

ORIGINAL STUDY

Microbiological study of nasal aerobic flora in patients with chronic rhinosinusitis

Mihaela Mitroi¹, Georgescu Monica², Alina Capitanescu¹, Marina Amarascu³, Elena Ionita¹

¹ENT Department, Emergency County Hospital, University of Medicine and Pharmacy of Craiova, Romania

²Turceni Hospital, Romania

³Morphology of the stomatognathic system, Dental Materials, University of Medicine and Pharmacy of Craiova, Romania

ABSTRACT

OBJECTIVE. The purpose of this study was to analyze the microbiology of patients with chronic rhinosinusitis (CRS).

MATERIAL AND METHODS. The study included a total of 106 patients diagnosed with chronic rhinosinusitis with nasal polyposis (CRSwNP) and without nasal polyposis (CRSsNP), hospitalized in our ENT Department. Nasal secretions from all the 106 patients involved in the study were sampled. These secretions were collected from their nasal fossa using sterile swab and were sent to the laboratory in insulated containers, within an interval of maximum 2 hours since collection.

RESULTS. Data on 106 patients were analyzed. There were 64 positive cultures (60.4%). The etiology of the positive samples was as follows: *Staphylococcus aureus* (53.1%), *Staphylococcus epidermis* (37.5%), *Escherichia coli* (4.7%), *Klebsiella* (3.1%), *Proteus* (1.6%).

CONCLUSION. The most prevalent microorganisms in patients with CRS are *Staphylococcus aureus* (*S. aureus*), followed by *Staphylococcus epidermis*. This suggests that persistent inflammation of the rhinosinusal mucosa could be influenced by the presence of staphylococci, not only *S. aureus*.

KEYWORDS: chronic rhinosinusitis, bacteriology, *Staphylococcus aureus*

INTRODUCTION

Chronic rhinosinusitis is a major health problem, not only due the impact they have on the lower respiratory tract and lung parenchyma, but also due the huge costs involved in treating these disorders.

Chronic rhinosinusitis is considered to be responsible for 18 million calls on the physician each year in the United States¹. In Canada, the IMS Health report² published in 2006 estimates the number of prescriptions for acute and chronic rhinosinusitis to 2.89 million, with a distribution of approximately 2.3 for acute rhinosinusitis and 3.1 for chronic rhinosinusitis.

Regarding the prevalence of chronic rhinosinusitis in Europe, in 2011, the Rhinology Journal published a

study signed by Hastan et al.³, which was based on the network GA2LEN (Global Allergy and Asthma European) and carried out in 19 centers in 12 countries. The above-mentioned study shows that there are large variations in the prevalence of chronic rhinosinusitis in different European countries involved in collecting these data. The highest prevalence of chronic rhinosinusitis was recorded in Portugal (Coimbra) - 27.1%; the lowest prevalence was found in Germany (Brandenburg) - 6.9%.

The cause of the infection chronicity of rhinosinusitis has not been well established yet. Many studies suggest that a small number of bacteria are common in the paranasal sinuses of subjects with CRS, whereas the sinuses of healthy individuals are lightly colonized by commensal microorganisms. Generally, it is observed

that species include aerobes and anaerobes germs, frequently localized in the nasal fossae and mouth^{4,5}.

The pathogens involved in CRS are difficult to identify due to low bacterial concentration rates and because most cultures are obtained after patients have been treated with antibiotic.

The aim of this study was to characterize the composition of microbial communities that colonize the nasal fossae of patients with CRS.

MATERIAL AND METHODS

In our study we included a total of 106 patients diagnosed with chronic rhinosinusitis with nasal polyposis (CRSwNP) and without nasal polyposis (CRSSNP), hospitalized in our ENT Department. All patients signed an informed consent for being involved in this study.

Chronic rhinosinusitis was diagnosed on the criteria established by the EPOS⁶:

- inflammation of the nose and paranasal sinuses characterized by two or more symptoms, one of which being nasal obstruction / nasal congestion or rhinorrhea (anterior or posterior)
- with / without cephalalgia / sensation of tension at the facial level
- with / without hypo- / anosmia
- endoscopic changes in the nasal fossae
 - nasal polyps and / or
 - purulent rhinorrhea starting from the middle meatus and / or
 - oedema / obstruction congregation in the middle meatus.

Nasal secretions from all the 106 patients involved in the study were sampled. These secretions were collected from their nasal fossa using sterile swab and were sent to the laboratory in insulated containers, within an interval of maximum 2 hours since collection.

Initially, the examination was a direct, macroscopic one (with a magnifying glass), followed by a microscopic examination of the biological product. To this end, fresh samples between the blade and the lamella and samples which were fixed and stained by specific methods were used. All these operations were carried out within a microbiological safety field.

The fresh samples between the blade and the lamella were examined under the optical microscope.

The samples which were fixed by means of heat from a Bunsen gas lamp were stained with methylene blue using the Gram method and staining bath with appropriate reagents kits. Staining times were monitored with a stopwatch.

To ease the growth and multiplication of bacteria, the seeded environments were incubated aerobically for 24-48 hours at 37° C in the thermostat. The macroscopic appearance of the samples (examined with the

naked eye, with simple magnifying glasses or stereoscopic glasses) oriented the next steps of our investigation. The cultures obtained in liquid or solid environment were identified according to the following criteria: morphological, biochemical, antigenic, pathogenic.

RESULTS

We conducted a statistical analysis of the group made up of 106 patients – 45 of them were females (42%) and 61 males (58%); 45 patients from rural areas (42%) and 61 from urban areas (58%) (Chart 1). The average age of the entire group was 52.5 ± 14.6 years, by gender the average age was 53 ± 13.1 years for women and 52.1±15.7 for men. By the area of origin (Chart 2), the average age of the patients from the rural areas was 52.3 ± 14.7, and from urban areas 52.6 ± 14.7 years.

It is worth noticing group homogeneity both in terms of age mean and variation.

The hospitalization duration was another feature we statistically analyzed. The results showed as follows: the patients belonging to the rural areas generally required a longer hospitalization period compared to those from urban areas, without noticing significant differences between men and women (Table 1).

The bacteria identified in our study were the following: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella*, *Proteus*.

Of the 106 samples (nasal discharge) collected, 64 were positive (60.4%). The etiology of the positive samples was as follows:

- 34 strains of *Staphylococcus aureus* (53.1%);
- 24 strains of *Staphylococcus epidermidis* (37.5%);
- 3 strains of *Escherichia coli* (4.7%);
- 2 *Klebsiella* strains (3.1%);
- 1 strain of *Proteus* (1.6%).

It appeared that Gram-positive cocci (58 strains - 90.6%) had the highest incidence, followed to a lesser extent by Gram-negative bacilli (6 strains - 9.4%).

Among Gram-positive cocci, *Staphylococcus aureus* (34 strains - 53.1%) was in the first place, followed by *Staphylococcus epidermidis* (37.5%).

The statistical analysis of the group showed the following correlations between the germs isolated in the bacteriological examination and the area of origin of the patients, as seen in Chart 3, and the gender of the patients (Chart 4).

A remark about the germs involved in the development of chronic rhinosinusitis was that the patients detected with Enterobacteriaceae in the nasal fossae at the bacteriological examination developed more aggressive forms of chronic rhinosinusitis, requiring longer periods of hospitalization (Table 2).

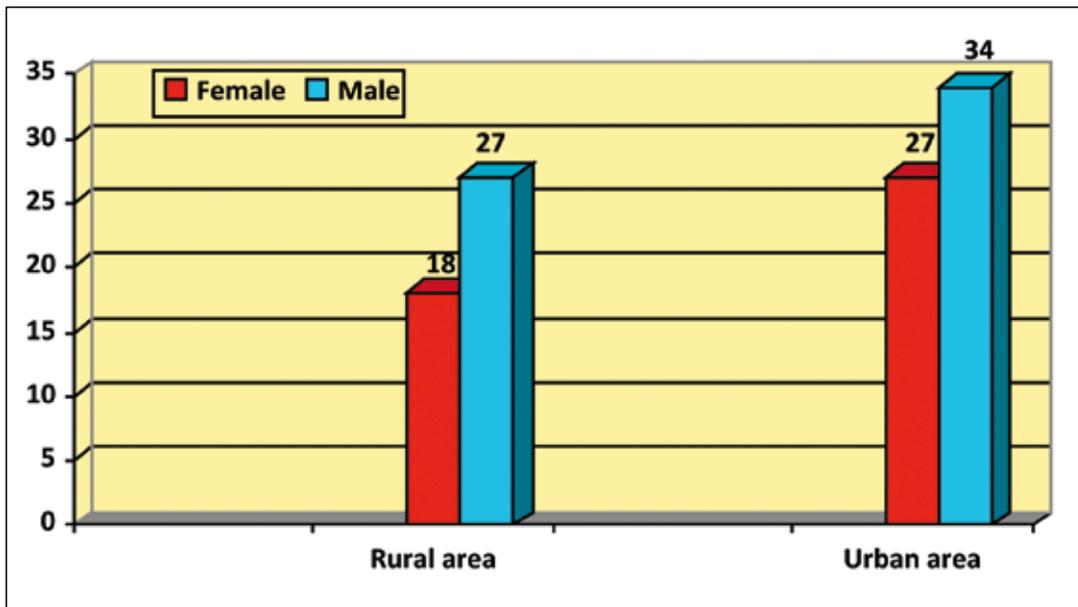


Chart 1 Distribution of the patients by gender and area of origin

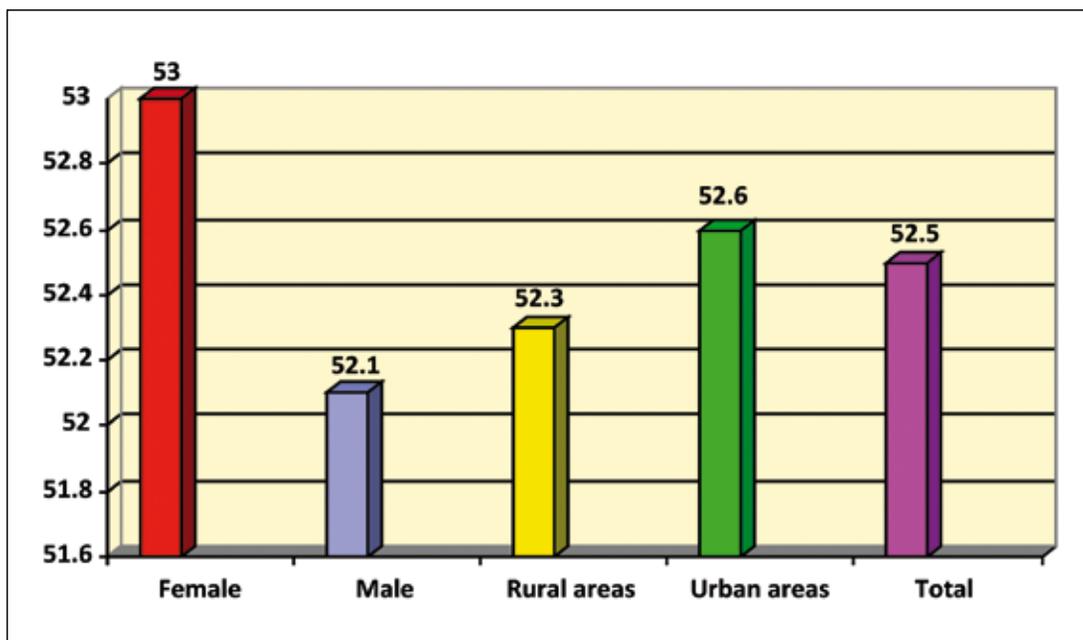


Chart 2 Distribution of age mean by gender and area of origin

Table 1
Distribution of the patients by gender, area of origin and average of hospital stay

	Female	Male	Rural	Urban	Total
Mean of duration of hospital stay	7.0	6.9	7.6	6.4	6.9
Standard deviation	4.1	3.5	3.6	3.8	3.7

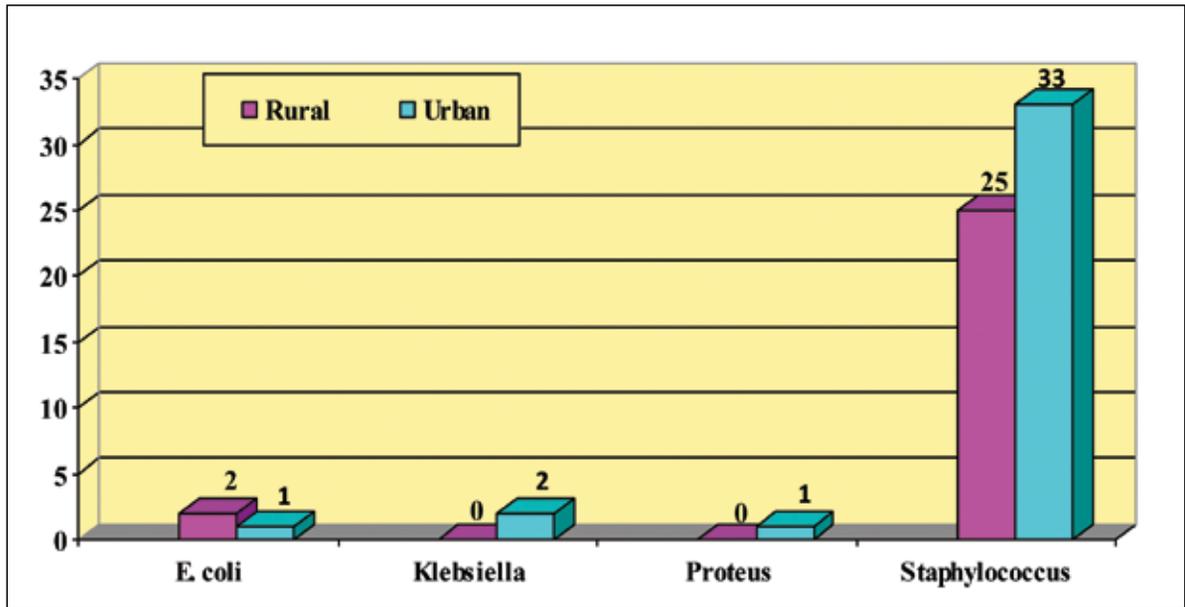


Chart 3 Distribution of the patients with chronic rhinosinusitis by the area of origin and the germs isolated at the bacteriological examination of the nasal secretion

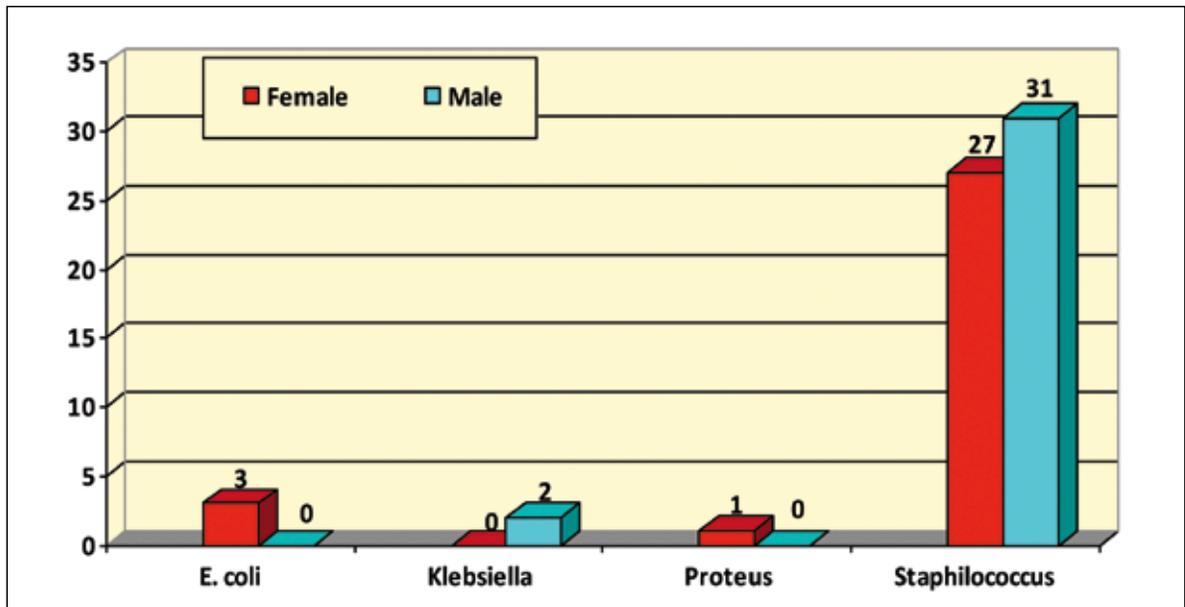


Chart 4 Distribution of the patients with chronic rhinosinusitis by the gender and the germs isolated at the bacteriological examination of the nasal secretion

Table 2
Distribution of the patients with chronic rhinosinusitis by hospitalization period in terms of the isolated germ during the bacteriological examination

	E. Coli	Klebsiella	Non-developed	Proteus	Staphylococcus	Total
Mean of hospital stay	8.3	9.0	7.3	3.0	6.6	6.9
Standard deviation	3.5	2.8	4.5	0.6	3.1	3.7

Table 3
Distribution of the patients with chronic rhinosinusitis by duration of hospital stay in terms of the type of *Staphylococcus* isolated during the bacteriological examination

	<i>S. white pathogen</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	Total
Mean of duration of hospital stay	10.0	6.8	6.6	6.9
Standard deviation	1.2	3.5	3.1	3.7

Depending on the type of *Staphylococcus*, the most severe forms of chronic rhinosinusitis with nasal polyposis were recorded in the patients with isolated strains of the white pathogenic *Staphylococcus* (Table 3).

DISCUSSIONS

Chronic rhinosinusitis is the most common cause of morbidity, with a prevalence of 14.2% (29.2 million people) in the adult population in the United States. It is, therefore, more common than arthritis (12.47%) and hypertension (11.44%), according to data issued by the National Center for Health Statistics in 2004.

The causes of persistent rhinosinusal mucosa inflammation, which determines subsequent irreversible histological changes, are not fully understood.

Our study includes a total of 106 patients diagnosed with chronic rhinosinusitis with nasal polyposis (CRSwNP) and without nasal polyposis (CRSSNP), hospitalized in our ENT Department. The statistical analysis of the group in terms of the classical parameters: area of origin, age and gender, pointed out a homogeneity of the lot according to both age mean and variation; the average age of the entire group is 52.5 ± 14.6 years, and by gender the average age is 53 ± 13.1 years for women and $52,1 \pm 15,7$ for men; according to area of origin, the average age of patients from the rural areas is 52.3 ± 14.7 , and of those from urban areas is 52.6 ± 14.7 years.

The group consisted of 106 patients, including 45 females (42%) and 61 males (58%); 45 patients from rural areas (42%) and 61 from urban areas (58%). In the patients with chronic rhinosinusitis with or without nasal polyps, no significant differences by the area of origin or gender were recorded.

Of the 106 samples collected, 64 were positive (60.4%). In our study, there was no case in which we detected the presence of more than one bacterial strain. The results reported in the literature by different studies show a higher percentage of positive bacteriological samples. Thus, in a study published in 2012, Feazel⁷ had positive bacterial culture in all the patients with CRS analysis, with an average of 2.8 strains iso-

lated per patients; Cleland⁸ – 83% (with 0.95 average number of isolates detected per patient); Coffey⁹, in a study carried out on 144 patients who had undergone functional endoscopic sinus surgery, identified bacteria in 90% of the biological samples.

In our study, the lower percentage of positive samples could be explained by the fact that it was conducted on the patients admitted to the ENT Clinic of Craiova who had definitely followed outpatient antibiotic treatments.

The bacteria identified in our study were the following: *Staphylococcus aureus* (53.1%), *Staphylococcus epidermidis* (37.5%), *Escherichia coli* (4.7%), *Klebsiella* (3.1%) and *Proteus* (1.6%).

The data obtained differed from the ones in the specialty literature. Cleland⁸, in the above-mentioned study, identified the following germs: *Staphylococcus aureus* (35%), followed by *Pseudomonas aeruginosa* (9%), *Haemophilus spp.* (7%), and *Streptococcus pneumoniae* (5%) in revision patients. No difference was determined between CRSwNP and CRSSNP patients for any of the bacterial outcomes. Kingdom¹⁰ identified *Staphylococcus aureus* (24% of cultures), Gram-negative rods (25% of cultures) and *Pseudomonas aeruginosa* in 9% of cultures.

The large number of strains of *Staphylococcus aureus* isolated was consistent with the specialty literature data⁸⁻¹⁰.

We could notice the more and more frequent involvement of the strains of coagulase-negative *Staphylococcus epidermidis* in the sinus pathology. They proved their conditional pathogenic ability (opportunistic infections). This new aspect of the etiology of staphylococci emerged particularly in the last three decades, in direct relation with the widespread use of the invasive medical procedure.

We also determined the involvement of certain Gram-negative bacilli as etiological agents, as follows: 3 strains (4.7%) of *Escherichia Coli*, 2 strains of *Klebsiella* (3.1%) and a *Proteus* strain (1.6%); these samples represented 9.4%. We have to specify that a few years ago these bacilli were incriminated as etiological factors in chronic rhinosinusitis at a very low extent; we also noticed that lately they had occurred increasingly more in this type of infection, probably due to the decreased

reactivity of the body. Few studies in the literature have revealed the presence of Enterobacteriaceae in the nasal fossa in such a high percentage. In 2004¹¹, Yildirim published a study in which he identified at the bacteriological examination of nasal secretions of patients with CRS that coagulase-negative staphylococci (45.8%) had the highest proportion, followed by *Streptococcus pneumoniae* (16.7%), Enterobacteriaceae (16.7%), *Staphylococcus aureus* (10.4%), and *Pseudomonas aeruginosa* (10.4%).

In our study, we did not identify strains of *Pseudomonas aeruginosa*, although studies carried out on this subject identified this germ in 9-10% of patients with CRS.

Another parameter that we examined was the average duration of hospitalization, which was directly proportional to the clinical form of chronic rhinosinusitis: even in this case, there were no significant differences by the area of origin of the patients and gender.

About the germs isolated from the nasal secretion during the bacteriological examination, we found that there was a difference in duration of hospital stay of the patients, in the sense that the patients with CRS and Enterobacteriaceae had longer hospitalization periods: in *Klebsiella* presence, the duration of hospital stay was of 9.0 days (standard deviation = 2.8) and in *Escherichia coli* was of 8.3 days (standard deviation = 3.5). Both results were more above the mean of the study group (6.0 days with a standard deviation of 3.7). This means that the Enterobacteriaceae infection required a difficult treatment, the germs being, in general, more resistant to the antibiotics used in the patients with CRS.

Regarding the remaining 42 samples of nasal secretion (39.6%), we did not get any bacterial culture (nothing was developed in the collected samples), probably due to previous antibiotic treatment (our group consisted of patients hospitalized in ENT clinic and subjected to surgery; it was therefore very possible that they had completed ambulatory treatment with antibiotics).

CONCLUSIONS

1. Chronic rhinosinusitis patients were characterized by greater abundance of microorganisms in the nasal cavities.
2. We did not notice a predisposition to the disease by the area of origin or gender in the patients with chronic rhinosinusitis: 45 women (42%) and 61 males (58%). 45 patients are from rural areas (42%) and 61 from urban areas (58%).
3. The average age of the entire group is 52.5 ± 14.6 years, and by age and gender the mean is 53.0 ± 13.1 years for women and 52.1 ± 15.7 for men; by

area of origin, the average age of the patients from rural areas is 52.3 ± 14.7 , and of those from urban areas is 52.6 ± 14.7 years. We noticed group homogeneity in terms of both age mean and variation.

4. Out of the 106 samples (nasal discharge) collected, 64 were positive (60.4%); the absence of germs in the bacteriological examination of nasal secretion is due to the ambulatory antibiotic treatments taken by the patients with chronic rhinosinusitis.
5. The etiology of the positive samples was as follows: 34 strains of *Staphylococcus aureus* (53.1%), 24 strains of *Staphylococcus epidermis* (37.5%), 3 strains of *Escherichia coli* (4.7%), 2 strains of *Klebsiella* (3.1%), 1 *Proteus* strain (1.6%). We noted the high frequency of *Staphylococcus aureus*.

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REFERENCES

1. Lee L.N., Bhattacharyya N. - Regional and specialty variations in the treatment of chronic rhinosinusitis. *Laryngoscope.*, 2011;121(5):1092-1097.
2. IMS Health. Canadian Disease and Therapeutic Index (CDTI) Database. 2006.
3. Hastan D., Fokkens W.J., Bachert C., et al. - Chronic rhinosinusitis in Europe – an underestimated disease. A GA(2)LEN study. *Allergy.*, 2011;66(9):1216-1223.
4. Bhattacharyya N. - Bacterial Infection in Chronic Rhinosinusitis: A Controlled Paired Analysis. *American Journal of Rhinology.*, 2005;19:544-548.
5. Stephenson M.F., Mfuna L., Dowd S.E., et al. - Molecular characterization of the polymicrobial flora in chronic rhinosinusitis. *J Otolaryngol Head Neck Surg.*, 2010;39(2):182-187.
6. Fokkens W.J., Lund V.J., Mullol J., Bachert C., et al. - European Position Paper on Rhinosinusitis and Nasal Polyps, *Rhinology.*, 2012;supplement 23:1-298.
7. Feazel L.M., Robertson C.E., Ramakrishnan V.R., Frank D.N. - Microbiome complexity and *Staphylococcus aureus* in chronic rhinosinusitis. *Laryngoscope.*, 2012 February;122(2):467-472.
8. Cleland E.J., Bassiouni A., Wormald P.J. - The bacteriology of chronic rhinosinusitis and the pre-eminence of *Staphylococcus aureus* in revision patients. *Int Forum Allergy Rhinol.*, 2013 Aug;3(8):642-6. doi: 10.1002/alr.21159. Epub 2013 Mar 6.
9. Coffey C.S., Sonnenburg R.E., Melroy C.T., Dubin M.G., Senior B.A. - Endoscopically guided aerobic cultures in postsurgical patients with chronic rhinosinusitis. *Am J Rhinol.*, 2006 Jan-Feb;20(1):72-6.
10. Kingdom T.T., Swain R.E. Jr. - The microbiology and antimicrobial resistance patterns in chronic rhinosinusitis. *Am J Otolaryngol.*, 2004 Sep-Oct;25(5):323-8.