Increased carriage of virulence genes mediates enhanced pathogenicity of chronic rhinosinusitis-related S. aureus strains

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Abstract

Background There is growing evidence to implicate *Staphylococcus aureus* (*S. aureus*) in the pathogenesis of recalcitrant chronic rhinosinusitis (CRS). Our group has demonstrated the ability of *S. aureus* to internalise within mast cells in nasal polyps and this may mediate disease recalcitrance. We investigated carriage of virulence genes in CRS-related *S. aureus* strains and its influence on the bacteria's ability to localise and survive intracellularly. **Methods** *S. aureus* strains isolated from non-CRS controls (n=5), CRSsNP (n=4) and CRSwNP patients (n=4) were sequenced using short read paired sequencing and interrogated for carriage of virulence genes. A representative control and CRSwNP isolate were tested for intracellular survival in the LAD2 mast cell line to investigate phenotypic differences. **Results** Fifty percent of the CRSwNP group had deletion of the hld gene which may promote small colony variant formation or reduced enterotoxin production, and seventy-five percent expressed virulence genes associated with invasive disease. The CRSwNP isolate had a superior ability to localise intracellularly at 6 and 9 hours and showed a higher burden of *S. aureus* colony forming units at 24 hours. **Conclusions** When compared to non-CRS controls, CRS-related *S. aureus* strains demonstrate increased carriage of virulence genes. This appears to facilitate intracellular localisation of the bacteria conferring a survival advantage and enhancing pathogenicity. The latter may be partly due to a reduction in enterotoxin production and the acquisition of serine proteases splA and B and leukocidins E/D genes. This pathogenic *S. aureus* phenotype may manifest clinically with disease recalcitrance and refractoriness to antibiotics.

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Short title: Enhanced pathogenicity of CRS-related S. aureus

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ABSTRACT

Background

There is growing evidence to implicate *Staphylococcus aureus*(*S. aureus*) in the pathogenesis of recalcitrant chronic rhinosinusitis (CRS). Our group has demonstrated the ability of *S. aureus* to internalise within mast cells in nasal polyps and this may mediate disease recalcitrance. We investigated carriage of virulence genes in CRS-related *S. aureus* strains and its influence on the bacteria's ability to localise and survive intracellularly.

Methods

S. aureus strains isolated from non-CRS controls (n=5), CRSsNP (n=4) and CRSwNP patients (n=4) were sequenced using short read paired sequencing and interrogated for carriage of virulence genes. A representative control and CRSwNP isolate were tested for intracellular survival in the LAD2 mast cell line to investigate phenotypic differences.

Results

Fifty percent of the CRSwNP group had deletion of the hld gene which may promote small colony variant formation or reduced enterotoxin production, and seventy-five percent expressed virulence genes associated with invasive disease. The CRSwNP isolate had a superior ability to localise intracellularly at 6 and 9 hours and showed a higher burden of *S. aureus* colony forming units at 24 hours.

Conclusions

When compared to non-CRS controls, CRS-related *S. aureus* strains demonstrate increased carriage of virulence genes. This appears to facilitate intracellular localisation of the bacteria conferring a survival advantage and enhancing pathogenicity. The latter may be partly due to a reduction in enterotoxin production and the acquisition of serine proteases splA and B and leukocidins E/D genes. This pathogenic*S. aureus* phenotype may manifest clinically with disease recalcitrance and refractoriness to antibiotics.

KEY WORDS

Chronic rhinosinusitis

Nasal polyps

Staphylococcus aureus

Quorum-Sensing

Virulence Factors

INTRODUCTION

Staphylococcus aureus (*S. aureus*), a gram-positive commensal bacterium, is associated with a wide spectrum of pathology ranging from asymptomatic colonisation of the nares to being the leading cause of nosocomial

bacteraemia with an associated mortality of 15-60%/.[1] Due to the diverse phenotypic behaviour of*S. aureus* it has been difficult to characterise its involvement in diseases such as chronic rhinosinusitis (CRS). *S. aureus*colonises the nasal cavity in 64% of patients with nasal polyps (CRSwNP) compared with 33% of those without polyps (CRSsNP) and 20% in those without CRS.[2,3] A higher proportion of patients with CRSwNP demonstrate IgE towards *S. aureus* enterotoxins in their serum than those without CRS (22.6-32.5 % vs 6.7-14.3% in controls).[4] Culture of *S. aureus* pre- and post-operatively in patients with CRS is a poor prognostic indicator for disease recurrence and recalcitrance.[5] However, the factors responsible for the enhanced pathogenicity of *S. aureus*strains prevalent in difficult-to-treat CRS disease remain poorly understood.

S. aureus can persist in the nasal cavity of CRS patients, evading the immune system and the effects of antimicrobials.[6] It can achieve this through internalisation within host cells by localising within the intracellular space or creating extracellular biofilms.[7, 8] In 2015, our group made the novel observation that *S. aureus* internalises within mast cells in nasal polyps which could act as a reservoir of bacteria seeding into the extracellular space and driving chronic inflammation in CRSwNP patients.[9] To transform from a free-floating planktonic *S. aureus* phenotype into a biofilm or intracellular bacteria requires the expression of virulence genes.[10] These fall into three main categories including pore forming toxins known as exfoliative toxins, enzymatic toxins and superantigens.[11] Exfoliative toxins including α haemolysin and bi-component leukocidins are involved in lysing phagocytes and have been shown to be essential for intracellular survival and phagosome escape.[12,13] Exoenzymes promote biofilm formation and disruption of cell function.[14]Superantigens such as Staphylococcus enterotoxin B (SEB) have been shown to promote *S. aureus* uptake and degranulation in mast cells within CRS sinonasal tissue.[15] Most exert their toxicity by activation of T and B cells via binding human leukocyte antigen molecules which communicate with the variable β chain of the T cell receptor causing widespread inflammatory, type 2 cytokine release.[11]

Notably, *S. aureus* has limited ability to survive intracellularly. It has been reported that *S. aureus* from only seven out of twenty-three clinical isolates from patients' nares were able to survive intracellularly in a keratinocyte cell line.[16] Furthermore, recent evidence demonstrates an increased number of prophages (bacterial viruses) within the genome of*S. aureus* cultured from CRSwNP patients, providing additional virulence genes.[17] Given these findings, we prospectively set out to investigate the differences in virulence factor gene carriage in *S. aureus* isolates cultured from controls, CRSsNP and CRSwNP patients using short read genome sequencing and bioinformatics techniques. We then used a representative strain from the control and CRSwNP group to compare intracellular localisation in a mast cell model and determine which genes may be linked with intracellular survival.

METHODS

Subjects

Patients undergoing nasal endoscopic examination in Rhinological clinics and those undergoing endoscopic sinus surgery procedures by the senior authors (HASJ, PGH and RJS) at University Hospital Southampton between 8/12/2020-7/2/2022 were invited to participate. Subjects which met the EPOS 2020 criteria[18] for diagnosis of chronic rhinosinusitis were stratified into CRSsNP and CRSwNP, and those who with other diagnoses including allergic rhinitis, nasal masses and anatomical nasal obstruction were placed in the control group. Exclusion criteria included patients under 18 years of age, cystic fibrosis, primary ciliary dyskinesia, immune deficiency syndromes, inability to provide informed consent and those with blood borne viruses which could put the research team at risk including hepatitis B, C and human immunodeficiency virus. Demographic data including age, sex, atopic status, antibiotic and steroid use in the past month, medical history, history of asthma, and smoking habits was collected. Nasal swabs were taken either intraoperatively or under topical local anaesthetic in clinic from the middle meatal region.

Specimen testing and genomic sequencing

Swabs were spread onto *S. aureus* 24-hour brilliance agar plates (Oxoid, Basingstoke, UK). Blue coagulase positive colonies were selected and tested for the presence of catalase and DNAse. Coagulase, catalase and DNAse positive colonies were subjected to matrix assisted laser desorption/ionisation time of flight spectrometry to confirm the Staphylococcus genus. Positive *S. aureus* cultures were grown to the logarithmic growth phase in Rosewell Park Memorial Institute Medium 1640 (RPMI 1640; Life Technologies, Paisley, UK) at 37°C and frozen in the presence of 25% glycerol (VWR, Leicestershire, UK) at -80°C. All 11 collected*S. aureus* strains and 2 well characterised strains previously collected from a control and CRSwNP patient were prepared in RNA Shield (Zymo Research, Freiburg im Breisgau, Germany) and underwent 30x Illumina short read sequencing (MicrobesNG, Birmingham, UK).

Bioinformatics approach

Paired end DNA sequencing reads (contigs) were assembled using the Bactopia pipeline using *S. aureus* specific datasets from NCBI AMRFinderPlus, Ariba's getref reference datasets, RefSeqMashSketch and Genbank Sourmash Signatures.[19, 20] Post alignment, the assembled sequences were interrogated using AMRFinder to determine virulence factor presence.[21] Only genes demonstrating greater than 90% sequence coverage and 90% sequence homology were included in the results.

Intracellular survival assays

Well characterised reference *S. aureus* were grown in RPMI1640 at 37°C in the presence of 5% CO_2 until the exponential growth phase was reached. Absorbance at 600nm was calculated and extrapolated to a known colony forming unit (CFU) concentration.

LAD2 cells (a kind gift from Dr AS Kirshenbaum, Laboratory of Allergic Diseases, Bethesda, MD) were grown in antibiotic free STEMPRO-34 media (Life Technologies, Paisley, UK) containing 0.1 mM stem cell factor (SCF; PreproTech, London UK). *S. aureus* ($1x10^6$CFUs) was added at an MOI ratio of 1:1 LAD2 cells in 2 ml cell culture medium. The co-cultures were incubated for 3, 6 or 9 hours then centrifuged at 250 g for 10 minutes. The cell pellet was then resuspended with 0.5ml 20µg/ml lysostaphin (Sigma Aldrich, Dorset, UK) for 60 minutes. LAD2 cells were then pelleted at 250g for 10 minutes and washed in 1ml antibiotic free media three times. The washed LAD2 cells were then centrifuged at 250g for 10 minutes and the supernatant was streaked on a Columbia blood agar plate (Oxoid, Basingstoke, UK) to ensure no growth. The remaining pellet was resuspended in STEMPRO-34 media containing 0.1 mM SCF and 0.5% Triton X (Sigma Aldrich, Dorset, UK) and vortexed for 10 minutes. The remaining supernatant was used to perform serial CFU assessments.

Analysis of intracellular survival at 24 hours was conducted by infecting LAD2 cells as above, and culturing for 6 hours. Cells were then washed with 20µg/ml lysostaphin for 60 minutes to eradicate extracellular bacteria as above and the cells washed in antibiotic free media three times. Co-culture continued until the 24-hour timepoint at which point the cells were resuspended in medium with 0.5% Triton X-100 and vortexed for 10 minutes. The lysate was subjected to serial CFU enumerations as described above.

Confocal microscopy

LAD2 cells were co-cultured with the reference control and CRSwNP*S. aureus* strains as above for 6 hours. Cells were resuspended in 0.5ml 20 µg/ml lysostaphin for 60 minutes and washed in calcium and magnesium free phosphate buffered saline (PBS) three times. Cells were then resuspended in 15 µM Syto9 and 40µM propidium iodide in 1 ml PBS (Thermo-Fisher, Basingstoke, UK). A 50µl aliquot of each suspension was placed on an Ibidi 8-well glass bottom slide (Thistle Scientific, Glasgow, UK) and imaged using a Leica TCS SP5/8 inverted confocal microscope (Lecia Microsystems, Milton Keynes, UK) using a 63x glycerol immersion lens. Images were collected with Leica LAS-AF software and analysed with Fiji 2 [(22)].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software Inc, San Diego, Ca, USA) and SPSS (IBM, Portsmouth, UK). Data was assessed for normality using histogram plots and normality tests. Pearson Chi-squared and one-way Anova tests were used to compare demographic data. Paired t-tests were used for to compare the differences between *S. aureus* strain co-cultures.

Study approval

All methods were carried out in accordance with relevant guidelines and regulations. Ethical approval was obtained for the study via the London- Hampstead Research Ethics Committee (REC reference: 20/PR/0183). Informed written consent was obtained from all participants prior to inclusion in the study. Relevant non-infected control samples were used for all experimental techniques as a means of validating observed trends.

RESULTS

Patient Demographics

Nasal swabs were taken from Forty-four patients who were enrolled in the study. In total 17 control, 10 CRSsNP and 17 CRSwNP patients participated. *S. aureus* was cultured in 4 control, 4 CRSsNP and 3 CRSwNP patients. There was a higher proportion of asthmatic patients within the CRSwNP group which was found to be statistically significant at 58.8% compared with 17.6% in the control and 20% in the CRSsNP group (p=0.023). There was a larger number of smokers in the control group (23.5%) with no smokers in the CRSsNP or CRSwNP groups(p=0.034). Other demographics showed no statistically significant differences between the groups (Table 1). A subgroup analysis was performed on subjects from whom *S. aureus* was cultured. There was no significant difference in demographics including age, procedure, antibiotic use, steroid use, airborne allergies, asthma presence and smoking status (Table 2).

Bacterial genome sequencing

Illumina short read sequencing was completed for 11 *S. aureus*strains identified from 4 control subjects, and 4 CRSsNP and 3 CRSwNP patients. Two previously collected, well characterised *S. aureus*strains from a patient without CRS (Control 1) and a CRSwNP strain (CRSwNP 1) were also sequenced. Each paired read sequence was reconstructed and aligned using bacterial alignment and analysis pipeline, Bactopia. Each assembled genome spanned between 2.67 and 2.77 mega base pairs.

Virulence genes

Bi-component gamma haemolysin, *hlgA/B* and *hlgB/C* , polysaccharide intracellular adhesin biosynthesis/export protein (*icaC*) and aureolysin (*aur*) genes were ubiquitous throughout the groups. Reduced carriage of delta haemolysin (*hld*) and staphylococcal complement inhibitor (*scn*) genes were observed in the CRSwNP group when compared with the control and CRSsNP cohort. CRSsNP strains appeared to have reduced carriage of virulence genes with none exhibiting leukocidin E/D (*lukD/E)* , serine protease A/B (*splA, splB)* , enterotoxins n,u (*sen, seu*) or enterotoxin like protein X (*selX*), and only one exhibiting *Sei/m* . In contrast 75% of CRSwNP strains carried *lukE/D* genes and serine protease (*splA, splB)* (Figure 1).

Intracellular localisation and survival

Control 1 and CRSwNP 1 which closely matched the virulence gene carriage patterns of control and CRSwNP groups were selected for further study of intracellular survival in a *S. aureus* -LAD2 mast cell infection model. Intracellular survival increased until 6 hours after which there were less viable bacteria. There were marked differences between the two strains, with no viable bacteria noted at 3 hours in the reference strain but growth in the CRSwNP strain. There was a significant increase in the CRSwNP strain survival at 6 and 9 hours of 1.89 (p=0.0087) and 9.84 (p=0.0088).

Given the reduction in intracellular bacteria after 6 hours which appeared to be due to mast cell death (unpublished data), we eradicated extracellular bacteria using lysostaphin and cultured cells for 24 hours. We noted persistence and a significant increase in intracellular CFUs in both the control (97-fold) and CRSwNP (2570-fold) strains. The CRS strain showed an 11.4-fold increase in intracellular survival when compared with that of the control strain (p=0.035) (Figure 2).

Confocal microscopy

Following infection for 6 hours, cells were stained with BacLight Live/Dead stain and imaged using confocal microscopy (Figure 3). The images confirmed intracellular localisation of the bacteria within the cytoplasm of mast cells. For the CRSwNP strain there was a large number of bacteria within both live mast cells and apoptotic cell bodies when compared to the control strain (Figure 3). The commensal organism infected between 14-25% of cells while the pathogenic strain infected 52-57% of cells (p=0.18).

DISCUSSION

Our findings suggest that there are differences in virulence gene carriage between *S. aureus* cultured from control subjects and patients with CRSsNP or CRSwNP. In the case of CRSwNP isolated *S. aureus* these differences may translate into the ability to localise and replicate intracellularly, thus conferring a bacterial survival advantage.

Phenol soluble modulins such as delta haemolysin gene (*hld*) are specific to *S. aureus* bacteria and induce neutrophil and erythrocyte lysis.[23] We noted its absence in 50% of CRSwNP isolates but presence in all other isolates. Deletion of*hld* has been shown to nearly abolish transcription of the accessory gene regulator (*agrA*) transcription and downstream virulence genes including alpha toxin, beta haemolysin and enterotoxin B and gamma haemolysin.[24] Given that mutations in the accessory gene regulator locus have been shown to create senescent bacteria known as small colony variants with reduced virulence gene expression and superior intracellular translocation in epithelial cells, the absence of *hld* may allow for similar pathogenic behaviour thus establishing an intracellular source of infection.[25-27]

Immune modulators including aureolysin which have been shown to cleave the C3 protein of the classical and alternative complement pathways were well preserved within the groups. Staphylococcal complement inhibitor (scn) was less frequently carried by the CRSwNP strain. Complement lowers the threshold for B-cell activation and antibody formation; hence the absence of staphylococcal complement inhibitor could lead to greater antibody formation towards *S. aureus* as seen in CRSwNP.[3, 28]

Exfoliative virulence factors involved in pore formation in host cells including bi-component gamma haemolysin components (*hlgA, hlgB HlgC*) were well conserved throughout all isolate groups. However, leukocidin E/D (*LukE/D*), which is not under control of the accessory gene regulator locus, was carried by 40% of control and 75% of CRSwNP isolates but no CRSsNP isolates. Both toxins have been shown to create pores within target neutrophils and erythrocytes while leukocidin E/D has been shown to induce calcium channel activation in neutrophils independent of its pore forming function leading to cell death.[29] Furthermore, leukocidin E/D can deplete the adaptive immune response by lysing polymorphic neutrophils leading to failure of local immunity and was essential for bacterial growth and seeding in a mouse model of bacteraemia.[30] Often found in invasive *S. aureus* disease, serine proteases (splA, splB and splE) have been shown to cleave the mucin 16 glycoprotein which forms a mucosal barrier. There carriage was abundant in the CRSwNP group (75%) but not seen in the CRSsNP group and only in 40% of the control group.[31] Furthermore, serine proteases (splA, splB) and leukocidins E/D (*lukE/D*) were absent in presence of the enterotoxin gene cluster and may suggest different phenotypic behaviours between these strains. This data would be in keeping with the findings of Nepal *et al* who recently demonstrated greater number of CRSwNP isolates carrying the Sa3int prophage which contains lukE/D genes, when compared with controls and CRSsNP patients.[17]

Superantigens including the enterotoxin gene cluster sei, sem, sen, seo seu were carried by 60% of control isolates, 25% of CRSwNP isolates and only minimally present in a CRSsNP isolates. These enterotoxins

are associated with long term colonisation in the nasal airways, cystic fibrosis lung and atopic dermatitis wounds, and were found at a similar carriage frequency in our control group.[32,33]Furthermore, they have been shown to stimulate T cell proliferation, but conversely act like decoy targets for antibodies protecting *S. aureus* from attack.[33]

As greater numbers of intracellular bacteria have been observed within CRSwNP than CRSsNP tissue, we selected a strain from the control group and CRS group which represented the virulence gene pattern observed.[7] When investigating intracellular survival, we confirmed that a representative CRSwNP *S. aureus* strain carrying serine proteases (splA, splB) and leukocidins E/D without the enterotoxin gene cluster was better able to internalise, survive and replicate within the LAD2 cell line than a representative control strain carrying the enterotoxin gene cluster with absent serine protease (splA, splB) and leukocidinE/D genes. Confocal microscopy confirmed the intracellular localisation of the bacteria and demonstrated a large number of apoptotic mast cells with a number of live bacteria residing within them when compared to the control group. Hence the CRSwNP*S. aureus* phenotype may further support its own survival by using dead cell bodies to protect itself from the effects of antibiotics and the immune system.

There may be a survival advantage for *S. aureus* in a CRSwNP environment to shed the *hld* gene and enterotoxin gene cluster allowing a more senescent phenotype with reduced virulence factor production and increased intracellular survival. Furthermore, the carriage of *LukE/D* may well promote the Th2 environment as this has been shown to skew the immune response, depleting memory T lymphocytes thereby reducing the number of IL-17 and IFN-gamma producing cells, and impairing bacterial clearance.[13,34] The CRSsNP group *S. aureus* genomes did not carry *lukE/D* or*splA/B* genes, with only one isolate demonstrating minimal gene carriage from the enterotoxin gene cluster. This may lead to lower levels of IgE raised towards *S. aureus* enterotoxins, and could explain why CRSsNP patients have lower serum IgE levels towards *S. aureus* enterotoxins than CRSwNP patients.

Sequencing large numbers of virulence factors from genomic samples is likely to demonstrate significant heterogeneity which makes statistical significance testing difficult without large cohorts. Nevertheless, our findings complement existing sequencing data and further our understanding of mechanisms of enhanced pathogenicity for *S. aureus* in CRS.

AUTHOR CONTRIBUTIONS

SPG, AFW, RJS contributed to study conception and design. RJS, PGH, HASJ were responsible for tissue sample and data collection. Sample preparation, data collection and analysis were performed by SPG, LCL. The first draft of the manuscript was written by SPG and all authors advised on previous drafts. All authors approved the final manuscript. All authors have read and approved the final version of the manuscript.

DECLARATIONS OF INTEREST

None

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FIGURE LEGENDS

Figure 1. Virulence gene carriage in control, CRSsNP and CRSwNP*S. aureus* isolates

Heatmap demonstrating the presence (red) or absence (white) of virulence genes from each isolate.

Figure 2. *S. aureus* intracellular invasion, localization and replication within LAD2 mast cells.

Intracellular survival of *S. aureus* co-cultured with LAD2 mast cells. A control *S. aureus* strain (blue) was compared with a CRSwNP *S. aureus* strain (yellow) at time points of 3, 6, 9 hours (n=9, mean + SEM,*p[?]0.05, ** p[?]0.01). Extracellular bacteria was eradicated at 6 hours for the control (blue/grey checkboard) and CRSwNP isolates (yellow/grey checkboard) using lysostaphin and the culture continued to 24 hours (n=6, mean + SEM, *p[?]0.05).

Figure 3. Confocal imaging of BacLight Live/Dead staining of LAD2 co-cultures with a control and CRSwNP *S. aureus* strain.

Control *S. aureus* strain . Two images of co-infection of LAD2 cells with a control *S. aureus* strain. Few *S. aureus*bacteria appear within the intracellular compartment (red arrows) with some dead bacteria seen within the cell (purple arrows).

CRSwNP *S. aureus* strain . Two images of co-infection of LAD2 cells with a CRSwNP *S. aureus* strain. Significant numbers of*S. aureus* appear within live cells (red arrows) with multiple apoptotic cell bodies containing a large number of live and dead bacteria (blue arrows).

Live bacteria and live cell nuclei compartments stain green while dead bacteria and dead nuclei stain red.

TABLES

Table 1 Demographic profile of study population

Disease type	Control	CRSsNP	CRSwNP	p value
Number of subjects	17(38.6%)	10(22.7%)	17(38.6%)	-
Gender (M:F)	10:7	6:4	9:18	.917 *
Mean Age (SD)	52.4 (15.7)	50.8 (17.4)	46.5 (16.0)	.563 +
Clinic review/ procedure	Clinic review 1 Biopsy 3 Septoplasty/PETS 9 Limited FESS 4 Full house FESS 0	Clinic review 2 Biopsy 0 Septoplasty/PETS 1 Limited FESS 5 Full house FESS 2	Biopsy 0 Septoplasty/PETS 0 Limited FESS 0 Full house FESS 16 Clinic review 1	N/A
Antibiotic use in past month	1 (5.88%)	1 (10%)	4 (23.5%)	.302*
Steroid use in past month	1 (5.88%)	0	4 (23.5%)	.117*
S. aureus culture	4 (23.5%)	4 (40%)	3 (17.6%)	.563*
Airborne allergies	6 (35.2%)	3 (30%)	8 (47.0%)	.637*
Asthma	3 (17.6%)	2 (20%)	10 (58.8%)	.023*
Ex-smokers	2 (11.8%)	0	4 (23.5%)	.243*
Current Smokers	4 (23.5%)	0	0	.034*

* Pearson Chi-squared, + one-way Anova, PETS (Powered Endoscopic Turbinoplasties), FESS (Functional Endoscopic Sinus Surgery)

Table 2 Demographic profile based on sub-analysis *S. aureus* culture status

Culture of *S. aureus*	No growth	Growth	p value
Number of subjects	33 (75%)	11 (25%)	-
Gender (M:F)	21:11	4:8	.054*
Age	49.3	51.0	.75+
Clinic review/ procedure	Biopsy 1 Septoplasty/PETS 6 Limited FESS 9 Full House FESS 14 Clinic review 2	Biopsy 2 Septoplasty/PETS 4 Limited FESS 0 Full House FESS 4 Clinic review 1	N/A
Antibiotic use in past month	5	1	.53*
Steroid Use in past month	4	1	.69*
Airborne allergies	12	5	.80*
Asthma	11	4	.948*
Ex-smokers	5	1	.508*
Current smokers	3	1	.89*

* Pearson Chi-squared, + one-way Anova, PETS (Powered Endoscopic Turbinoplasties), FESS (Functional Endoscopic Sinus Surgery)