plates with McConkey agar (Becton Dickinson, Maryland, USA), blood agar (enriched with 10% of sheep blood) and chocolate agar (incubated at 10% co<sub>2</sub>) and incubated at 37°C for 24 hours. If no bacterial growth was seen, the media were re-incubated for 24 hours more before being deemed negative. After isolation and confirmation of its nature using Gram stain, the microorganism was identified using the API system for aerobes (Bio Merieux, France).

Anaerobe culture was carried out in sheep blood agar, having as base the brucella blood agar (Difco, Detroit, USA) with incubation for the most of 72 hours in

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anaerobiosis atmosphere provided by the following system: Gaspak (Becton Dickinson, Maryland, USA).

Mycology studies were carried out through direct material examination on slide; and material culture in Sabouraud's dextrose agar medium with chloramphenicol and with or without cycloheximida. Incubation was carried out under 25°C and 35°C, and the cultures were observed for up to 30 days before being considered negative for fungi. Fungi identification was carried out through macroscopic and microscopic morphology using lacto phenol blue stain.

Laboratorial procedures followed those advocated by the National Committee for Clinical Laboratory Standards (NCCLS), considering the bacterial species and the site of infection.

## RESULTS

Postoperative cultures were obtained from 48 patients with recalcitrant sinusitis after ESS. Between January 2012 and January 2014. Antibiotics were stopped 7 days before cultures.

*Pseudomonas aeruginosa* was isolated in 5 (10.4%) patients, *Staphylococcus aureus* was isolated in 12 (25%) patients and *Aspergillus* was isolated in 3 (6.2%) patients. 28 (58.3%) patients showed no bacterial or fungal growth on culture (Table 1).

**Table 1**

Number of patients	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Aspergillus Fumigatus</i>
28 (58.3%)	-	-	-
5 (10.4%)	+	-	-
12 (25%)	-	+	-
3 (6.3%)	-	-	+

## DISCUSSION

In our department, SA and *Pseudomonas* were the most common pathogens cultured from patients with CRS who have been previously treated surgically.

Our data showed an interesting finding, despite the findings of purulence on endoscopic examination, a considerable portion of patients have no growth from culture specimens after ESS. Several possible explanation

of this exist, including the absence of bacteria, infection with atypical or difficult to culture bacteria, the presence of bacteria intracellularly or biofilm.

28 (58.3%) patients showed no bacterial or fungal growth on culture, this means that there is another factor in chronicity and resistance and this may be due to inflammatory mucosa or biofilm formation. (Bassiouni et al, 2012) examined the "inflammatory load hypothesis" as a possible explanation. They hypothesized that the grade of the inflammation is the most important predictor of long-term outcomes. Surgery, therefore, has a significant role not only in reestablishing ventilation, but also with removing the inflammatory load in the affected sinuses.

They suspect that in these severely diseased patients, a more radical removal of local proinflammatory factors during surgery may improve patient outcomes.<sup>(11)</sup>

Another study has shown that patients with biofilms have more severe disease preoperatively and persistence of postoperative symptoms, ongoing mucosal inflammation, and infections. This study strengthens the evidence for the role that biofilms may play in recalcitrant CRS.<sup>(6)</sup>

Patients who have persistent symptoms are often found to have Sino-nasal colonization by *Staphylococcus aureus* (SA). This organism is known to produce toxins, a number of which have the capacity to act as super antigens. Recently, it has been suggested that such toxins acting as super antigens may have a role in the pathogenesis of CRS with polyps.

Experience with treating SA related disease using appropriate oral antibiotics has been disappointing and all patients included had failed multiple previous courses of culture directed antibiotics. This may be due to inadequate drug penetration of Sino-nasal mucoperiosteum and bone, or because SA is able to exist as a biofilm, and thus remain relatively resistant to antibiotic treatment. They have therefore been interested in developing alternative strategies to manage these patients. Topical antiseptics were initially tried but it was found that povidine-iodine was poorly tolerated because of severe intranasal discomfort experienced immediately as the solution contacted the nasal mucosa.<sup>(12)</sup>

Different biofilm species are associated with different disease phenotypes. *H. influenzae* biofilms are typically found in patients with mild disease, whereas *S. aureus* is associated with a more severe, surgically recalcitrant pattern.<sup>(13)</sup>

A high percentage of CRS patients (28.6%) whose sinonasal mucopurulence has been examined have biofilm-forming capacity. Postsurgical patients had a high prevalence of biofilm-forming bacteria, a possible

reflection of the severe nature of their disease. Additional studies are warranted.<sup>(14)</sup>

Over the last five years, research has progressed rapidly since biofilms were first identified on the surface of diseased sinonasal mucosa. Their presence there has now been associated with more severe disease that is often recalcitrant to current management paradigms. Technological advances are allowing accurate characterization of the bacterial and fungal species within these biofilms, which would appear to be an important step in improving our understanding of how these bacterial communities might interact with the host to cause disease. This is an unanswered, yet highly important, question in this field of research that will undoubtedly be an area of investigation in the near future. As the body of evidence suggesting biofilms may be involved in this disease grows, research interest has switched to the development of antibiofilm therapies.<sup>(15)</sup>

Another study in Maryland, USA should that *Pseudomonas aeruginosa* and Gram-negative aerobic bacilli (GNAB) were more often isolated in patients who had surgery (9 of 33 patients had *P aeruginosa* and 17 had GNAB) than in patients who did not have surgery (3 of 75 had *P aeruginosa* and 7 had GNAB;  $p < .001$ ).<sup>(16)</sup>

There is considerable controversy concerning the role of fungi as the cause of "chronic eosinophilic rhinosinusitis" and whether this even represents a distinct clinical entity.<sup>(17)</sup>

It was suggested that fungi might be an important cause of most cases of chronic rhinosinusitis. This hypothesis remains controversial, and there is mounting evidence to support the multifactorial nature of chronic rhinosinusitis, which may include fungus. In fact, etiologic factors for all forms of fungus-related sinus disease are still poorly understood. The prevalence of the disease and the dominant fungal pathogen appear to vary in different geographic regions and probably are related to individual host conditions. Since sinus fungi are rarely invasive in immunocompetent individuals, it is not clear whether the effects of the antifungal treatments are a result of the antifungal action itself, or due to additional properties these drugs possess.<sup>(18)</sup>

Bhattacharyya and Kepnes<sup>(19)</sup> indicated that a wide range of bacteria may be present in the infected post ESS sinus cavity, with a considerable population of Gram negative organisms, including *Pseudomonas* species.

## CONCLUSION

- *Pseudomonas aeruginosa* was isolated in 5 (10.4%) patients, *Staphylococcus aureus* was isolated in 12 (25%) patients and *Aspergillus* was isolated in 3 (6.2%) patients.
- 28 (58.3%) patients showed no bacterial or fungal growth on culture.
- Limited information is available regarding the microbiology of chronic rhinosinusitis in the postoperative setting.
- Inflammatory load hypothesis and biofilms should be put in mind when treating recalcitrant sinusitis.
- Intracellular bacteria should be taken into consideration when designing novel treatment strategies to lessen the chance of reinfection.
- More research is needed to know contributing factors in chronicity and resistance.

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