

1 **Antibiotic concentrations in the sinonasal secretions and tissue in CRS**
2 **patients after oral therapy: a randomized trial**

3 Short title: Sinonasal antibiotic concentrations in CRS

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22 Financial disclosures: This study was supported by a grant from the Garnett Passe and Rodney

23 Williams Memorial Foundation

24 Transparency declarations: None to declare

25

26 Keywords: Sinusitis; Bacteria; Microbiota; Antibiotics; Antibiotic resistance; Macrolides; Tetracyclines

27 Abstract

28 **Background:** Despite the widespread prescription of antibiotics for patients with
29 chronic rhinosinusitis (CRS), the extent to which drug distribution to the sinonasal
30 mucosa influences their efficacy remains largely undefined.

31 **Methods:** Thirty subjects undergoing functional endoscopic sinus surgery (FESS) for
32 bilateral CRS were randomized to one of three groups: 1) doxycycline (100 mg daily
33 for seven days) 2) roxithromycin (300 mg daily for seven days) and 3) control (no
34 antibiotics given). Drug levels were measured using liquid chromatography-tandem
35 mass spectrometry in sinonasal secretions, sinonasal tissues and serum at steady
36 state. Nasal endoscopy (Modified Lund-Kennedy) and Gastrointestinal Symptom
37 Rating Scale (GSRS) scores were recorded.

38 **Results:** Antibiotic concentrations in the nasal secretions were significantly lower
39 compared to those in the serum and tissue (mean mucus/serum ratio at steady state
40 = 0.16 and 0.37 for doxycycline and roxithromycin respectively; $p < 0.01$). A short
41 course of antibiotic intake did not correlate with any difference in clinical outcomes
42 except where slightly higher GSRS scores were reported in the roxithromycin group
43 ($p = 0.04$).

44 **Conclusions:** Although the efficacy of doxycycline and roxithromycin in sinonasal
45 mucus *in vivo* cannot be predicted solely from reported minimum inhibitory
46 concentrations, given the added complication of bacterial biofilm antimicrobial
47 tolerance, these results suggest that low mucosal penetration of antibiotics may be
48 one of the factors contributing to the limited efficacy of these agents in the treatment
49 of CRS.

50

51 INTRODUCTION

52 Despite the widespread use of antibiotics in the treatment of chronic rhinosinusitis
53 (CRS), their efficacy for this indication remains debatable. There is increasing
54 evidence that the repeated use of broad-spectrum antibiotics is associated with both
55 microbial dysbiosis and the emergence of resistant bacterial strains(1-6). The
56 changes that occur in the sinonasal microbiota during oral antibiotic treatment in
57 CRS patients are poorly understood, and the microbiological effect of antibiotics at a
58 molecular level have not been correlated with clinical outcome measures.

59 Chronic rhinosinusitis (CRS) represents a spectrum of disorders that result from a
60 variety of immunopathological mechanisms that lead to persistent inflammation of
61 the paranasal sinus mucosa. Its clinical classification in two broad groups: CRS with
62 nasal polyps (CRSwNP) and CRS without NPs (CRSsNP) provides insight into the
63 severity of disease, extensiveness of surgery required, and the efficacy of medical
64 therapies. On a cellular level, both of these phenotypes are characterized by
65 epithelial disruption, ciliary dysfunction, mucus gland hyperplasia, bacterial
66 overgrowth and the formation of biofilms(7). The role of microbes in the
67 pathogenesis of CRS is largely unknown, but bacteria probably contribute to the
68 persistence and severity of the disease(8). The International Consensus Statement
69 on Allergy and Rhinology recommends the use of oral macrolides as an option in the
70 treatment of CRS without nasal polyps (CRSsNP) on the basis that they have shown
71 at least temporary benefit in some studies, reducing endoscopy scores and
72 improving symptoms(9). A reduction in polyp size with doxycycline has been
73 demonstrated in patients with polyps (CRSwNP), but no difference was found in
74 patient-reported outcomes(10). The benefit of a short-term period (<3 weeks) of oral
75 antibiotics is particularly unclear(9). Several studies report that a short-term use of

76 antibiotics from various antibiotic classes improve clinical symptoms such as nasal
77 discharge and nasal blockage but there is a lack of clinical trials showing a direct
78 benefit in improving the patient's intraoperative condition(11-13).

79 The extent to which drug distribution to the sinonasal mucosa influences the efficacy
80 of oral antibiotics in patients with CRS remains largely undefined. In vitro bacterial
81 susceptibility testing does not take into account the pharmacokinetics of the
82 antimicrobial agent and the variability in drug distribution to various sites in the
83 body(14). Current analytical methodologies and instrumentation allow accurate
84 quantitative analysis of drug concentrations in nasal secretions and tissue by
85 chromatography and spectrometry(15-30). However, few studies have used such
86 methods to determine sinonasal concentrations of antibiotics used in CRS,
87 particularly those from the macrolide and tetracycline groups(26, 31, 32). Although
88 the studies performed to date have suggested a therapeutic concentration of
89 antibiotics greater than reported minimum inhibitory concentrations (MIC) was
90 achieved, there is little evidence supporting their efficacy *in vivo*.

91 This randomized control trial aimed to determine the concentrations of two
92 commonly prescribed antibiotics, doxycycline and roxithromycin, in the nasal
93 secretions, serum and sinonasal tissues of CRS patients following a one-week
94 course. The clinical impact of a short-term duration of antibiotics on nasal endoscopy
95 scores (Modified Lund-Kennedy), and adverse gastrointestinal symptoms as
96 measured by the Gastrointestinal Symptom Rating Scale (GSRS) were secondary
97 endpoints. Finally, further analysis was performed on a subset of five patients,
98 initiating an investigation into the relationship between drug concentrations and
99 patient-specific antibiotic susceptibility from measured MIC's of predominating
100 sinonasal bacteria. The data presented in the sub analysis are part of an ongoing

101 study aiming to test our hypothesis that MIC's may have limited relevance to CRS
102 patients in the setting of biofilms.

103

104 MATERIALS AND METHODS

105 **Study design and sample collection**

106 Thirty subjects undergoing functional endoscopic sinus surgery (FESS) for extensive
107 bilateral CRS were recruited for this study (Table 1). This clinical population was
108 deemed most suitable for the measurement of antibiotic concentrations in various
109 tissue sites which could be sampled at the time of their operation.

110 Patients aged <16 years, with acute exacerbations, smokers, or who had been
111 prescribed oral antibiotics or systemic corticosteroids during the four weeks prior to
112 recruitment were excluded. Patients with a diagnosis of cystic fibrosis, fungal
113 sinusitis, chronic kidney disease, impaired liver function, immunodeficiency,
114 congenital mucociliary problems, systemic vasculitis and granulomatous disease,
115 chronic gastrointestinal inflammatory or immune-mediated diseases were also
116 excluded. This study was approved by the New Zealand Health and Disability Ethics
117 Committee (17/NTB/228) and written informed consent was obtained from all
118 participants and in the case of a single participant aged <18 (16 years) in the
119 company of his parents.

120 Eligible patients were randomised using a random number generator to one of three
121 groups: 1) doxycycline (100 mg orally with food daily for seven days) 2)
122 roxithromycin (300 mg orally at least 30 minutes before food daily for seven days)
123 and 3) control (no treatment). Patients were diagnosed according to the 2012
124 European Position Paper (EPOS) definition of CRS(33). Patients in the medication

125 groups started their medications seven days before surgery, taking one dose every
126 morning up to and including the morning of surgery. All patients were recruited
127 during a pre-operative consultation fewer than four weeks before FESS surgery
128 (timepoint 1). During FESS, multiple specimens were collected from the patients in
129 the medication groups (timepoint 2). Samples collected include blood, bilateral
130 middle meatal mucus samples, inferior turbinates and ethmoid bulla mucosa.
131 Patients completed a gastrointestinal symptom rating scale (GSRS)(34), and were
132 assessed endoscopically at both timepoints 1 and 2. The GSRS is a 15-item
133 questionnaire for patients with gastrointestinal symptoms validated for various
134 gastrointestinal disorders(34). The sum of the scores ranging from 15 to 105 is
135 regarded as the total score. Nasal endoscopy scores are derived from the Modified
136 Lund-Kennedy System (MLK) for polyps, discharge, and oedema on a scale of 0–
137 12(35). Baseline rhinosinusitis symptoms(35) (SNOT-22), radiological Lund-Mackay
138 scores(36) and patient demographic data were collected (Table 1).

139 One patient underwent limited FESS (bilateral uncinectomy, middle meatal
140 antrostomy, anterior ethmoidectomy) and the remaining underwent comprehensive
141 FESS including ethmoidectomy, sphenoidectomy, and frontal sinus dissection. Four
142 of these were revision operations and five of them included frontal sinus drill out
143 surgery. All study investigators apart from those conducting drug assays of biological
144 specimens were kept blinded to randomization until completion of the study. Those
145 conducting drug assays required knowledge of the drug since the assays were drug
146 specific. To maximize patient compliance, it was elected not to blind patients to their
147 treatment group. The benefit of this in optimizing the determination of drug
148 concentrations outweighed the potential impact on clinical correlation with the GSRS
149 score, considered one of the secondary endpoints in the study. Patients were asked

150 to keep their medication packets which were checked for compliance during the
151 consultation at timepoint 2. In accordance with standard treatment for CRS, all
152 patients continued to use daily topical corticosteroid nasal sprays and performed
153 regular sinonasal saline lavage, excluding the day of FESS surgery.

154 Samples were collected in the operating room prior to the application of topical
155 vasoconstrictors and anaesthetic solutions. Undiluted nasal secretions from both
156 sides of the sinonasal cavity were obtained by aspiration and collected in a mucus
157 trap extractor. The extractor was weighed before and after the collection of
158 secretions in order to calculate the weight of sample collected for each patient.
159 Tissue samples (ethmoid bulla and inferior turbinates) were collected bilaterally
160 using standard surgical techniques as part of the standard FESS procedure. Blood
161 was withdrawn using a 4 mL serum tube without clot activator. All sinonasal
162 specimens and blood specimens were transported on ice to the laboratory within 2 h.
163 Blood was centrifuged in order to retain the serum component for storage. All
164 specimens were stored at -80 °C until drug assay.

165

166 **Determination of antibiotic concentrations by LC-MS/MS**

167 A sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay
168 was employed to determine antibiotic concentrations in human nasal mucus,
169 sinonasal tissue and serum. Samples were separated with a Luna C18 column
170 (Phenomenex, CA, USA) and antibiotic levels were detected with a triple-quadrupole
171 mass spectrometer using electrospray ionization in positive mode and multiple
172 reaction monitoring. QTRAP® 6500 and API 4000™ systems (SCIEX, MA, USA)
173 were employed for doxycycline and roxithromycin respectively. Methacycline was

174 used as the internal standard for doxycycline and clarithromycin was used as the
175 internal standard for roxithromycin. Calibration curves were obtained from partial
176 method validation (lower limit of quantification (LLOQ) for doxycycline = 32ng/mL in
177 serum; 1ng/mL in mucus and tissue, LLOQ for roxithromycin = 0.2µg/mL in serum;
178 5ng/mL in mucus and tissue). The determination of both drugs in these biological
179 matrices was reliable and reproducible according to pharmaceutical guidelines(15,
180 37, 38).

181 *Sample preparation*

182 Serum sample preparation for the determination of doxycycline concentrations
183 involved deproteinization of serum sample with ACN, drying the sample under a
184 stream of nitrogen before reconstitution of the sample with ACN:B.P. water (15:85,
185 v/v) containing 0.1% (v/v) formic acid. Serum sample preparation for the
186 determination of roxithromycin concentrations involved deproteinization with
187 acetonitrile (ACN) then further dilution of the sample with ACN:B.P. water (30:70,
188 v/v) containing 0.1% (v/v) formic acid. An additional step of dilution with
189 methanol:B.P. water (50:50, v/v) was performed initially for nasal mucus samples,
190 while dilution and homogenization in ACN:B.P. water (50:50, v/v) containing 0.05%
191 of formic acid was performed initially for the sinonasal tissue samples.

192

193 **Subgroup analysis of bacterial isolates and their antibiotic susceptibilities**

194 A random block of five patients taking either doxycycline or roxithromycin had further
195 samples taken for bacterial culture analysis and antibiotic susceptibility testing. This
196 included a right-sided middle meatus swab at timepoints 1 and 2 as well as a right
197 sided ethmoid bulla tissue sample at timepoint 2. The presence or absence of
198 *Staphylococcus aureus* and beta-haemolytic *Streptococci* on these samples were

199 reported using colonial appearances and confirmed using Matrix-Assisted Laser
200 Desorption/Ionization-Time of Flight (MALDI-TOF). All other organisms were only
201 isolated for genus identification using MALDI-TOF if they were considered a
202 dominant organism. A dominant organism grew two times more than the other
203 organisms present. Growths were defined as light (growth only in the initial
204 inoculum), moderate (growth in the initial inoculum and streak lines but not over the
205 entire plate) or heavy (growth over entire plate). The minimum inhibitory
206 concentration required to inhibit the growth of 90% of organisms (MIC⁹⁰) of all
207 identified organisms to doxycycline, erythromycin and amoxicillin-clavulanic acid
208 were tested using MIC strips (Liofilchem®) according to manufacturer instructions.
209 Bacterial susceptibilities were interpreted using the European Committee on
210 Antimicrobial Susceptibility Testing (EUCAST) criteria(39). Erythromycin was
211 selected over roxithromycin as the closest macrolide drug due to the unavailability of
212 roxithromycin test strips.

213

214 **Statistical analyses**

215 Significance levels were set to $p < 0.05$ (two-sided). Patient data were summarised
216 descriptively, for continuous and categorical variables. The student's t test was
217 applied to analyse differences in continuous variables between groups. The chi-
218 squared test was similarly used for categorical variables. Linear regression analyses
219 were conducted to determine correlations between drug concentrations and clinical
220 scores (MLK and GSRS scores) in the medication groups. Based on an α value of
221 .05, a sample size estimate indicated that a correlation coefficient of 0.77 could be
222 detected with a sample size of 10 at a power of 0.80.

223

224 RESULTS

225 All patients complied with trial protocol and completed the study. There were no
226 statistically significant differences in gender, age, disease type (CRSwNP vs.
227 CRSsNP), presence of asthma, baseline clinical scores (Lund-Mackay, MLK, SNOT-
228 22 and GSRS) or number of antibiotic courses taken in the 12 months preceding
229 recruitment between all three patient groups. The roxithromycin group had a slightly
230 worse GSRS score change compared with the control group (2.3 vs. -1.9; $p=0.04$).
231 No other significant differences were observed within or between study groups for
232 changes in clinical scores (MLK and GSRS).

233 Regression analyses were performed comparing clinical score changes (MLK, and
234 GSRS) with antibiotic concentrations in the nasal mucus, tissue and blood. These
235 analyses demonstrated a reduction in the GSRS score ($R^2 = 0.45$, $p=0.04$) with
236 increasing mucus antibiotic concentrations in the doxycycline group (Figure 1). No
237 other significant correlations were found.

238

239 **Drug penetration in nasal secretions and sinonasal tissue**

240 Mean antibiotic concentrations and penetration are summarized in Table 2 and
241 Figure 2. Individual data are included in Supplementary Table S1 (for reviewers'
242 information only). The median time of specimen sampling from the last dose of
243 medication was 6.5 h (range, 2-15). There was no correlation between time of
244 sampling to drug concentration in the serum, mucus or sinonasal tissue specimens.
245 The mean concentrations (\pm SD) of doxycycline detected were – serum: 1.6 ± 0.9
246 $\mu\text{g/mL}$ (range, 0.5-3.0); mucus: $0.27 \pm 0.18 \mu\text{g/mL}$ (range, 0.09-0.69); turbinates: 1.4

247 $\pm 0.48 \mu\text{g/mL}$ (range, 0.49-2.1); ethmoid bullae: $1.6 \pm 0.5 \mu\text{g/mL}$ (range, 0.7-2.4). The
248 mean concentrations ($\pm\text{SD}$) of roxithromycin detected were – serum: $4.3 \pm 1.1 \mu\text{g/mL}$
249 (range, 2.0-5.5); mucus: $1.6 \pm 1.7 \mu\text{g/mL}$ (range, 0.1- 4.8); turbinates: 2.8 ± 0.9
250 $\mu\text{g/mL}$ (range, 0.9-4.3); ethmoid bullae: $2.6 \pm 1.1 \mu\text{g/mL}$ (range, 0.7-4.8).

251 In the doxycycline group, the mean mucus-to-serum ratio was 0.2 (range, 0.08-0.7).
252 This difference was highly statistically significant ($p<0.001$). The mean tissue-to-
253 mucus ratio was 5.6 (range, 3.1-7.5) while the mean tissue-to-serum ratio was 0.9
254 (range, 0.6-1.6). There were significantly higher levels of drug measured in the tissue
255 compared to mucus ($p<0.0001$) but not compared to the serum ($p=0.2$). In the
256 roxithromycin group, the mean mucus-to-serum ratio was 0.4 (range, 0.02-1.1). This
257 difference was statistically significant ($p=0.002$). The mean tissue-to-mucus ratio was
258 1.7 (range, 0.7-30) while the mean tissue-to-serum ratio was 0.6 (range, 0.4-1.1).
259 These differences were highly statistically significant ($p<0.001$).

260 There was no significant difference in drug concentrations between left and right
261 sinonasal tissue specimens nor between tissue sites (ethmoid bulla versus inferior
262 turbinate).

263

264 **Antibiotic susceptibilities of bacterial isolates**

265 A subgroup analysis of bacterial isolates was performed in five randomly selected
266 patients assigned to an antibiotic treatment group (Table 3). A light growth of *S.*
267 *aureus* was identified in baseline swabs for two patients, which were sensitive to
268 both doxycycline and roxithromycin. These patients (patients 19 and 27) had
269 therapeutic concentrations of antibiotics in the mucus and tissue according to the
270 susceptibility results and *S. aureus* was not detected in any of their post-drug

271 samples. Two patients in the doxycycline group (patients 19 and 23) had bacteria
272 predominating the culture plates for their post-drug samples despite one week of
273 medication. These were moderate growths of *Propionibacterium* and *Moraxella*
274 species respectively and were present despite both being sensitive to doxycycline.
275 Serum, mucus and tissue doxycycline concentrations for the two patients were at
276 least two times greater than the MIC thresholds of the respective organisms.

277

278 DISCUSSION

279 The results of this study suggest that the levels of doxycycline and roxithromycin in
280 the mucus of patients with CRS is substantially lower than the level measured
281 simultaneously in the sinonasal mucosa and serum. This finding may provide part of
282 the explanation why antibiotics have not proven to be particularly effective in the
283 treatment of CRS.

284 Measuring antibiotic concentrations from paranasal tissue and secretions is
285 challenging due to difficulties in various stages of bioanalysis related to small volume
286 samples and the heterogeneous, protein-rich nature of the samples. Few studies
287 have examined antibiotic concentrations in human sinonasal tissues and/or
288 mucus(18-32, 40-44). The majority of these studies were performed in patients with
289 acute sinusitis, acute exacerbations of CRS or upper respiratory tract infections. As a
290 result, data for various antibiotics commonly used for the treatment of CRS are
291 lacking, particularly those from the macrolide and tetracycline groups, compared with
292 fluoroquinolone and beta-lactam drugs(26, 31, 41, 44).

293 The results from this study showed that doxycycline and roxithromycin
294 concentrations in the nasal secretions were significantly lower compared to that in

295 the serum. The mean ratios at steady state were 0.16 and 0.37 respectively. These
296 values are comparable to those found in bronchial secretions in lung studies(45-48).
297 However, much higher penetration in human nasal secretions are reported for other
298 antibiotics that have been studied(18-32, 40-44). Notably, sinonasal secretion to
299 blood ratios of other macrolides such as telithromycin, clarithromycin and
300 azithromycin have been reported as ≥ 1.0 (26, 31). Our results suggest that
301 penetration of doxycycline and roxithromycin in sinonasal tissues were much higher
302 compared to that in the nasal secretions, although tissue levels were still ≤ 1.0
303 compared to serum, contrary to the published existing studies of several different
304 antibiotics(19, 21, 22, 24, 26, 30). In one study of telithromycin, the ratio of the
305 tissue versus plasma for area under the curve (AUC) was 5.9 for nasal mucosa and
306 1.6 for ethmoid bone(26). Regardless of drug class, different antibiotics display high
307 variability in tissue site versus intravascular ratios. Nevertheless, there are limitations
308 in the interpretation and comparison of existing studies including small sample sizes,
309 heterogeneity of study populations, specific antibiotic and dosage regimens, nasal
310 sampling methods, time of sampling in relation to the time of dose,
311 pharmacodynamic outcomes and methods of drug analysis.

312 The analysis of drugs or their metabolites in extravascular compartments such as the
313 mucus and mucosa can improve our understanding of penetration and likely efficacy
314 at the site of disease(17). This statement has been supported by existing studies of
315 pharmacodynamics and pharmacokinetics of antibiotics used to treat respiratory
316 infectious diseases(45-50). It is unknown whether the ability of an antibiotic to kill
317 bacteria above MIC thresholds is more important at the tissue or mucus level within
318 the sinonasal cavity, however existing respiratory studies conclude that epithelial
319 lining fluid is the site where pathogenic bacteria reside(45-50). Given that antibiotic

320 intervention has not been proven to be generally effective in CRS and our results
321 showing that concentrations in the sinonasal mucus were significantly lower
322 compared to those in the serum and tissue we hypothesize that the mucus may be a
323 more important target compartment for antibiotics. This is further supported by our
324 findings suggesting that pathogens commonly associated with acute sinusitis and
325 upper respiratory infection, such as *Staphylococcus aureus*, *Streptococcus*
326 *pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Moraxella*
327 *catarrhalis*, are susceptible to tissue drug levels in relation to MIC⁹⁰ levels for
328 susceptible strains reported in the literature(51-54). On the other hand, for the
329 majority of patients the concentrations we found in mucus was less than the MIC of
330 these species, with the exception of *Streptococcus pneumoniae* and *Streptococcus*
331 *pyogenes* in the roxithromycin group(51-54). Roxithromycin also exceeded the MIC
332 in mucus of *Corynebacterium spp.*, *Lactobacillus spp.* and *Propionibacterium*
333 *acnes*(51), the presence of which may be important in maintaining a stable sinonasal
334 bacterial community in CRS patients(55). The implications for diversity depletion and
335 microbial dysbiosis should be considered when prescribing these broad spectrum
336 antibiotics(1-6). Additionally, interpretation of the MIC may be misleading as many of
337 the bacteria in the sinonasal mucosa may be living in biofilms and have a
338 substantially higher resistance to antibiotics than the in vitro MIC against planktonic
339 organisms would suggest(7). This is supported by our small subgroup analysis of
340 bacterial isolates in which two patients taking doxycycline were found to have
341 moderate dominant growths of certain organisms despite susceptibility testing
342 showing the MIC for doxycycline was exceeded. In addition, serum, mucus and
343 tissue antibiotic concentrations for these patients were at least two times greater the
344 MIC⁹⁰ thresholds of the respective organisms.

345 In this study, a positive linear correlation was found between doxycycline
346 concentrations in the nasal mucus and score reduction in the GSRS questionnaires.
347 This suggests a reduction in gastrointestinal effects with increasing penetration of
348 doxycycline into the mucus. Further study is required to investigate the possibility
349 that an increased distribution of antibiotics to the mucus is accompanied by a
350 reduction in the distribution of antibiotics to the gastrointestinal tract. Antibiotic intake
351 did not correlate with any significant difference in objective clinical assessment by
352 nasal endoscopy nor gastrointestinal symptom scores between groups overall
353 except where slightly less favourable gastrointestinal scores were found in patients
354 taking roxithromycin. A larger double-blinded study with a longer medication period is
355 required to validate these clinical correlations, and any differences among patient
356 subgroups such CRSwNP compared to CRSsNP, where the penetration of drugs
357 into polypoid tissue compared with non-polypoid tissue would be of additional
358 interest.

359

360 **Limitations**

361 There are some limitations associated with this study. Since patients were not
362 blinded, the interpretation of patient reported GSRS scores is limited. However, the
363 primary outcome of the study was to investigate drug distribution and whether tissue
364 or mucus levels reached reported MICs' of the antibiotic for common pathogens
365 associated with CRS, while correlation with clinical scores (MLK and GSRS) was
366 considered a secondary endpoint. In addition, major clinical changes were not
367 expected with a brief course of antibiotics. A further secondary analysis was
368 performed on a subset of five patients adding objective data from bacterial swabs.
369 This aimed to provide an insight into the relationships between drug concentrations,

370 patient-specific MIC's, and bacterial eradication. A follow-up study is required to
371 establish these relationships, since our preliminary results support the hypothesis
372 that MIC's may have limited relevance to CRS patients in the setting of biofilms

373 Since samples for drug assays were obtained at the time of surgery, each subject
374 was only available for one sample collection. To best overcome changing
375 penetration ratios over time, sampling was performed at steady state. Methods in
376 this study can be applied in future studies using serial nasal secretion collections to
377 evaluate important pharmacodynamic indices linked to efficacy, for example
378 percentage of time that free drug remains above the MIC over a 24-hour period, the
379 ratio of free drug area under the concentration-time curve (AUC) to MIC over a 24-
380 hour period, and the ratio of maximum concentration to MIC.

381 A drawback of drug concentrations reported from whole tissues or secretions is the
382 assumption that antibiotics are uniformly distributed within tissue compartments
383 (intracellular, interstitial, intravascular). Newly developed methods have been applied
384 to a number of respiratory studies to evaluate the distribution of antibiotics across
385 these compartments, which may represent different sites of infections. Future studies
386 should evaluate intracellular levels of antibiotics in sinonasal secretions, since
387 polymorphonuclear leucocytes and macrophages have a high uptake of macrolides
388 and tetracyclines(47, 56, 57).

389 This study does not evaluate the factors influencing drug penetration of antibiotics
390 but considers them by using a prospective randomized control design. A relatively
391 larger unexplained variation is seen in the concentration of doxycycline in the serum
392 and the concentration of roxithromycin in the mucus. Drug penetration of antibiotics
393 is dependent on both drug-related and host-related factors(45, 49, 50, 57). Drug-

394 related factors include pKa, lipophilicity, protein binding, molecular weight and mode
395 of transport(45, 57). Host-related factors include macrophage uptake, bio-inactivation
396 from various sources including bacterial or leucocyte enzymes and elemental ions,
397 or elimination mechanisms including lymphatic drainage and mucociliary
398 transport(45, 49, 50, 57). Inflammation is also an important factor since it increases
399 the amount of tissue binding, which will inhibit movement of antibiotics across the
400 mucosa into the mucus(57). The post-antibiotic effect which quantifies the
401 persistence of bacterial suppression after short exposure to the drug is also
402 unknown.

403

404 CONCLUSIONS

405 The concentration of doxycycline and roxithromycin in nasal mucus was less than
406 those in the sinonasal mucosa or systemic circulation. Based on the MIC of
407 individual bacterial species associated with CRS these were therapeutic in the tissue
408 and serum but not in the mucus. However, effective distribution to the infection site
409 cannot be assumed alone on predicted bacterial susceptibilities since antibiotic
410 resistance is variable and microbes exist in complex communities that may increase
411 their tolerance to antibiotics. A short course of antibiotic intake did not correlate with
412 any significant difference in endoscopic assessment nor gastrointestinal symptom
413 scores between groups except where slightly less favourable gastrointestinal scores
414 were found in patients taking roxithromycin. Further research is required in order to
415 determine the factors influencing drug penetration in the mucus, and more
416 importantly whether this is clinically relevant.

417

418 Acknowledgements: Laboratory and technical assistance were provided by the
419 microbiology laboratory in Labtests Auckland, and the research and development
420 team in Zenith Technology Corporation. This study was supported by a grant from
421 the Garnett Passe and Rodney Williams Memorial Foundation.

422 Transparency declarations: None to declare.

423

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580 Table 1: Demographics, disease state and average clinical scores of study cohort.

Treatment group	M (n=)	F (n=)	Mean age (range)	CR Sw NP	CR Ss NP	Average n. of antibiotic courses in 12 months (range)	Mean Lund Mackay score (range)	Mean SNOT- 22 score (range)	Mean Modified Lund-Kennedy score (range)		Mean GSRS ⁵⁸¹ score (range) ⁵⁸²	
									T1	T2	T1	T2 ⁵⁸³
Controls	5	5	50 (17-74)	7	3	2 (0-5)	16 (10-24)	42 (20-68)	7 (3-12)	7 (3-12)	22 (16-37)	21 ⁵⁸⁴ (15-31) ⁵⁸⁵
Doxycycline	6	4	41 (21-73)	6	4	2 (0-5)	12 (5-19)	47 (12-72)	6 (2-8)	6 (2-12)	25 (15-48)	25 (15-38)
Roxithromycin*	7	4	52 (26-72)	6	5	3 (0-7)	15 (7-24)	48 (19-78)	6 (3-10)	7 (3-11)	19 (15-30)	21 ⁵⁸⁷ (16-35)

588 *One patient in this group was unable to have specimens obtained in the operating theatre due to logistical reasons on the day therefore sinonasal
589 microbiological data and drug measurements at the second time-point were not available for this patient. An additional patient was recruited into the
590 roxithromycin group in order to obtain a complete data set for 10 patients. M = males, F = females, CRSwNP = CRS with nasal polyposis, CRSsNP = CRS
591 without nasal polyposis, n. = number, T1 = timepoint 1, T2 = timepoint 2.

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604 Table 2. Mean antibiotic concentrations and penetration ratios.

	Doxycycline	<i>p</i> value	Roxithromycin	<i>p</i> value
Time of sampling (hrs since last dose) ± SD	8 ± 3 (range, 6-15 ^a)		6 ± 3 (range, 2-9)	
Serum concentration (µg/mL) ± SD	1.62 ± 0.91 (range, 0.46-2.99)		4.28 ± 1.12 (range, 2.07-5.52)	
Mucus concentration (µg/mL) ± SD	0.27 ± 0.18 (range, 0.09-0.69)		1.60 ± 1.67 (range, 0.08-4.76)	
Turbinates tissue concentration (µg/mL) ± SD	1.38 ± 0.48 (range, 0.49-2.05)		2.76 ± 0.90 (range, 0.85-4.29)	
Ethmoid bulla tissue concentration (µg/mL) ± SD	1.59 ± 0.54 (range, 0.68-2.42)		2.62 ± 1.13 (range, 0.72-4.83)	
Mucus-to-serum ratio	0.16 (range, 0.08-0.67)	<0.001	0.37 (range, 0.02-1.06)	0.002
Tissue-to-mucus ratio	5.58 (range, 3.09-7.52)	<0.0001	1.68 (range, 0.66-29.7)	<0.001
Tissue-to-serum ratio	0.91 (range, 0.56-1.61)	0.2	0.63 (range, 0.37-1.08)	<0.001

605 ^aThis patient had a much longer sampling time since their operation was scheduled for the morning
 606 and they were instructed to take the last dose of doxycycline with their meal the night before surgery.

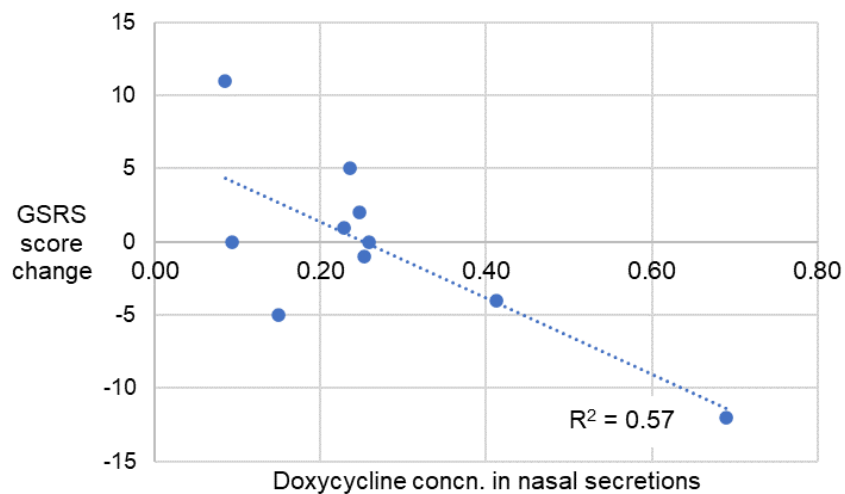
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629 Table 3. Bacterial isolates cultured from random subgroup and respective bacterial susceptibilities to
 630 doxycycline, erythromycin and amoxicillin/clavulanic acid.

Patient #	Drug	Serum drug concn	Mucus drug concn	Mean tissue concn	Bacterial isolate (MM swab at T1)	MIC ⁹⁰	Bacterial isolate (Sample at T2)	MIC ⁹⁰
19	D	2.67	0.25	1.82	Light growth S. aureus	Dox = Sens. (0.125) Ery = Sens. (0.125) Aug = Res. (0.50)	Moderate growth Proponiobacterium species (ethmoid bulla tissue)	Dox = Sens. (0.064) Ery = Sens. (0.016) Aug = Sens. (<0.016)
23	D	2.89	0.24	1.61	-	-	Moderate growth Moraxella species (MM swab)	Dox = Sens. (0.125) Ery= Res. (0.75) Aug = Sens. (<0.016)
25	R	3.58	3.80	3.85	-	-	-	-
27	R	4.32	1.33	2.52	Light growth S. aureus	Dox = Sens. (0.064) Ery = Sens. (0.125) Aug = Res. (0.50)	-	-
31	R	5.07	4.76	3.13	-	-	-	-

631 D = doxycycline; R = roxithromycin; MM = middle meatus; MIC⁹⁰ = minimum inhibitory concentration
 632 required to inhibit the growth of 90% of organisms; Dox = Doxycycline; Ery = Erythromycin; Aug =
 633 Augmentin (amoxicillin/clavulanic acid); sens. = sensitive; res. = resistant

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658 Figure 1. Gastrointestinal symptoms rating scale (GSRS) score change vs. doxycycline
659 concentrations in nasal secretions.

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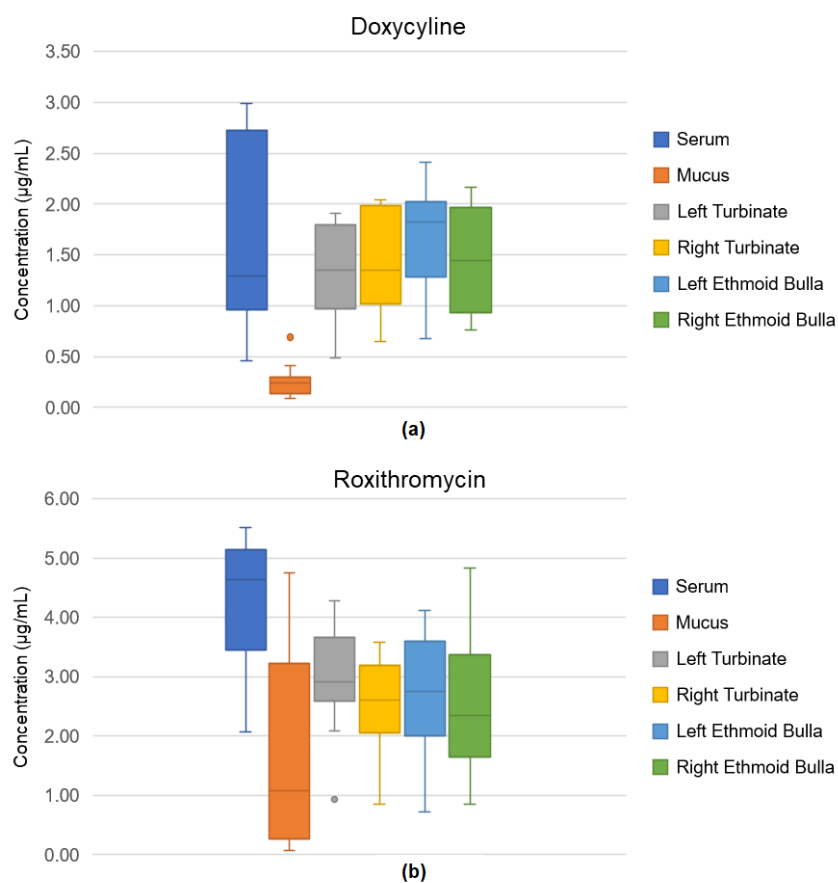
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669 Figure 2. Antibiotic concentrations in the serum, mucus and different sinonasal tissue sites in the
670 doxycycline (a) and roxithromycin (b) groups.

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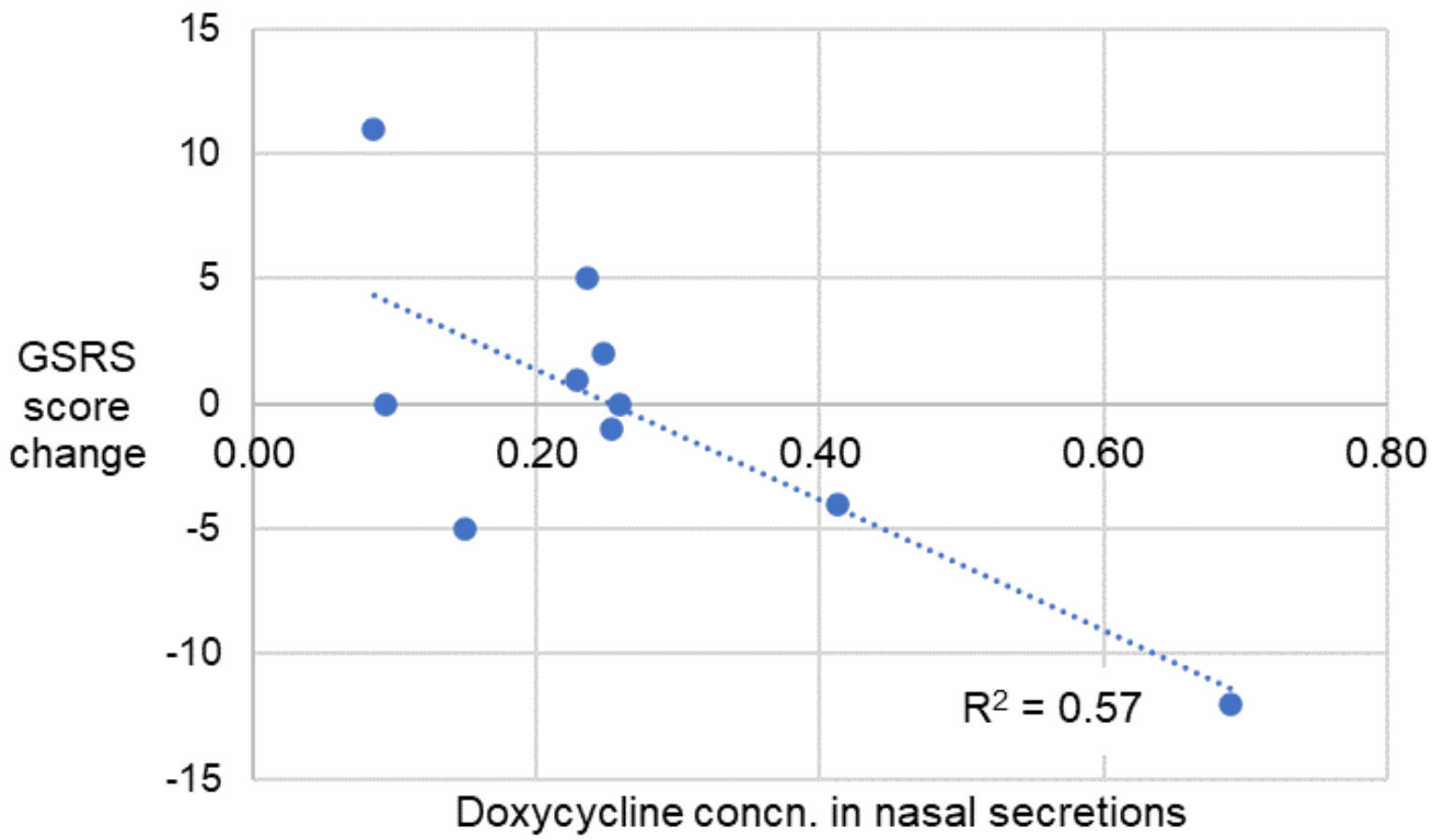
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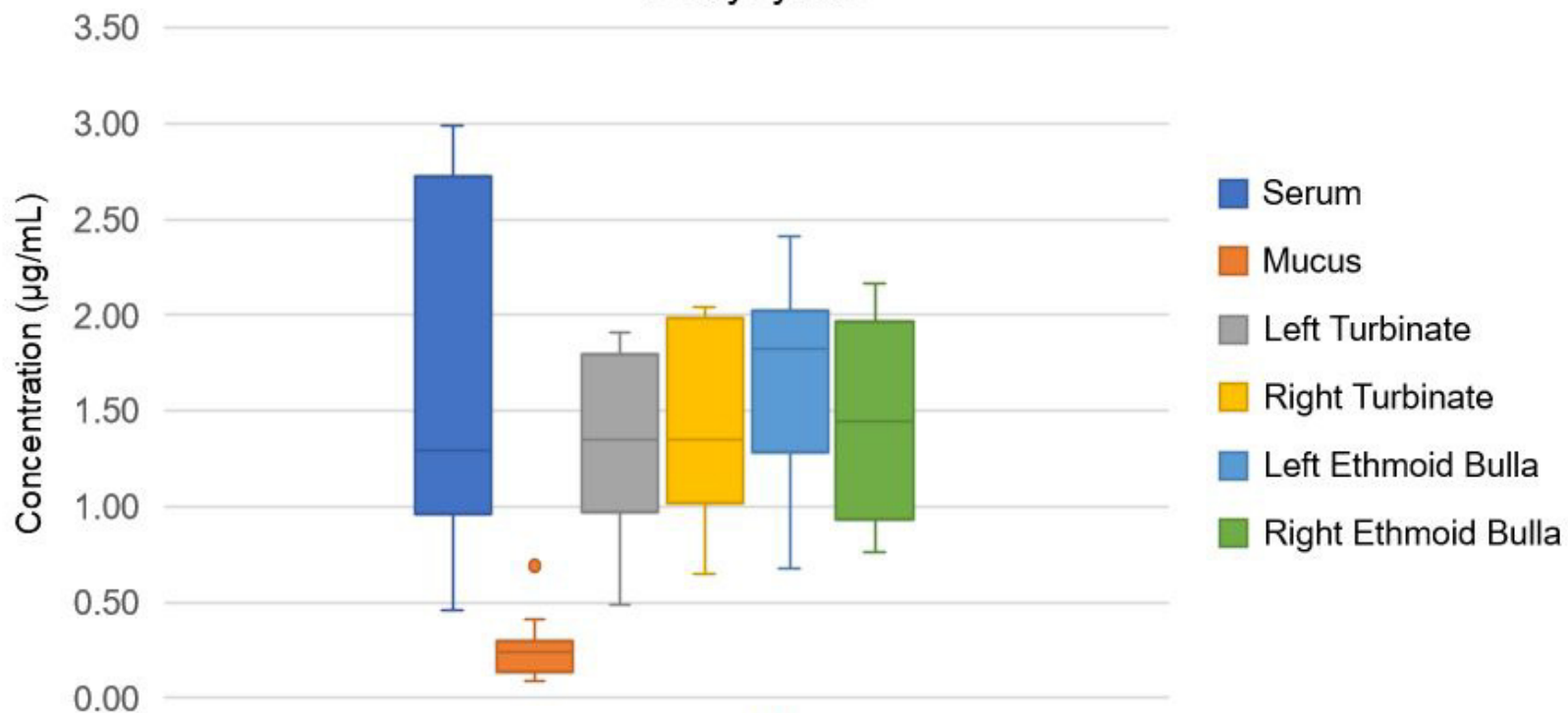
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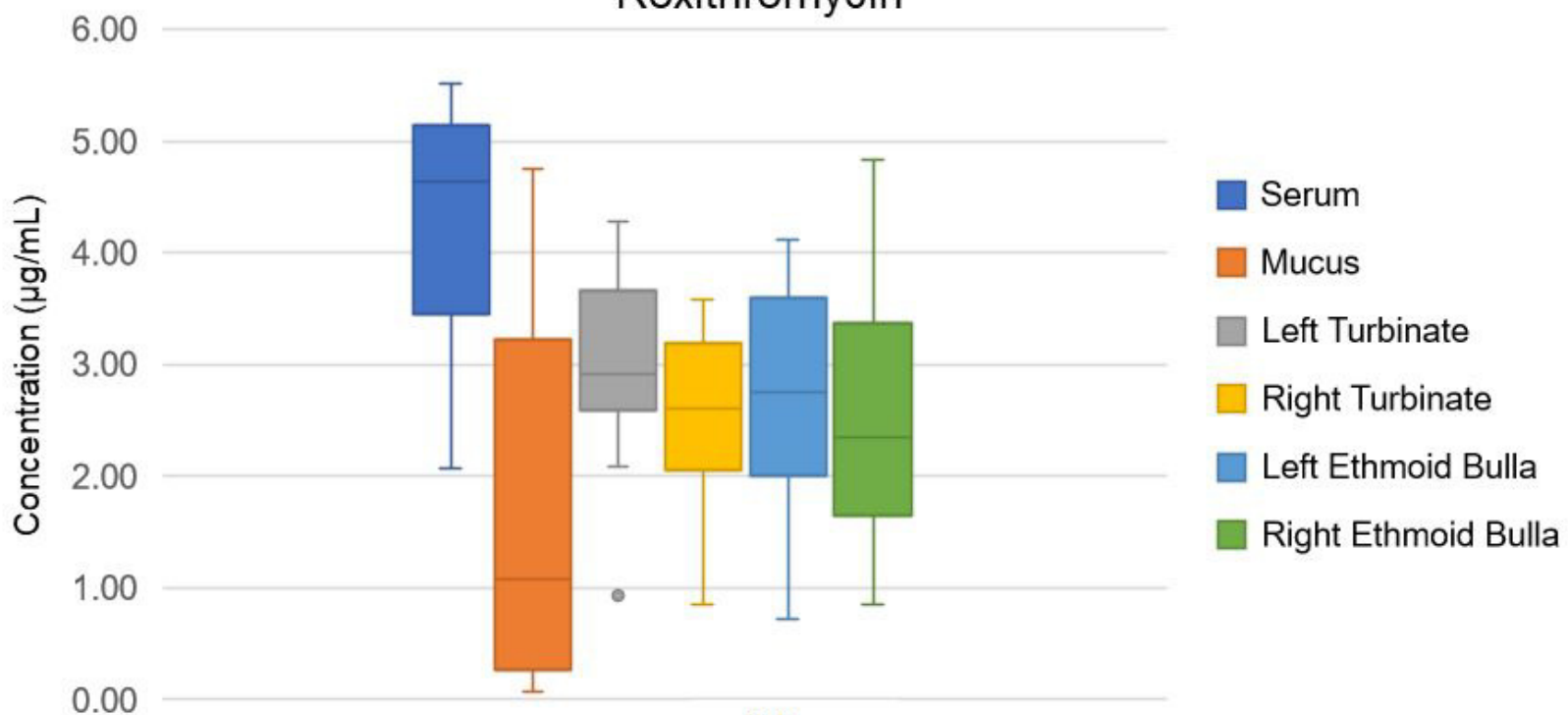


Doxycycline



(a)

Roxithromycin



(b)