

The bacteriology of chronic rhinosinusitis and the pre-eminence of *Staphylococcus aureus* in revision patients

Edward John Cleland, MBBS, Ahmed Bassiouni, MBBCh and Peter-John Wormald, MD

Background: The role of bacteria in the etiopathogenesis of chronic rhinosinusitis (CRS) remains an area of interest. The impact of surgery and factors such as the presence of polyps, asthma, and aspirin sensitivity on the bacterial state are poorly understood. To determine the effect of these factors, this study examines the culture results from a large cohort of CRS patients.

Methods: This retrospective study used the culture results from 513 CRS patients, which were analyzed for species growth and compared to factors such as previous surgery, presence of polyps, aspirin sensitivity, and asthma. Univariate and multivariate logistic regression models were used for statistical analysis.

Results: Eighty-three percent (83%) of patients had a positive culture result. The average number of isolates detected per patient was 0.95. *S. aureus* was the most frequently cultured organism (35%), followed by *P. aeruginosa* (9%), *Haemophilus* spp. (7%), and *S. pneumonia* (5%). Revision patients were more likely to grow *S. aureus* ($p = 0.001$), *P. aeruginosa* ($p = 0.044$) and have a positive culture

($p = 0.001$). Asthma was correlated with a positive culture ($p = 0.039$). No difference was determined between polyp and nonpolyp patients for any of the bacterial outcomes.

Conclusion: This study highlights important factors in the bacteriology of CRS patients. *S. aureus* was the most prevalent species identified in our cohort, followed by *P. aeruginosa*. *S. aureus* rates of isolation were also significantly higher in patients undergoing revision surgery. No association was found between the presence of nasal polyposis and culture rates. © 2013 ARS-AAOA, LLC.

Key Words:

chronic rhinosinusitis; CRS; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; microbiology; primary; revision

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The role of bacteria as an environmental factor in the etiopathogenesis of chronic rhinosinusitis (CRS) is an area of ongoing interest. A definitive representation of the various organisms involved, and their importance, continues to be disputed. Previous studies have suggested that a number of specific organisms are particularly linked to disease recalcitrance. The species most commonly

implicated are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.¹⁻⁵ Surprisingly, while *P. aeruginosa* has been shown to be more frequently cultured in patients who progress to revision surgery,⁶⁻⁸ this relationship has not been conclusively demonstrated for *S. aureus*.

The detection of bacteria through culture-independent techniques such as 16S ribosomal RNA (rRNA) gene sequencing, is increasingly being used to investigate the microbiome of CRS patients.^{9,10} These detection techniques provide a sensitive means by which bacteria resident in a CRS patient can be identified. This is best highlighted by rates of species detection using a molecular technique that demonstrated an increased diversity of bacterial species by a magnitude of 10,⁹ when compared to standard cultures, and is also consistent with other reports in which it is proposed that only 1% to 10% of bacteria encountered in the environment are able to be cultured in the laboratory.¹¹ While 16S rRNA gene sequencing methods have the ability to reveal a greater biodiversity in samples, traditional cultures have been shown to correlate well with

Department of Surgery–Otorhinolaryngology, Head and Neck Surgery, University of Adelaide, Adelaide, Australia

Correspondence to: Peter-John Wormald, MD, Department of Otorhinolaryngology, Head and Neck Surgery, The Queen Elizabeth Hospital, 28, Woodville Road, Woodville, SA 5011, Australia; e-mail: peterj.wormald@adelaide.edu.au

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species abundance.¹² The cost benefit of traditional cultures vs molecular detection techniques, which demand specific equipment and laboratory skill sets must be considered.¹³ As a result, traditional cultures continue to play an important role in bacterial detection for both the clinical and research settings.

The objective of this research is to study, through standard cultures, the bacteriology of CRS patients undergoing primary and revision surgery. Through the power of a large cohort, and by controlling for clinical factors, we aim to investigate the prevalence and clinical associations of bacteria in CRS.

Patients and methods

Study design

This retrospective study uses prospectively collected data, from the tertiary referral practice of the senior author (P.J.W.). The local Human Research Ethics Committee gave approval for the study (2012052). All patients who underwent endoscopic sinus surgery (ESS) in the senior author's (P.J.W.) practice had cultures obtained at the time of surgery. Patients who underwent ESS for CRS between 2003 and 2011 as defined by the Rhinosinusitis Task Force¹⁴ were identified through the practice database. Patients were excluded if less than 18 years of age, immunocompromised, or diagnosed with cystic fibrosis or Kartagener's syndrome. Patients without microbiological investigations were not included.

The extent of surgery varied from middle meatal antrostomies with anterior ethmoidectomies (mini-functional ESS [mini-FESS]), to complete fronto-spheno-ethmoidectomies (full-house FESS). A number of revision patients with extensive severe disease received a Draf-III/frontal drillout procedure and/or canine fossa trephination (CFT) of the maxillary sinus. Before proceeding to surgery, all patients underwent a standard trial of medical therapy. This included a 3-week course of systemic prednisolone and a 2 month course of topical nasal steroid and saline washes.

Data collection

Samples for microbiological determination were harvested intraoperatively under endoscopic guidance by 2 means: a swab from the sinus cavity and/or direct retrieval of mucous (if present) and mucosal tissue from within the sinus cavity. Intraoperative samples were processed with standard techniques as set out by the Clinical and Laboratory Standards Institute.¹⁵

A database was created to collate demographics and microbiological results. These results were recorded for growth, and by species as indicated by the microbiology laboratory report. For several organisms identification did not extend beyond the genus level. In these cases this has been indicated as appropriate by convention. Isolates were recorded per person irrespective of the number of samples harvested.

TABLE 1. Demographics and disease characteristics of study population

Characteristics	Primary surgical patients (n = 162)	Revision surgical patients (n = 351)	Total (n = 513)
Mean age (SD)	51 (16)	51 (14)	51 (15)
Male/female	96/66	184/167	280/233
Nasal polyps, n (%)	53 (33)	192 (55)	245 (48)
Asthma, n (%)	47 (29)	160 (46)	207 (40)
Aspirin sensitivity, n (%)	3 (2)	35 (10)	38 (7)
Smoking, n (%)	10 (6)	23 (7)	33 (6)
Mean CT scores ^a	13	15	14

^aTotal Lund-Mackay score.

CT = computed tomography; SD = standard deviation.

Data analysis

Statistical analysis used a univariate logistic regression model. This was applied to the 2 patient groups (primary and revision) that were compared with respect to each bacteria outcome for the 5 most commonly cultured pathogenic bacteria and also for a positive culture result. When a significant result was determined, a multivariate logistic regression model was then applied to control for the potential confounders: age, sex, presence of nasal polyps, asthma, aspirin sensitivity, and smoking. All calculations were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC) and a *p* value <0.05 was considered significant.

Results

A total of 515 patients satisfied the selection criteria; 2 patients were excluded due to incomplete demographic data, reflecting the high standard of prospectively collected data. The demographic data for these patients is represented in Table 1. The total culture rate was 83%, with the predominant organisms being *S. aureus*, *P. aeruginosa*, *Haemophilus* spp., *S. pneumonia* and *E. coli*. A full list of organisms cultured including floras is shown in Table 2. The univariate logistic regression model yielded significant results between primary and revision patients for *S. aureus* (*p* = 0.001), *P. aeruginosa* (*p* = 0.024), and positive culture (*p* < 0.001) (Table 3). The results for *Haemophilus* spp., *S. pneumonia*, and *E. coli* did not achieve significance. After controlling for confounders with the multivariate logistic regression model (Table 4), the results for *S. aureus*, *P. aeruginosa*, and positive culture remained significant (*p* = 0.001, *p* = 0.044, and *p* = 0.001, respectively).

Discussion

This study is a retrospective review of the bacteriology results of CRS patients presenting for primary or revision ESS. The key findings are the significantly higher rates of

TABLE 2. Bacterial culture results

	Primary (n = 162)	Revision (n = 351)	Total (n = 513)
Total positive cultures	119	306	425
Average number of species cultured per patient	0.84	1.01	0.95
Bacteria			
Aerobic and facultative anaerobic bacteria			
Gram positive			
<i>Bacillus</i> sp.	0	1	1
<i>Corynebacterium</i> sp.	1	0	1
<i>Staphylococcus aureus</i>	40	138	178
<i>Streptococcus intermedius</i>	1	4	5
<i>Streptococcus pneumonia</i>	9	17	26
<i>Streptococcus Group G</i>	0	1	1
<i>Microaerophilic streptococci</i>	1	0	1
Gram negative			
<i>Acinetobacter</i> sp.	0	2	2
<i>Citobacter</i> spp.	2	1	3
<i>Coliform</i> sp.	0	1	1
<i>Enterobacter</i> spp.	4	7	11
<i>Escherichia coli</i>	11	12	23
<i>Haemophilus</i> spp.	7	28	35
<i>Klebsiella</i> spp.	2	12	14
<i>Moraxella catarrhalis</i>	4	6	10
<i>Proteus mirabilis</i>	8	11	19
<i>Pseudomonas aeruginosa</i>	7	37	44
<i>Serratia marcescens</i>	1	2	3
Flora			
Oral, skin and respiratory	36	68	104
Anaerobic bacteria			
Mixed anaerobe sp.	0	1	1
Other			
Miscellaneous organisms not otherwise identified	2	4	6

S. aureus (39% vs 25%) and *P. aeruginosa* cultures (11% vs 4%) in the revision group compared to primaries. A significant relationship was also shown in the revision group when considering all bacteria, where positive cultures (87% vs 73%) were more frequently observed.

For any discussion related to the rates of bacterial isolation, it is important to be mindful of what is represented by the culture rate for a specific species. Nadel et al.⁶ noted that culture rates can be described in a variety of ways. For example, some authors report species rates per total num-

ber of isolates. This can be misleading, particularly when multiple specimens from the same patients are obtained. To maintain clinical relevance in this study, species culture rates are expressed as the number of patients who cultured a particular species per total number of patients.

The total positive culture rate of 83% was comparable to Klossek et al.¹⁶ and Finegold et al.¹⁷ Another study by Desrosiers et al.¹⁸ had a considerably lower positive culture rate at 46%. The higher rate of positive culture in revision vs primary patients (87% vs 73%) was an

TABLE 3. Results of the univariate logistic regression model (revision vs primary)

Outcome variable	Odds ratio (revision vs primary)	95% Confidence interval	Chi-square	p
<i>S. aureus</i>	1.98	1.30–3.00	10.28	0.001
<i>P. aeruginosa</i>	2.61	1.14–5.99	5.12	0.024
<i>Haemophilus spp.</i>	1.92	0.82–4.49	2.26	0.133
<i>S. pneumonia</i>	0.87	0.38–1.98	0.12	0.733
<i>E. coli</i>	0.49	0.21–1.13	2.83	0.092
Positive culture	2.46	1.54–3.93	14.14	<0.001

TABLE 4. Results of the multivariate logistic regression models (revision vs primary)*

Outcome variable	Odds ratio (revision vs primary)	95% confidence interval	Chi-square	p
<i>S. aureus</i>	2.09	1.35–3.22	11.09	0.001
<i>P. aeruginosa</i>	2.39	1.03–5.59	4.07	0.044
Positive culture	2.41	1.47–3.94	12.19	0.001

*Controlling for age, sex, presence of nasal polyps, asthma, aspirin sensitivity, and smoking.

interesting finding in our study and may be indicative of an increased bacterial load in those patients with recalcitrant disease. This finding remained significant after controlling for confounders, which were subsequently tested separately to explore any further relationships. Of these, only asthma was found to be significantly associated with a positive culture in the univariate and multivariate models (in this instance revision surgery was also controlled); 87% of asthmatics had a positive culture vs 79% of nonasthmatics ($p = 0.039$).

Asthma itself has been identified as a predictor of poor outcome post-ESS,^{19,20} and in our cohort, 46% of revision patients were asthmatics. Nadel et al.⁶ reported no significant differences in sinus cultures between asthmatics and nonasthmatics. Others have shown higher rates of *S. aureus* colonization in a subgroup of polyp patients with asthma and also aspirin sensitivity.²¹ The role of *S. aureus* in this setting and its ability to provoke a proinflammatory response through superantigens has already been described.²² The systemic effect of this may be the exacerbation of asthma. Locally the heightened inflammatory state due to *S. aureus* superantigen activity, may be providing a hospitable environment for bacteria and thus be a driving force for the higher positive culture rates in these patients.

S. aureus was the most commonly detected species in our patients (35%). This correlated with the results of Nadel et al.⁶ who reported rates of *S. aureus* detection of 31% in a

cohort of 265 patients. Gittelman et al.²³ reported similar rates of *S. aureus* detection in an outpatient setting. Our detection rates of *S. aureus* contrasted with the results of Niederfuhr et al.,²⁴ who identified it in only 19% of CRS patients with biopsy and lavage samples. Higher rates of *S. aureus* detection (50%) have been seen in a recent molecular study using 16S rRNA gene sequencing. Interestingly though, in this work, the rate of *S. aureus* isolation by standard culture remained in the order of 18%.⁹

P. aeruginosa was another commonly isolated organism (9%). The rates of prevalence reported in the literature vary widely, with culture rates between 0%²⁴ and 17%.⁶ This is in stark contrast to the detection rate reported using a molecular technique. These authors demonstrated *P. aeruginosa* in a remarkable 93% of polyp, 92% of mucus, and 90% of turbinate samples.¹⁰

A large proportion of the study population were revision surgery candidates (68%) reflecting the nature of referrals seen in this tertiary rhinology practice. Significantly higher rates of *P. aeruginosa* were found in revision patients as previously described by Brook and Frazier.⁷ Other studies have also confirmed this relationship.^{6,8} Interestingly though, while *S. aureus* has been shown to be a predominant species in postsurgical infections,^{1,2,25–27} no study to our knowledge has identified a significantly higher rate of *S. aureus* detection in revision patients. It is likely the power of our large cohort has enabled us to elucidate this finding. If we consider revision surgery as a marker of disease recalcitrance, the higher rates of *S. aureus* and *P. aeruginosa* seen in this group are of great interest, and parallel the findings related to *S. aureus* biofilms and disease severity.^{4,5,20}

Comparisons between the data presented here and that found in control patients in other studies is limited due to methodological differences. The study already referenced by Niederfuhr et al.²⁴ included 21 controls. This group showed no significant differences in the bacteriologic features between those with and without polyps, and control patients. The major limitations of this study were the alternative inferior turbinate site from which these patients were sampled and the selection criteria for a normal control. In this case, patients being treated for nasal structural abnormalities were selected as controls, yet they had not been cleared of sinus disease radiographically. Klossek et al.¹⁶ also described the middle meatus bacteriology of a large cohort of controls ($n = 139$). They found the isolation of *Streptococcus* spp, *H. influenzae*, *S. pneumoniae*, *Prevotella* spp, *Fusobacterium* spp, and *S. aureus* to be significantly associated with CRS. Another study using a rigorous inclusion criteria and collection technique in a group of 14 patients undergoing Lefort I osteotomies revealed a sinus positive culture rate of only 18%. The organisms cultured in their cohort were coagulase negative staphylococci, *Citrobacter fundii* and polymorphic floras.²⁸ Key pathogens such as *S. aureus* and *P. aeruginosa* were notably absent.

When considering floras, we could not reproduce the findings of Schlosser et al.,²⁹ who found that coagulase negative staphylococcus was found with increased frequency

in patients with previously operated on frontal sinuses. Our results were not sinus-specific, which may have limited this comparison. Furthermore, no significant difference was found between polyp and nonpolyp patients for all of the bacterial outcomes, which was comparable with other studies.^{24,30} In particular though, *S. aureus* was not seen more frequently in polyp patients, which contrasted with the finding of Van Zele et al.²¹

Other considerations relating to the microbiology that have not been measured in this study include the effect of recurrent courses of antimicrobial agents. Patients with CRS frequently receive multiple courses of antibiotics. The impact of these antibiotics in selecting for organisms outside their spectrum of cover to create superinfections in CRS is not yet fully understood; it may, for example, contribute to higher rates of *P. aeruginosa* detection in some patients. The effect of topical intranasal corticosteroids is also of interest. The influence of these medications on culture rates is potentially widespread given their use in preop-

erative and postoperative patients, and is supported by one study which has shown that intranasal corticosteroids used preoperatively are associated with lower rates of bacterial recovery in revision patients.¹⁸

Conclusion

Several important relationships in regards to the microbiology of CRS patients have been recognized by this study. Revision patients are more likely to have a positive culture. *S. aureus* and *P. aeruginosa* are predominant pathogens in CRS patients, and for the first time, significantly higher rates of *S. aureus* have been demonstrated in a revision population. Its relationship with recalcitrance should therefore see ongoing attention in future research. ④

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