

## ORIGINAL ARTICLE

# A Comparative Study of Microbiology of Chronic Rhinosinusitis in Smokers and Nonsmokers

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## ABSTRACT

**Aim:** Comparative study of microbiology of chronic rhinosinusitis (CRS) in smokers and nonsmokers.

**Materials and methods:** This study was carried out on 700 patients diagnosed with CRS attending the ear, nose, and throat outpatient department (OPD) at Era's Lucknow Medical College, Lucknow, India, between January 2015 and June 2016. These patients were divided into two groups (smokers and nonsmokers). All patients underwent diagnostic nasal endoscopy. Two samples were collected and antimicrobial sensitivity test was done. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Chi-squared test and independent samples t-test were used to compare the data. A p-value <0.05 indicated a statistically significant association.

**Results:** Of 700 patients included in the study, smokers constituted 333 (47.57%) patients and nonsmokers constituted 367 (52.43%) patients. Out of the 700 patients, bacterial isolates of 585 (83.57%) were found to be positive, of which aerobes were 485 (82.91%) and the rest 100 were anaerobes. After antimicrobial therapy, all the symptoms were higher in smokers as compared with nonsmokers. Proportion of improvement in nonsmokers (90.19%) was higher as compared with smokers.

**Conclusion:** Microbiology of CRS is highly influenced by smoking habit. On evaluating the treatment response in terms of repeat sampling after 3 months, we found that pathogen positivity rate was much higher in smokers as compared with nonsmokers, thus implying that smoking exposure *in vivo* does alter the efficacy of antibiotics.

**Keywords:** Aerobes, Anaerobes, Chronic rhinosinusitis, Fungal, Microbiology, Rhinosinusitis, Smokers and nonsmokers.

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**Conflict of interest:** None

## INTRODUCTION

Rhinosinusitis (RS) is a group of disorders characterized by inflammation of the nose and the paranasal sinuses. The RS is classified into acute RS (ARS) (7 days to ≤4 weeks), subacute (4–12 weeks), recurrent acute ≥4 episodes of ARS per year, chronic (≥12 weeks), and acute exacerbation of chronic (sudden worsening of CRS with return to baseline after).<sup>1,2</sup> According to extrapolated figures, in India, the prevalence rates of CRS are close to 12.8% of the total population,<sup>3</sup> which is similar to the prevalence rate of 12.5% observed in US population.<sup>4</sup> The RS develops in relationship to infections or inflammation that occurs secondary to fungal or bacterial colonization<sup>5,6</sup>; trauma—primary or secondary, tobacco smoke exposure,<sup>7</sup> chronic or acute irritants or noxious chemicals, or iatrogenic factors including surgery, medication, nasal packing, or nasogastric tube placement. Smoking affects the normal mucociliary defense mechanisms. Smoke particles together with the substances like aldehydes, particularly formaldehyde and acrolein affect the cilia, decreasing mucociliary clearance. The microbiology of CRS includes both aerobic and anaerobe bacterial flora as well as fungi, influenced by exposure to direct or indirect smoking, presence or absence of nasal polyps, exacerbations, and administration of antimicrobials.<sup>8</sup> A preliminary study has shown an extraordinary rise in antibiotic-resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) in smokers than in nonsmokers.<sup>9</sup> The present study was carried out with an aim to evaluate and understand the microbiology of CRS between smokers and nonsmokers and see the effect of antimicrobial therapy on smokers and nonsmokers.

## MATERIALS AND METHODS

This longitudinal cross-sectional study was carried out on 700 diagnosed patients of CRS attending the OPD in the Department of Otorhinolaryngology and Head and Neck Surgery at Era's Lucknow Medical College and Hospital, Lucknow, India, between January 2015 and June 2016. These diagnosed patients of CRS were divided into two groups:

1. *Group I:* Smokers
2. *Group II:* Nonsmokers

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A predesigned questionnaire and pro forma were used to record the relevant information like patient's particulars, clinical findings, and investigation reports. Patients were exposed to complete history taking and thorough clinical examination. Written and informed consent was taken from patients. This study had been approved by the Ethical Committee of the college.

Patients diagnosed as cases of CRS according to the definition given by the European position paper on rhinosinusitis and nasal polyps 2012 (EPOS 2012)<sup>10</sup> and those who were active smokers were included in the study. Patients of ARS, using intranasal steroids and antihistamines, with active diseases of nose, with benign and malignant diseases of nose and paranasal sinus, and with systemic diseases like diabetes mellitus, tuberculosis, leukemia, and bleeding disorders were excluded from the study. All the patients of diagnosed CRS underwent diagnostic nasal endoscopy and specimens were collected through endoscopic-guided middle meatus swab. Two samples were collected, one for fungal isolation and the second for bacterial isolation. The time period between the collection and receiving of sample did not exceed 30 minutes. The specimen was sent to the Department of Microbiology and was processed as per standard procedures.<sup>11</sup> Antimicrobial sensitivity test was done by Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratories Standard Institute.<sup>12</sup> Antimicrobial therapy was given as per the standard protocol of EPOS 2012.<sup>10</sup>

**STATISTICAL TESTS USED**

The data were analyzed using SPSS version 23.0. Chi-squared test and independent samples t-test were used to compare the data. The confidence level of the study was kept at 95% and a p-value <0.05 indicated a statistically significant association.

**RESULTS**

All the 700 diagnosed patients of CRS attending the OPD in the Department of Otorhinolaryngology and Head & Neck Surgery were divided into two groups based on their smoking status: (i) smokers: those who were active smokers; (ii) nonsmokers: those who were neither active or passive smokers nor were exposed to smoke industrial or domestic.

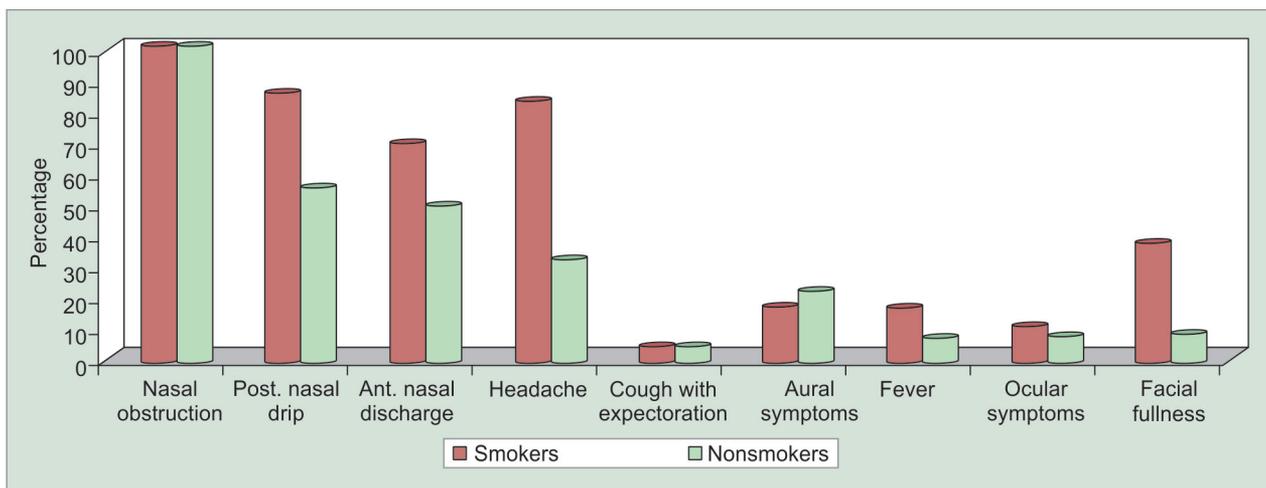
Of 700 patients included in the study, smokers constituted 333 (47.57%) patients and nonsmokers constituted 367 (52.43%) patients; the majority were males (n = 395; 56.43%) and the rest 305 (43.57%) were females. Male:female ratio was 1:0.77. Difference in gender of smoker and nonsmoker patients of CRS was found to be statistically highly significant (p < 0.001).

Comparison of symptomatology in smokers and nonsmokers showed all the patients, irrespective of smoking status, reported nasal obstruction. Posterior nasal drip, headache, facial fullness, and fever were more common in smokers, whereas cough with expectoration, aural symptoms (decreased hearing and heaviness and itching in ears) were higher in nonsmokers (Graph 1).

In majority of the patients of CRS (96.29%), fungal culture was found to be negative. Fungal culture of 26 patients was found to be positive. *Aspergillus flavus* was the most common fungal isolate (46.15%) while *Aspergillus fumigatus* (3.85%) was the least common isolate.

Out of 700 patients, bacterial isolates of 585 (83.57%) were found to be positive, of which aerobes were 485 (82.91%) and the rest 100 were anaerobes. Proportion of aerobic bacterial isolates was higher in smokers, while proportion of anaerobic bacterial isolates was higher in nonsmokers. This difference was found to be statistically significant (p < 0.001).

Before antimicrobial therapy, the proportion of aerobic bacterial isolates higher in smokers as compared with



**Graph 1:** Comparison of symptomatology in smoker and nonsmoker cases of chronic sinusitis

**Table 1:** Distribution of aerobic bacterial isolates in clinical cases of chronic sinusitis before antimicrobial therapy

Type of bacterial isolate	Total (n = 485)		Smokers (n = 283)		Nonsmokers (n = 202)	
	No.	%	No.	%	No.	%
<i>S. aureus</i>	180	37.11	123	43.46	57	28.22
<i>S. pneumoniae</i>	52	10.72	41	14.49	11	5.45
<i>S. viridans</i>	43	8.87	30	10.60	13	6.44
<i>Pseudomonas aeruginosa</i>	86	17.73	41	14.49	45	22.28
<i>Klebsiella</i> spp.	60	12.37	24	8.48	36	17.82
<i>Citrobacter</i> spp.	14	2.89	5	1.77	9	4.46
<i>Enterobacter</i> spp.	13	2.68	0	0	13	6.44
<i>Acinetobacter</i> spp.	16	3.30	8	2.83	8	3.96
<i>Moraxella catarrhalis</i>	21	4.33	11	3.89	10	4.95

$\chi^2 = 52.954$  (df = 8);  $p < 0.001$

nonsmokers were *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus viridans*, while proportion of aerobic bacterial isolates higher in nonsmokers as compared with nonsmokers were *Pseudomonas aeruginosa*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Moraxella catarrhalis*. This difference was found to be statistically significant ( $p < 0.001$ ; Table 1).

Before antimicrobial therapy, the proportion of anaerobic isolates higher in smokers as compared with nonsmoker patients was *Bacteroids*, *Clostridium* spp. and *Prevotella melaninogenica*, while proportion of nonsmokers was higher as compared with smoker patients for *Eubacterium*, *Fusobacterium*, and *Peptostreptococci*. This difference was not found to be statistically significant ( $p = 0.538$ ; Table 2).

After antimicrobial therapy, all the symptoms were higher in smokers as compared with nonsmokers, which was statistically significant (Graph 2).

After antimicrobial therapy, only 279 specimens of patients of CRS were isolated with aerobic bacteria, out of which 245 were smokers and only 34 were nonsmokers. Difference in aerobic bacterial isolates among smoker and nonsmoker patients of CRS after

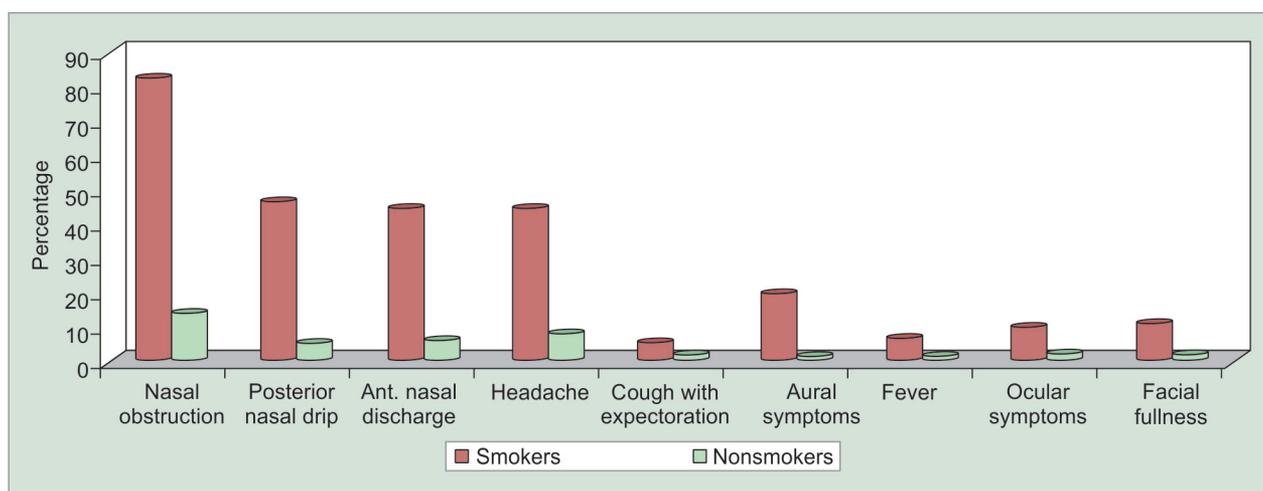
**Table 2:** Distribution of anaerobic bacterial isolates in clinical cases of chronic sinusitis before antimicrobial therapy

Type of bacterial isolate	Total (n = 100)		Smokers (n = 39)		Nonsmokers (n = 61)	
	No	%	No	%	No	%
<i>Bacteroids</i>	30	30.00	12	30.77	18	29.51
<i>Clostridium</i> spp.	8	8.00	4	10.26	4	6.56
<i>Eubacterium</i>	13	13.00	4	10.26	9	14.75
<i>Fusobacterium</i>	11	11.00	4	10.26	7	11.48
<i>Peptostreptococci</i>	36	36.00	13	33.33	23	37.70
<i>Prevotella melaninogenica</i>	2	2.00	2	5.13	0	0

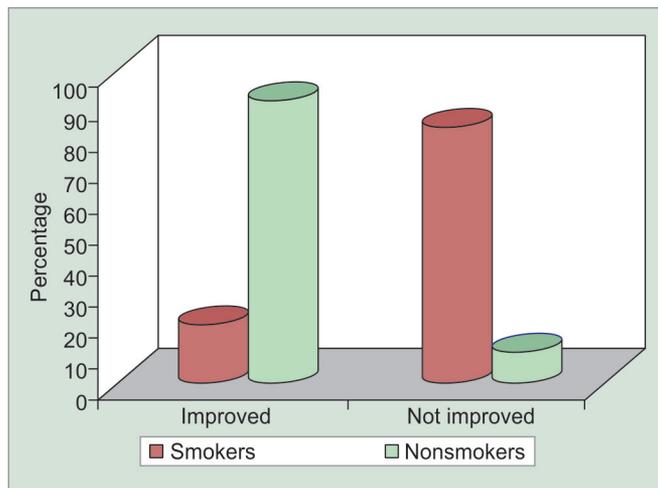
$\chi^2 = 4.076$  (df = 5);  $p = 0.538$

antimicrobial therapy too was found to be statistically significant ( $p < 0.001$ ).

After antimicrobial therapy, only 41 specimens were found to be positive for anaerobic bacteria. Of these, 36 (87.8%) were smokers and 5 (12.2%) were nonsmokers. Proportion of nonsmoker was higher as compared with smoker patients for isolation of *Bacteroids* (60.00 vs 30.56%) and *Fusobacterium* (20.00 vs 11.11%), rest of the anaerobic bacteria were isolated in higher proportions of smokers as compared with nonsmokers. This difference was not found to be statistically significant ( $p = 0.707$ ).



**Graph 2:** Symptomatology in patients of chronic sinusitis after antimicrobial therapy



**Graph 3:** Comparison of outcome of smoker and nonsmoker cases of chronic sinusitis

Out of 700 patients, 393 (56.14%) improved and in the rest 307 (43.86%), no improvement was seen. Proportion of improvement in nonsmokers (90.19%) was higher as compared with smokers (18.62%). Difference was found to be statistically significant ( $p < 0.001$ ; Graph 3).

Cefoxitin (99.64%) was found to be the most sensitive antibiotic, followed by amoxicillin/clavulanate (96.73%) and imipenem (95.71%), while metronidazole (14.29%) was least sensitive followed by ceftizidime (30.00%). Antibiotic drugs sensitive in majority of patients were cephalaxin, erythromycin, ciprofloxacin, ampicillin, ceftazidime/sulbactam, ceftriaxone, amikacin, piperacillin/tazobactam, imipenem, and amoxicillin/clavulanate (Table 2).

## DISCUSSION

By definition, CRS is a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 weeks duration.<sup>5</sup> Smoking affects the normal mucociliary defense mechanisms. Smoke particles together with the substances like aldehydes, particularly formaldehyde, and acrolein affect the mucociliary clearance and, thus, bring about pathological changes that result in inflammation and, as a result, high prevalence of CRS in both men and women.<sup>13</sup> Tobacco smoke exposure<sup>7</sup> is a known risk factor for CRS. However, the effects of tobacco smoke on CRS are less well documented. According to Shi et al<sup>14</sup> smokers are 50% more likely to have CRS as compared with nonsmokers.

The primary consideration during the study was to understand the effect of smoking on microbiology and antibiotic susceptibility; however, an attempt was also made to understand the clinical presentation of the disease owing to presence of smoking.

Majority of enrolled subjects in this study were males (56.43%). Male-to-female ratio was 1:0.77. The

epidemiological studies have a variable gender ratio in studies carried out in different parts of the world. In India, prevalence of smoking is higher in males as compared with females. The male:female ratio of smokers stands at 23.9.<sup>15</sup> In the present study too, there was a high prevalence of males as compared with females among smokers (60:1).

A significant difference in symptomatic profiles of smokers and nonsmoker patients with CRS was observed in our study. These differences in symptomatology could mainly be attributed to the difference in pathogenesis of CRS among smokers as compared with nonsmokers. Tobacco smoke irritates the delicate mucous membrane lining of the nasal passages resulting in inflammation and increases the amount of mucus secreted. It also damages the cilia, which are responsible for moving the mucus.<sup>16</sup>

In our study, KOH culture for *Candida albicans* was found to be positive in significantly higher proportion of smokers as compared with nonsmokers, thus showing fungal etiology. Although there is no clinical study reporting effect of smoking on candidal opportunistic infection and growth in cases of CRS, Soysa and Ellepola<sup>17</sup> attempted to explain the interaction between smoking habit and fungal infections especially in the context of oral candidosis. In this conceptual model, they explained how smoking facilitates the opportunistic infections like *Candida*. Sinus environment and mucosa are similar to oral environment.

In this study, a total of 585 (83.6%) specimens were culture-positive for bacteria and 26 (3.7%) were positive for fungal isolates. The bacterial positivity rate in the present study is similar to that reported by Su and Jiang,<sup>18</sup> who reported culture positivity rate of 81.9 and 80% respectively, among patients of CRS with and without nasal polyposis. Bhattacharyya,<sup>19</sup> in his study, reported bacterial positivity rate to be slightly higher at 87.8%. In the present study, aerobes (485/585; 82.91%) predominated over anaerobes (100/585; 17.09%); however, Bhattacharyya<sup>19</sup> in his study reported anaerobes to be dominating (59.1%) over aerobes (40.9%). In another study, Boase et al<sup>20</sup> reported all their CRS samples to be culture positive. However, like our study, they also reported the prevalence of anaerobes to be lower than aerobes. Mantovani et al,<sup>21</sup> in their study, did not report presence of anaerobes in any of their cases.

Our study has shown an overall higher bacterial positivity rate in smokers (322/333; 96.7%) as compared with nonsmokers (263/367; 71.7%). However, anaerobe positivity rate was significantly higher among nonsmokers. In a recent study by Brook and Gober,<sup>22</sup> potential pathogen recovery was reported to be higher among smokers as

compared with nonsmokers, which is similar to the findings in present study. However, they did not report any difference with respect to aerobic/anaerobic status. The higher prevalence of anaerobes in nonsmokers might be attributed to possible change in bacterial balance of oral and sinus environments. It is reported that cigarette smoking induces an anaerobic environment in the oral cavity.<sup>23</sup> It may be possible that owing to unfavorable environment in the oral cavity of smokers, the aerobes make their way to the sinus and thus, change the aerobic/anaerobic balance there.

Incidentally, despite the reported inhibitory effect of cigarette smoke on Gram-positive pathogens like *S. aureus*, it was found to be the most common aerobic isolate in smokers (43.46%) followed by *S. pneumoniae* and *P. aeruginosa* (14.49% each). Among nonsmokers *P. aeruginosa* and *Klebsiella pneumoniae* were more common (22.28 and 17.82% respectively). Although it is difficult to explain this variance based on currently available evidence, the variance in microbial profile in oral, respiratory, and sinus locations between smokers and nonsmokers<sup>20,22-24</sup> is reported occasionally, which needs extensive exploration further in order to build more specific rather than generalized evidence.

In our study, smoking was found to be significantly associated with symptomatic manifestation. Reh et al,<sup>25</sup> in their study, reported that smokers can have symptoms similar to CRS even in the absence of CRS. Eye irritation, nasal irritation, nasal congestion, and rhinorrhea have been reported to be the most frequent symptoms after smoke exposure.<sup>28</sup> In another study, Lee et al<sup>26</sup> also reported that smoking promotes eosinophilic inflammation and, thus, results in more severe symptoms in patients of CRS. The findings of the present study are in agreement with these observations.

In the present study, following treatment too, a significant difference in both number as well as spectrum of aerobic microbes was observed. After treatment, the number of samples positive for aerobes was much higher among smokers (n = 245) as compared with nonsmokers (n = 34). With respect to impaired treatment effect of antimicrobials, there is divided opinion among researchers; some researchers are of the view that cigarette smoking has an adverse impact on the antibiotic efficacy,<sup>27,28</sup> whereas some others are of the view that the evidence on this aspect is not clear.<sup>29</sup> The findings of the present study, however, tend to indicate that antibiotic resistance is higher in smokers.

The proportion of those showing improvement following treatment was much higher in nonsmoker group (90.19%) as compared with the smoker group (18.62%) in our study. Cigarette smoking tends to bring about

permanent changes that affect the antibiotic susceptibility and as such reduce the immunity of an individual. Tobacco smoke has immunosuppressive effects by suppressing monocyte-derived macrophage function as well as by inhibiting inflammatory cytokines by suppressing toll-like receptor-mediated pathways in human bronchial epithelial cells.<sup>30</sup> The findings of the present study showed that the adverse impact of smoking on CRS is long-lasting and interferes with the treatment response too.

In this study, a substantial number of isolates were *S. aureus*; however, a high sensitivity of Cefoxitin showed that MRSA rate was quite low. Kamath et al<sup>31</sup> also showed an MRSA prevalence of 9% only. In the present study, sensitivity for erythromycin was found to be 50.55% only, which is in agreement with the reported increasing trends of antibiotic resistance against erythromycin.<sup>32</sup> In the present study among Gram-positive isolates, the sensitivity rates were 100% for vancomycin, which is in agreement with Hasehmi et al.<sup>33</sup> In the present study, ceftriaxone was found to have 81.9% sensitivity against Gram-negative isolates. Farahani et al<sup>34</sup> also showed ceftriaxone to be highly sensitive ranging from 71.4% (for *Acinetobacter baumannii*) to 100% (for *S. pneumoniae*, *Corynebacterium diphtheriae*, and *Haemophilus influenzae*).

## CONCLUSION

This study indicated that microbiology of CRS is highly influenced by smoking habit. On evaluating the treatment response in terms of repeat sampling after 3 months, we found that pathogen positivity rate was much higher in smokers as compared with nonsmokers, thus implying that smoking exposure *in vivo* does alter the efficacy of antibiotics. It is an issue to be explored further. The present study provided some useful information regarding the microbiology of CRS among smokers and its impact on the treatment outcome. This is perhaps the first study of its type and needs further exploration. Further studies on this issue are recommended.

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