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Microbiology and Antimicrobial Sensitivity Patterns of Chronic Rhinosinusitis – 2020

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Introduction

Chronic Rhinosinusitis (CRS) is an inflammatory condition of nose and paranasal sinuses that lasts for more than 12 weeks. CRS represents a significant disease burden worldwide, affecting at least 11% of the population and consequently carrying with it a substantial economic burden to healthcare systems, to patients and to the economy from loss of productivity in the workplace [1,2]. According to "clinical practice guidelines" (update 2015) [3], and European Position paper on Rhinosinusitis (EPOS) 2020 diagnostic criteria for chronic rhinosinusitis is:- Twelve weeks or longer of two or more of the following signs and symptoms: Mucopurulent drainage (anterior, posterior, or both), nasal obstruction (congestion), facial pain-pressure-fullness or decreased sense of smell. Inflammation is documented by one or more of the following findings: Purulent (not clear) mucus or edema in the middle meatus or anterior ethmoid region, polyps in nasal cavity or the middle meatus and/or radiographic imaging showing inflammation of the paranasal sinuses [3,4]. Chronic Rhinosinusitis (CRS) is phenotypically divided into those cases with polyps (CRSwNPs) and those without (CRSsNPs) based on endoscopic findings. CRS with Nasal Polyps (CRSwNP) and CRS without Nasal Polyps (CRSsNP) also imply different therapeutic approaches with CRSwNP being more responsive to therapy with corticosteroids [5]. CRS is certainly not a classic infectious disease, but has a complex, multifactorial etiology. Ostiomeatal complex obstruction has long been deemed a major factor, but its actual role might have been overestimated and seems to be more important in CRS without Nasal Polyps (CRSsNP) compared to CRS with Nasal Polyps (CRSwNP). Patients with CRS continue to show decreased "quality of life" scores, comparable with or lower than those patients with chronic obstructive pulmonary disease [6,7]. While the precise mechanism(s) underlying the pathogenesis of CRS currently remain poorly understood, several mechanisms, mostly related to microorganisms, have been proposed and investigated extensively [8]. Chronic Rhinosinusitis (CRS) is a prevalent multifactorial disease process in which bacteria are believed to play a role in the propagation of inflammation. Recognition of the unique microbiology of CRS is of great importance when selecting the antimicrobial therapy for this condition.

Material and Methods

Prospective observational study done in the department of ENT & HNS GMC Srinagar and Department of Microbiology, GMC Srinagar for period of 2 years. Sixty patients who reported to ENT & HNS OPD of Govt. Medical College Srinagar and diagnosed with CRS according to "clinical practice guidelines" 2015 criteria (which is also in line with European position paper on rhinosinusitis, EPOS, 2020), and who were willing to participate in the study. In addition to above 60 patients 10 patients who were fungal culture positive were excluded from the study as per the exclusion criteria of this study.

Inclusion criteria

a) Patients fulfilling the diagnostic criteria of CRS with or without nasal polyposis.

b) Age ≥ 18 to ≤ 60 Exclusion criteria:

i) Patients with fungal rhinosinusitis

ii) Patients with sinonasal malignancy

iii) Patients of CRS with medical comorbidities.

Samples from patients of CRS with polyposis (CRSwNP) were taken in the form of Polyps and/or Inflamed sinonasal mucosa/crusts and/or Swabs of nasal secretions from osteomeatal complex/

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nasal cavity under endoscopic guidance. Samples from patients of CRS without nasal polyposis (CRSsP) were taken as swabs of nasal secretions from osteomeatal complex under endoscopic guidance and/or crusts from nasal cavity. Polyps and inflamed mucosa/crusts (in sterile containers containing normal saline) and swabs of nasal secretions were transported immediately to microbiological laboratory.

Microbiological examination

Samples were inoculated on 5% sheep blood agar, MacConkey and chocolate agar for bacterial culture and on Sabouraud's Dextrose Agar (SDA) for fungal culture. Plates were incubated at 370°C for 24 h. Organisms were identified by standard microbiological procedures including various biochemical tests. All isolates were subjected to antimicrobial susceptibility testing on Mueller Hinton agar based on CSLI guidelines.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA). Categorical variables were summarized as percentage; continuous variables were summarized as mean and Standard Deviation (SD). ANOVA was used to analyze the difference in a continuous measurement across treatment groups. All P values were 2 sided and a P<0.05 will be considered statistically significant.

Observations and Results

In this study, 60 patients were enrolled. Maximum number i.e. 19 patients (31.67%) were in the age group of 21 to 30 years. Minimum number i.e. 6 (10%) patients were in the age group of 51 to 60. Youngest patient in our study was aged 18 years and eldest patient was aged 60 years. Mean age in years was 33.05 with standard deviation of ± 12.216. Thirty seven (61.7%) patients were males and 23 (38.3%) patients were females male: female ratio of 1.6:1. All the three sample types i.e. polyp, crusts/mucosa and nasal secretion swab were collected from 35 (58.3%) patients followed by two samples i.e. mucosa/crusts and nasal secretion from 15 (25.0%) patients and one sample only i.e. swab of mucopurulent nasal secretion from 10 (16.7%) patients. Out of 60 patients under study 46 (76.7%) patients were positive on routine culture whereas in 14 (23.3%) patients they were negative. on microbiological examination distribution of Gram positive and Gram negative bacterial isolates showed Gram positive agents in 25 (54.34%) patients and Gram negative agents in 21 (45.65%) patient. Total no of Gram positive bacteria were 25, out of which 16 (64%) were *Staphylococcus aureus*, 7 (28%) were Coagulase negative *Staph aureus* (CoNS) and 2 (8%) were *Pneumococcus.* Out of 21 bacterial isolates who were Gram negative the most common bacteria was *Pseudomonas* seen in 10 (47.6%) isolates, followed by *Enterobacteriaceae* in 8 (38.1%) [*Klebsiella* 6 (28.6%), *Citrobacter* 1 (4.8%), *Enterobacter* 1 (4.8%)] and *Acinetobacter* in 3 (14.3%). Gram positive bacteria isolates of CRS patients were tested for antibiotic susceptibility pattern for commonly used antibiotics. *Staph aureus* showed maximum susceptibility each for vancomycin and linezolid (93.75%) followed by levofloxacin (87.5%), clarithromycin (87.5%), Cotrimoxazole (87.5%), Gentamycin (68.7%), Erythromycin (50%). For Coagulase negative *Staph aureus* (CoNS) Maximum susceptibility was shown for vancomycin (100%) followed by linezolid (85.7%) followed by clindamycin (71.4%), levofloxacin (71.4%), clarithromycin (71.4%), Gentamycin (57.1%), tetracycline (57.1%). *Pneumococcus* was mostly susceptibility for vancomycin (100%),

Table 1: Nasal specimen type examined for bacterial culture/sensitivity (C/S) and Biofilm growth (N=60).

Nasal Specimen type	No of specimens examined	Percentage
Polyp + mucosa /crusts + nasal secretion	35	58.30%
Mucosa/crusts + nasal secretion swab	15	25.00%
Nasal secretion swab only	10	16.70%
Total	60	100%

Table 2: Distribution of patients on the basis of culture report (N=60).

Bacterial culture of nasal samples	No of patients	Percent (%)
Positive	46	76.7
Negative	14	23.3
Total	60	100

Table 3: Distribution of Gram positive and gram negative bacterial isolates (N=46).

Total No. of bacterial isolates	Gram positive	Gram negative
46	25 (54.34%)	21 (45.65%)

Table 4: Distribution of Gram positive bacteria (N=25).

Nature of Gram positive bacteria	No. of isolates, n (%)
Staphylococcus aureus	16 (64%)
Coagulase negative *Staph aureus* (CoNS)	7 (28%)
Pneumococcus	2 (8%)
Total	25 (100%)

Table 5: Distribution of Gram negative isolates in CRS patients (N=21).

Nature of gram negative bacteria	No. of isolates - n (%)
Pseudomonas	10 (47.6%)
Acinetobacter	3 (14.3%)
Enterobacteriaceae	8 (38.1%)
- Klebsiella	- 6 (28.6%)
- Citrobacter	- 1 (4.8%)
-Enterobacter	- 1 (4.8%)
Total	21 (100%)

Table 6: Distribution of antibiotic susceptibility pattern of Gram positive bacterial isolates in patients of CRS (N=25).

Antibiotics	*Staphylococcus aureus* (n=16)	CoNS (n=7)	*Pneumococcus (n=2)*
Azithromycin	3 (18.7%)	2 (28.6%)	2 (100%)
Erythromycin	8 (50.0%)	3 (42.8%)	
Clindamycin	7 (43.7%)	5 (71.4%)	2 (100%
Chloramphenicol	6 (37.5%)	4 (57.1%)	
Levofloxacin	14 (87.5%)	5 (71.4%)	1 (50.0%)
Ofloxacin	5 (31.2%)		
Linezolid	16 (100%)	7 (100%)	2 (100%)
Gentamicin	11 (68.7%)	4 (57.1%)	1 (50.0%)
Rifampicin	10 (62.5%)	3 (42.8%)	-
Tetracycline	5 (31.2%)	4 (57.1%)	2 (100%)
Vancomycin	15 (100%)	7 (100%)	2 (100%)
Clarithromycin	14 (87.5%)	5 (71.4%)	2 (100%)
Cotrimoxazole	14 (87.5%)	-	2 (100%)

CoNS: Coagulase Negative *Staph aureus*

Table 7: Antibiotic susceptibility pattern of Gram negative bacterial isolates in CRS patients (N=21).

Antibiotics	*Pseudomonas aeruginosa* (n=10)	*Acinetobacter baumannii* (n=2)	*Entero-bacteriaceae* (n=8)
Amikacin	8 (80.0%)	1 (50%)	6 (75.0%)
Amoxycillin/ clavulanic acid	7 (70.0%)	2 (66.7%)	
Ciprofloxacin	4 (40.0%)	3 (100%)	7 (87.5%)
Levofloxacin			
Cefepime	4 (40.0%)	1 (50%)	
Ceftazidime	4 (40.0%)		
Colistin	10 (100%)	2 (100%)	7 (87.5%)
Gentamicin	8 (80%)		5 (62.5%)
Imipenem	9 (90%)	3 (100%)	6 (87.5%)
Meropenem	6 (60%)	3 (100%)	6 (87.5%)
Ampicillin	4 (40%)		
Tobramycin	7 (70%)		6 (87.5%)
Tetracycline			5 (62.5%)
Piperacillin/ Tazobactam			6 (87.5%)
Cotrimoxazole	-		6 (75.0%)
Clarithromycin			7 (87.5%)

Azithromycin (100%), Clindamycin (100%), Linezolid (100%), Cotrimoxazole (100%), Tetracycline (100%), and Clarithromycin (100%), levofloxacin (50%). Gram negative bacterial isolates were tested for antibiotic susceptibility for commonly used antibiotics. *Pseudomonas*, most common Gram negative organism in this study, was most susceptible to colistin (100%), followed by imipenem (90%), amikacin (80%) gentamicin (80%), co-amoxiclav (70%), tobramycin (70%). Sensitivity of *Pseudomonas* to Cefepime, Ceftazidime and Ampicillin was 40% for each. Enterobacteriaceae was maximum sensitive to Clarithromycin (87.5%), Colistin (87.5%), Imipenem (87.5%), Meropenem (87.5%) Tobramycin (87.5%), Tazobactam/ Piperacillin (87.5%) and Ciprofloxacin (87.5%) followed by Amikacin (75.0%), Cotrimoxazole (75.0%). Gentamicin (62.5%), Tetracycline (62.5%), *Acinetobacter baumannii* was susceptible to most of the antibiotics with 100% sensitivity to Ciprofloxacin, Chloramphenicol, Imipenem and Meropenem.

Discussion

In our study both CRSsNP and CRSwNP were taken as a single group to find out the microbiology of CRS. In this study in 46 (76.7%) patients bacteria culture of nasal samples was positive on routine culture whereas in 14 (23.3%) patients it was negative. In a similar study by Zhang Z et al. [8], titled "Biofilms and mucosal healing in post-surgical patients with chronic rhinosinusitis", cultures were grown from 13 (65%) of the 20 patients. In another study done by Glowacki et al. [9] titled "The influence of bacterial biofilm on the clinical outcome of chronic rhinosinusitis: A prospective, double blind, scanning electron microscope study" out of 33 study subjects 24 (72.7%) grew bacteria on routine cultures. The percentage of culture positivity in our study was more or less similar to the above mentioned studies.

Gram positive and gram negative bacterial isolates

In this study the distribution of Gram positive and Gram negative bacterial Isolates showed the predominance of Gram positive agents in 25 (54.34%) patients and Gram negative agents in 21 (45.65%) patients. In a similar study done by Prince AA et al. [10] titled "Prevalence of biofilm forming bacteria in chronic rhinosinusitis" isolated 138 bacterial strains from their study population. Gram positive bacteria 64 (46.4%) and Gram negative bacteria 74 (53.6%) with Gram positive to Gram negative ratio of 0.8:1. In a similar study by Glowacki et al. [9], titled "The influence of bacterial biofilm on the clinical outcome of chronic rhinosinusitis: A prospective, double blind, scanning electron microscope study" Gram positive bacteria were 86.7% and Gram negative were 13.3%. In another study by Zhang et al. [11], titled biofilms and mucosal healing in post-surgical patients with chronic rhinosinusitis" the Gram positive bacteria were 61.5% and Gram negative bacteria were 38.4%. In our study predominance of Gram positive bacterial growth was similar to most of the above mentioned studies In this study total no of Gram positive bacteria were 25 out of which 16 (64%) were *Staph aureus*, 7 (28%) were CoNS and 2 (8%) were *Pneumococcus*. In similar study by Sanderson AR, titled "Bacterial Biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis" observed that out of 7 gram positive isolates 4 (57.1%) were *Staphylococcus* and 3 (42.9%) were *Pneumococcus* [12]. In another study by Brook I [13] titled "Microbiology of chronic rhinosinusitis" isolated 93 gram positive bacteria out of which *Staph aureus* were 67/93 (72%), *Streptococcus pyogenes* were 14/93 (15%) and *Streptococcus pneumonia* were 12/93 (13%). Similar to above mentioned studies *Staph aureus* was most common gram positive bacterial isolate in our study. In this study 21 bacterial isolates were Gram negative. The most common gram negative bacteria was *Pseudomonas* observed in 10 (47.6%), followed by Enterobacteriaceae 8 (38.1%): [*Klebsiella* 6 (28.6%), *Citrobacter* 1 (4.8%), *Enterobacter* 1 (4.8%)] and *Acinetobacter* 3 (14.3%). In a similar study done by Prince AA et al. [10], 11/40 titled "Prevalence of Biofilm forming bacteria in chronic rhinosinusitis" *Pseudomonas* was predominant Gram negative bacteria present in 19 (66.3%) patients whereas other Gram negative bacteria were observed in 11 (33.7%) patients. In another study by Zhang et al. [11] titled Biofilms forming bacteria and quality of life improvement after sinus surgery" among 24 gram negative isolates 10 (41.7%) were pseudomonas and 14 (58.3%) were other gram negative rods. In above mentioned studies pseudomonas was most predominant Gram negative bacteria as observed in our study.

Antibiotic susceptibility pattern of gram positive bacterial isolates in patients of CRS

In this study Gram positive bacteria isolates of CRS patients were tested for antibiotic susceptibility pattern for commonly used antibiotics. *Staph aureus* showed maximum susceptibility each for vancomycin and linezolid (100%) followed by levofloxacin (87.5%), clarithromycin (87.5%), Cotrimoxazole (87.5%), Gentamycin (68.7%), Erythromycin (50%). For Coagulase negative *Staph aureus* (CoNS) maximum susceptibility was shown for vancomycin (100%) followed by linezolid (100%) followed by clindamycin (71.4%), levofloxacin (71.4%), clarithromycin (71.4%), Gentamycin (57.1%), tetracycline (57.1%). *Pneumococcus* was mostly susceptibility for vancomycin (100%), Cotrimoxazole (100%), Azithromycin 100%), Clindamycin (100%), Linezolid (100%), Tetracycline (100%), Clarithromycin (100%), levofloxacin (50%). In a study by Dodeja et al. [14], titled "Bacterial profile and antibiotic sensitivity in patients with chronic rhinosinusitis undergoing functional endoscopic sinus surgery: A prospective study" found that Gram-positive Cocci were 95.5% sensitive to Linezolid, 82.2% sensitive to Doxycycline, 75.5% sensitive

to Tetracycline, 71.1% sensitive to Cefoxitin and Co-trimoxazole, 64.4% sensitive to Erythromycin, and 53.5% sensitive to Ampicillin and Clindamycin respectively. In another study by Musa et al. [15], titled antibiotic sensitivity pattern of bacterial isolates in patients with chronic rhinosinusitis in kudana Nigeria). *Staph aureus* was 100% sensitive to co-amoxiclav and ciprofloxacin followed by erythromycin (88%) and levofloxacin (77.8%). *Streptococcus pneumonia* was 100% sensitive to co-amoxiclav, ciprofloxacin and erythromycin followed by 80% sensitivity to levofloxacin. Antibiotic susceptibility pattern in our study was different then above mentioned studies that may be in part due to regional variation, overuse and self-medications.

Antibiotic susceptibility pattern of gram negative bacterial isolates in patients of CRS

In this study gram negative bacterial isolates were tested for antibiotic susceptibility for commonly used antibiotics. *Pseudomonas*, most common gram negative organism in this study, was most susceptible to colistin (100%), followed by imipenem (90%), amikacin (80%) gentamicin (80%), co-amoxiclav (70%), tobramycin (70%). Sensitivity of *Pseudomonas* to Cefepime, Ceftazidime and Ampicillin was 40% for each. Enterobacteriaceae was maximum sensitive to Clarithromycin (87.5%), Colistin (87.5%), Imipenem (87.5%), Meropenem (87.5%) Tobramycin (87.5%), Tazobactam / Piperacillin (87.5%) and Ciprofloxacin (87.5%) followed by Amikacin (75.0%), Cotrimoxazole (75.0%). Gentamicin (62.5%), Tetracycline (62.5%), *Acinetobacter baumannii* was susceptible to most of the antibiotics with 100% sensitivity to Ciprofloxacin, Chloramphenicol, Imipenem, Meropenem, and Mezlocillin. In a similar study Dodeja et al. [14], in their study titled "Bacterial profile and antibiotic sensitivity in patients with chronic rhinosinusitis undergoing functional endoscopic sinus surgery: A prospective study" their study showed that Gram-negative bacilli were found to be most sensitive to Piperacillin Tazobactam (100%), followed by Amikacin, Ceftazidime, and Imipenem, which showed sensitivity of 86.9%. The isolated organisms were least sensitive (60.81%) to Ciprofloxacin. In another study by Musa et al. [15] titled antibiotic sensitivity pattern of bacterial isolates in patients with chronic rhinosinusitis in Kudana (Nigeria). Observed *Pseudomonas* was 100% sensitive to co-amoxiclav, ciprofloxacin and erythromycin. Antibiotic susceptibility pattern in our study was different then above mentioned studies that may be in part due to regional variation, overuse and self-medications.

Conclusion

Both Gram positive and Gram negative bacteria exist on the sinonasal mucosa of chronic rhinosinusitis patients. *Staph aureaus* was the most common Gram positive bacteria while as *Pseudomonas* was the most common Gram negative bacteria. Both Gram positive and Gram negative bacteria were susceptible to commonly used antibiotics and an empirical therapy with these agents can be started while waiting for cultural reports.

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