The effect of diluted 1% baby shampoo on biofilm reduction in chronic rhinosinusitis with nasal polyposis (#104558)

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The effect of diluted 1% baby shampoo on biofilm reduction in chronic rhinosinusitis with nasal polyposis

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Background. Biofilm has been identified as the contributing factor for refractory chronic rhinosinusitis with nasal polyposis (CRSwNP). Nasal douching using baby shampoo was thought to be effective in patients with CRSwNP. We aimed to study the in-vitro reduction of biofilm using diluted 1% baby shampoo. **Methods.**Sixty nasal polyps taken from patients who met the inclusion and exclusion criteria were sent for histopathological examination using haematoxylin and eosin staining. Another portion of the same samples was sent for tissue culture and tissue culture plate assay to identify*S. aureus*and*P. aeruginosa*and determine their biofilm forming capacity. The efficacy of diluted 1% baby shampoo versus normal saline was tested on the biofilm*in vitro*where the optical density readings were compared pre- and post-treatment. **Results.**The prevalence of biofilm in patients with CRSwNP is 21.7%. Thirteen samples were positive for biofilm;*P. aeruginosa*23% (n=3),*S. aureus*15% (n=2), no bacterial growth 54% (n=7) and others 8% (n=1). Biofilm formation was significant in both*S. aureus*and*P. aeruginosa*(*p<0.001*) whilst a significant reduction biofilm was seen in diluted 1% baby shampoo (*p:0.043*). **Conclusion.**In conclusion, diluted 1% baby shampoo is an effective treatment in the reduction of biofilm for CRSwNP.

The Effect of Diluted 1% Baby Shampoo on Biofilm Reduction in Chronic Rhinosinusitis with Nasal Polyposis

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Abstract

Background. Biofilm has been identified as the contributing factor for refractory chronic rhinosinusitis with nasal polyposis (CRSwNP). Nasal douching using baby shampoo was thought

to be effective in patients with CRSwNP. We aimed to study the in-vitro reduction of biofilm using diluted 1% baby shampoo.

Methods. Sixty nasal polyps taken from patients who met the inclusion and exclusion criteria were sent for histopathological examination using haematoxylin and eosin staining. Another portion of the same samples was sent for tissue culture and tissue culture plate assay to identify *S. aureus* and *P. aeruginosa* and determine their biofilm forming capacity. The efficacy of diluted 1% baby shampoo versus normal saline was tested on the biofilm *in vitro* where the optical density readings were compared pre- and post-treatment.

Results. The prevalence of biofilm in patients with CRSwNP is 21.7%. Thirteen samples were positive for biofilm; *P. aeruginosa* 23% (n=3), *S. aureus* 15% (n=2), no bacterial growth 54% (n=7) and others 8% (n=1). Biofilm formation was significant in both *S. aureus* and *P. aeruginosa* ($p<0.001$) whilst a significant reduction of biofilm was seen in diluted 1% baby shampoo ($p: 0.043$).

Conclusion. In conclusion, diluted 1% baby shampoo is an effective treatment in the reduction of biofilm for CRSwNP.

Keywords Rhinosinusitis, biofilms, bacteria, nasal polyps, nasal irrigation, surface-active agents, surfactants

Introduction

Chronic rhinosinusitis (CRS) is characterized by mucosal inflammation of the nose and paranasal sinuses with sinonasal symptoms which persist for more than 12 weeks (Cain & Lal, 2013). CRS is a polymicrobial disease with a wide range of pathogens involved. It was difficult to determine which pathogens are predominant because of various sampling techniques (e.g. swab, biopsy, irrigation and aspiration), unable to maintain sterility through which the nasoendoscope is passed and different methods of culture (Meltzer *et al*., 2004). Pathogens which are commonly found in patients with CRSwNP are *S. aureus, H. influenzae*, and *P. aeruginosa* and fungus (Smith, Buchinsky & Post, 2011). According to study done by Zahedi *et al*, commonest pathogens cultured from swabs taken from middle meatus were *Pseudomonas* sp., *Enterobacter* sp. and followed by Coagulase-negative staphylococcus (CONS).

Biofilm is an organized, heterogeneous bacterial community, embedded in extracellular polymeric substances (EPS) which are predominantly water and rich in polysaccharides, nucleic acid and proteins. It is a complex three-dimensional structure which contains either single species or different species of microbial, organized in patches of separate colonies and each sub-specialized into different function (Costerton *et al*., 1995). The free-floating planktonic bacteria adhered to a biological surface to form microcolonies which eventually progressed to form a biofilm. Over the years, studies have revealed the possibility of bacterial biofilms formation which was postulated to be the driving cause of the disease being refractory to treatment (Tomooka, Murphy & Davidson, 2000). Biofilms are more commonly found in patients with chronic rhinosinusitis with nasal polyposis. Studies have shown that CRSwNP patients who failure of maximum medical therapy and surgery have a biofilm positive rate of 20-100% (Li *et al*., 2012).

Nasal irrigation is one of a few treatment options used for CRSwNP. Various studies have been carried out to compare different substances used for nasal irrigation such as normal saline, antibiotic, steroid, loop diuretics and surfactants. Normal saline nasal irrigation has been popularized over the years and achieve worldwide acceptance as an adjunctive therapy (Macassey & Dawes, 2008). Promising results from a study reported that diluted 1% baby shampoo improved nasal symptoms and scope findings in a group of patients following surgery generated further interest of diluted 1% baby shampoo nasal irrigation as a treatment option (Chiu *et al*., 2008).

Various imaging modalities have been used in different studies to detect the presence of biofilms. Imaging modalities include scanning and transmission electron microscopy, fluorescent *in situ* hybridization (FISH) and confocal scanning laser microscopy (CSLM). Detection of biofilms using hematoxylin and eosin (H&E) staining was first reported in 2010 by Hochstim *et al*. Tóth *et al* (2011) also reported that gram stain has a strong correlation with H&E staining and is a reliable predictor of the presence or absence of biofilm. There are a few microbiological methods used for biofilm detections in vitro. These include Tissue Culture Plate (TCP), Tube Method (TM) and Congo Red Agar (CRA). Among the three methods, Tissue Culture Plate has been demonstrated as the most sensitive and specific in detecting biofilms (Deka, 2014).

Many previous *in-vitro* studies explored the efficacy of surfactants in inhibiting the biofilm formation, such as using citric acid/zwitterionic surfactant (CAZS), SinuSurf, and baby

shampoo. But from all the studies, none was done on nasal polyps' specimens taken from CRSwNP patients (Desrosiers, Myntti & James, 2007; Chiu *et al*., 2008; Kofonow & Adappa, 2012). A study done by Chiu *et al* (2008) reported that Johnson & Johnson baby shampoo of 1% concentration was effective in inhibiting the formation of biofilm with favourable clinical improvements as compared to other concentration, thus making it as a determined concentration for clinical study. Although the study reported the efficacy of the 1% concentration baby shampoo in inhibiting the biofilm formation in mostly Chronic Rhinosinusitis patients, there is lack of data exploring this effect specifically on patient with Chronic Rhinosinusitis with Nasal Polyposis.

Hence, the aims of this study were to determine the prevalence of biofilm, investigate the biofilm forming capacity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and define the effect of diluted 1% baby shampoo on biofilm reduction using nasal polyp specimens of patients with CRSwNP. To the best of our knowledge, this is the first study conducted to compare the effects of diluted 1% baby shampoo in reducing biofilms *in vitro* on nasal polyp specimens of patients who underwent ESS for CRSwNP.

Materials & Methods

Study population

This study received the Research Ethical Committee, Universiti Kebangsaan Malaysia (REC UKM) approval with the approval number of JEP-2016-510. All patients underwent Endoscopic Sinus Surgery (ESS) for CRSwNP within the study period of 2 years who fulfilled both the inclusion and exclusion criteria were recruited into the study. The inclusion criteria were age 18 years old and above who underwent ESS for CRSwNP. Patients who have sinonasal tumour and/or diagnosed with granulomatous nasal diseases were excluded from the study. Informed consent was taken from patients who agreed to be involved in this study.

Methodology

Two samples of nasal polyp were obtained from each patient and samples were sent to the histopathology and microbiology lab as per laboratory protocols on the same day.

H&E and Gram Stain study on Biofilm

One section was stained with H&E using standard protocol. The section was deparaffinized in xylene twice for 5 minutes (2 × 5 minutes) and rehydrated with successive 1-minute washes in 100%, 96%, 80%, and 70% ethanol. Subsequently, the specimen was stained with haematoxylin for two minutes, rinsed with distilled water, rinsed with 0.1% hydrochloric acid in 50% ethanol, rinsed with tap water for 15 minutes, stained with eosin for one minute, and rinsed again with distilled water. The slide was dehydrated with 95% and 100% ethanol successively followed by xylene twice for five minutes (2 × 5 minutes) and mounted with coverslips. Slides were examined and evaluated by two researchers. Presence of Irregularly shaped groupings of small basophilic bacterial clusters one third of the size of the surrounding epithelial or inflammatory cells, a biofilm seen over the epithelial lining, not in or under the epithelial lining, biofilm seen tightly adherent to the surface epithelium or pulled away slightly; or a dense extracellular polysaccharide substance (EPS) material with embedded basophilic bacteria, occasionally entrapped erythrocytes, leukocytes and partially sheared from epithelial surface substance coating the epithelial surface indicating biofilm-positive (Tóth *et al.,* 2011). Gram staining of the samples following the protocols of Gram stain kit Bio-Optica 04-100802 was employed to complement the HPE findings of biofilm.

Tissue Culture Plate Assay

The tissue specimens obtained from the operating theatre were immediately transported to the microbiology lab. The tissue specimens were homogenized. Specimens were inoculated on blood agar, MacConkey agar, mannitol salt agar and cetrimide selective agar and incubated at 37°C for 24 hours. Pure colonies from the isolates above were then inoculated on to nutrient agars until further testing. Yellow colonies grew on mannitol salt agar were subjected to DNase and coagulase test. DNase positive and coagulase positive colonies were considered as *S. aureus*. Green-pigmented colonies which grew on cetrimide agar were subjected to the API 20 NE test to confirm the identification of *P. aeruginosa*. Three to four colonies of bacterial isolates were suspended in Mueller-Hinton broth until the turbidity matches a standard of 0.5 McFarland and were incubated aerobically at 37°C for 24 hours. One mL of the bacterial suspension was diluted 1:100 with fresh Mueller-Hinton broth (10^6 CFU.mL). One mL of this 1:100 bacterial suspensions was added to 1ml of sterile Mueller-Hinton broth to achieve a final concentration of 5 x 10^5 CFU/mL. Reference strain of biofilm-forming *S. aureus* ATCC 25923 and *P. aeruginosa*

ATCC 27853 obtained from American Tissue Culture Collection (ATCC) were used as a positive control in this study. Control strains were cultured with the same method. Biofilm presence of the tissue specimen was confirmed using H&E and Gram staining. Only tissues with biofilm and culture positive for *S. aureus* or/and *P. aeruginosa* were included in the analysis. All organisms were tested for biofilm-forming properties and subsequently, the amount of biofilm left after subjecting to diluted 1% baby shampoo versus normal saline were evaluated. 1% shampoo concentration was determined for this clinical study based on previous literature adapted (Chiu *et al*., 2008).

Three 96-well tissue-culture treated microplates were filled with 200µL of Mueller-Hinton broth and 10 µL of the final inoculums ($5x10^5$ CFU/mL) and were incubated it at 37°C for 8 days without agitation for biofilm growth. 50 µL of media were removed from each well and replaced with 50-150 µL of fresh media on alternate days to achieve maximal biofilm growth. After incubation for 8 days, the wells were washed twice with distilled water to remove planktonic forms of bacteria and were left to dry. One of the 96-well tissue-culture treated microplates was filled with 250 µL of methanol and left for 15 minutes for fixation of adherent bacteria. Subsequently methanol was removed, and the microplate was air dried for another 15 minutes. Subsequently 200 µL of 0.1% crystal violet (CV) solution was applied to stain the adherent bacteria for 5 minutes. Then, the CV solution was decanted, and the wells were washed three times with distilled water. The stained wells were filled with 250 µL of 95%ethanol and were incubated for 1 hour on a rocking platform at room temperature to solubilize the adherent material. Quantification of biofilm growth was determined by reading the optical density (OD) of each well at 595nm (OD595nm) using a microplate reader. Reference strains were used as control. Negative control wells were filled with only sterile broth. Test was carried out in a triplicate manner, and the mean OD was recorded. A cut-off optical density (ODc) needs to be established to note the presence or absence of biofilm. The cut-off optical density (ODc) was defined as 3 standard deviations above the mean OD of the negative control (culture medium): ODc=average OD of negative control+(3×SD of negative control). The mean OD of the strains will be compared to the OD of the negative control. Analysis was done as described by Stepanović *et al*., (2007) whereby strains were classified as follows: $OD \leq ODc$ no biofilm producer, $ODc < OD \leq 2 \times ODc$ weak biofilm producer, $2 \times ODc < OD \leq 4 \times ODc$ moderate biofilm producer and $4 \times ODc < OD$ strong biofilm producer.

An amount of 0.1ml of Johnson & Johnson baby shampoo was diluted in 10mls of normal saline to achieve a 1% concentration which was used as nasal irrigation (Joss *et al*., 2016). This concentration was determined and adapted based on previous literature (Chiu *et al*., 2008). Solution was prepared 30 minutes prior to test.

Once the biofilm had been established and confirmed on the first microplate on day 8, wells on the second and third microplates containing the same inoculums were filled with 100 µL of 1% baby shampoo diluted in normal saline and normal saline separately. The microplates were incubated for another 24hours at 37°C. Positive control wells contained only the inoculums and the media without the test component, and negative control wells contained only test component and media, without the inoculums. The microplates were analysed using a crystal violet assay and optical density and measured with a microplate reader as described earlier. A flow chart of the methodology is shown in *Fig. 1*. All tests were carried out in a triplicate fashion. The relative inhibition of biofilm was calculated as described by Ha *et al*., (2008), as follows.

Percentage of biofilm inhibition = 100 – [OD_{595} of treated well/OD_{595} of untreated well] x 100

Statistical analysis

Data was entered and analysed using SPSS 25.0. Wilcoxon Signed Rank test was employed to analyse the reduction of biofilm using diluted 1% baby shampoo and normal saline only and to compare biofilm reduction between normal saline and diluted 1% baby shampoo. Fisher Exact test was used to determine the statistical difference in biofilm formation and biofilm forming capacity of *S. aureus* and *P. aeruginosa.* $p < 0.05$ considered as statistically significant.

Results

Demographic Data

A total of 60 samples were collected from 30 patients who underwent endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. The age ranged from 26 to 78 years old whereby the mean ± standard deviation (SD) age 58±15 years. There were 21 male and 9 female which comprised of 70% and 30% respectively, and 13(43.3%) of them were Malays, 13(43.3%) were Chinese and 4(13.3%) were Indian. All patients were treated with intranasal corticosteroids and normal saline nasal douche. Half of the population underwent repeated ESS.

Data on biofilms

Biofilms were detected in 13 out of 60 samples (21.7%) using H&E complimented with Gram stain method (Figure 2). Out of these 13 samples, 23% (n=3) comprised of P. *aeruginosa*, 15% (n=2) for S. *aureus*, 54% (n=7) had no bacterial growth and 8% (n=1) which was others (Table 1). All samples which grew S. *aureus* or P. *aeruginosa* were bio-film-positive. There was no sample which grew S. *aureus* and P. *aeruginosa* simultaneously. There is statistical significance in biofilm formation of S. *aureus* or P. *aeruginosa* with the p-values of 0.0002 ($p<0.05$) (Table 2).

Biofilm forming capacity

All samples which grew S. *aureus* or P. *aeruginosa* with biofilm seen on H&E staining were subjected for tissue culture plate assay to determine the bacteria biofilm forming capacity. They were all graded after a cut-off point of optical density of the negative control was determined. All samples with P. *aeruginosa* (100%) isolates were strong biofilm forming in capacity whereas 1 sample with S. aureus has strong biofilm forming capacity and 1 weak biofilm forming capacity (50%). There is no statistical difference in biofilm forming strength between S. *aureus* and P. *aeruginosa* with the p-value of 0.4.

Effects of diluted 1% baby shampoo on biofilm mass

An average of 26.87% reduction of biofilm with normal saline alone and a mean of 57.31% reduction of biofilm after treated with diluted 1% baby shampoo (Table 3). There is significant reduction of biofilm with the treatment of diluted 1% baby shampoo with the p-value of 0.043 however there was no significant reduction of biofilm with normal saline with the p-value of 0.080 (Table 4). Overall analysis, diluted 1% baby shampoo is significantly more effective in biofilm reduction than normal saline with the p-value of 0.043 ($p<0.05$) (Table 4, *Fig. 3*).

Discussion

Chronic rhinosinusitis has been reported to affect quality-of-life more than those suffering from chronic obstructive pulmonary disease, congestive heart failure, ischemic heart disease and back pain. It remains to be a challenging disease in terms of treatment whereby disease was

inadequately controlled despite a combination of maximal medical therapy and ESS (Hong *et al*., 2014). One out of many postulations for the chronicity of the disease is the involvement of bacterial biofilms which made the planktonic bacteria resistant to the conventional treatment strategies (Tomooka, Murphy & Davidson, 2000).

The prevalence of biofilm in CRSwNP in our study is 22%, detected using a combination of H&E and Gram stain method. Morphologic features observed in all biofilms positive specimens in this study are in consistent with the findings reported by Hong *et al*. (2014) which are irregular groupings of small basophilic bacterial clusters, presence of a biofilm over the epithelial lining, biofilm tightly adherent on the surface epithelium and a dense extracellular polysaccharide substance with embedded basophilic bacteria. There is no standard methodology in biofilms detection ranging from light microscopy to crystal violet tissue culture plate assay. Hence the wide range of accuracy in biofilm-positive re-ports were observed in previous studies of patients with failure of maximal medical therapy and ESS (Li *et al*., 2012). Among the commonly used imaging modalities in biofilm studies are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The advantages over H&E method are that these methods able to showcase the structure, developmental stages and polymicrobial nature of biofilms, but the disadvantages are the difficult in fixation process and to speciate individual bacterial species. Currently, confocal laser scanning microscopy (CLSM) is the most used technique in biofilm studies as it provides a three-dimensional view of biofilm structures and also able to speciate the bacteria visualized using Bac light technique (Suh, Cohen & Palmer, 2010). However, CSLM is time consuming, incurs much higher cost and not readily available.

There were 15% of S. *aureus* biofilm and 23% of P. *aeruginosa* biofilm detected in this study. It is also evidenced in this study that S. aureus and P. aeruginosa were significantly associated with biofilm formation. This is consistent with previous studies which demonstrated S. aureus and P. aeruginosa were predominantly found in biofilms positive specimens of patients with CRSwNP. In a study group of 157 patients, S. aureus and P. *aeruginosa* found in 71% of samples which showed biofilm growth (Hong et al., 2014). Bendouah et al reported 14 out of 19 (73%) patients had isolates of S. *aureus* or P. *aeruginosa* biofilm. Many at times the individual or mixed species of bacteria or fungi which formed the bio-films were unable to be isolated or cultured by the conventional microbiological methods; hence the diagnosis is usually uncertain (Tóth *et al* (2011). This is because the bacteria in the biofilms have significant low metabolic rate which

renders the low culturability (Suh, Cohen & Palmer, 2010). Studies have reported there were significant associations of biofilms with nasal polyps. However, there were another group of authors reported no associations of biofilms and nasal polyps [Karosi et al., (2013); Tóth et al., (2013); Yan et al., (2012); Bezerra et al., (2011); Mladina, & Skitarelić, (2010); Mladina et al., (2010)]. There were studies which reported the heterogeneity of microbial community at different locations in the nasal cavity which demonstrated approximately 25% of micro-biome variations within-patient in CRS [Joss et al., (2016); Biswas et al., (2015); Yan et al., (2013)]. TCP assay with CV was used for in vitro study of biofilms on nasal mucosa specimens from patients with CRSwNP whereby OD of the wells which biofilms were read using TECAN Infinite F50 microplate reader at the wavelength of 590nm. In our study, the cut-off OD for S. *aureus* and P. *aeruginosa* was 0.72 and 0.74 respectively. The OD of the reference strain of biofilm-forming S. *aureus* ATCC 23923 and P. *aeruginosa* ATCC 27852 which were used as positive controls were 3.97 and 1.69 respectively. Although we observed that all P. *aeruginosa* biofilm had strong biofilm forming capacity as compared to S. *aureus* which demonstrated only 1 out of 2 has strong biofilm forming capacity, however we were unable to prove the statistical difference in the strength of biofilm forming capacity be-tween these 2 organisms. This is most likely attributed to the low number of sample size which are positive for biofilm forming S. *aureus* and P. *aeruginosa*. A study done by Prince et al reported that P. *aeruginosa* has higher propensity to biofilm formation as compared to the other bacteria found in the study such as, S. aureus, CONS and H. influenza. It is also re-ported that P. *aeruginosa* is an organism with strong biofilm forming capacity which is consistent with our results (Prince et al., 2008). Conversely, Foreman & Wormald reported that S. *aureus* has higher propensity for biofilm formation as compared to P. *aeruginosa* (Foreman, & Wormald, 2010). A study done by Bendouah et al., (2006) reported that the presence of biofilm forming S. *aureus* or P. *aeruginosa* also predicts an unfavorable outcome of ESS.

Due to the recalcitrant nature of the dise, adjunctive therapy with topical application of various substances had been used and studied. Different methods of applications were popularized such as nasal irrigation, douching, spray, and rinsing. In our study, we compared the reduction of biofilm mass in vitro using diluted 1% baby shampoo and normal saline alone on nasal mucosa specimens. We observed that diluted 1% baby shampoo showed significant reduction of biofilm mass of nasal mucosa specimens as compared to normal saline. This

corresponds to the study done by Chiu et al., (2008) who saw 46.6% symptomatic improvement and 63% olfaction improvement of postsurgical patients after using diluted 1% baby shampoo for 4 weeks together with the reduction of mucous thickness, postnasal drips and improved endoscopic findings. An in vitro study of S. *aureus* and P. aeruginosa biofilm done by Desroisiers et al., (2007) found that surfactant delivered by hydrodynamic force further enhanced the reduction of biofilm mass.

The implication of our findings towards clinical practice is as follows. Gram stain can be used to enhance biofilm detection in histopathological examination and Chronic Rhinosinusitis with Nasal Polyposis patients diagnosed as biofilm-positive can be treated by diluted 1 % baby shampoo nasal irrigation.

The main limitation of this study was due to the budget limitation. Biofilm detection was done without CSLM method with BacLight methods or FISH analysis due to high cost and hence possibly the low number of samples. Only 2 most found organisms in CRSwNP were included in this study also due to budget limitation in purchasing the ATCC strains. Besides that, it was our first-time experience of biofilm detection using H&E with Gram stain method and TCP assay in our center.

We suggest using CSLM method with BacLight methods or FISH analysis with species-specific oligonucleotides probes in the next study on biofilm, which is currently most widely used for biofilm detection and to speciate the organisms involved. Sampling methods should be improved by taking samples from all involved paranasal sinuses in view of the reported heterogenicity of organism community in the nasal cavity. We recommend a prospective multi center studies in the future to evaluate the effectiveness of diluted baby shampoo 1% in biofilm reduction comparing populations of urban and rural areas to achieve improved tailored management in our local population.

Conclusions

The prevalence of biofilm in patients with CRSwNP in our study is 21.7%. There are significant effect of biofilm reduction following the use of diluted 1% baby shampoo and significant association of S. *aureus* & P. *aeruginosa* with biofilm formation. Our findings suggest a role of screening patients with CRSwNP for biofilms in selecting patients that can benefit from 1% diluted baby shampoo treatment.

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Table 1(on next page)

Bacteria isolates cultured from biofilm-positive samples

Table 1.

Bacteria isolates cultured from biofilm-positive samples.

Bacteria	Frequency (n)	Percentage (%)
No Growth	7	54
S.aureus	2	15
P.aeruginosa	3	23
Others	1	8

Table 2(on next page)

Distribution of organisms forming biofilm

Table 2.

Distribution of organisms forming biofilm.

	Biofilm	No Biofilm	
S.aureus/*P.aeruginosa*	5	0	5
Others	8	47	55
		Total	60
		p-value	0.0002

Table 3(on next page)

Comparison of biofilm reduction between normal saline and diluted 1% baby shampoo

Table 3.

Comparison of biofilm reduction between normal saline and diluted 1% baby shampoo.

	Untreated (OD)	Treated with Normal Saline (OD)	Reduction with Normal Saline (OD)	%Reduction with Normal Saline
N	5	5	5	5
Mean	3.02	2.15	0.90	**26.87**
Median	3.46	2.11	1.37	30.35
Std. Dev	1.38	1.06	0.72	23.46
	Untreated (OD)	**Treated with Baby Shampoo (OD)**	**Reduction with Baby Shampoo (OD)**	**%Reduction with Baby Shampoo**
N	5	5	5	5
Mean	3.02	1.15	1,87	**57.31**
Median	3.46	1.35	2.02	58.41
Std. Dev	1.38	0.51	1.07	18.96

Table 4(on next page)

Effectiveness of biofilm reduction between normal saline and diluted 1% baby shampoo

Table 4.

Effectiveness of biofilm reduction between normal saline and diluted 1% baby shampoo

		N	Mean Rank	Sum of Ranks	Z	P
Normal Saline	Negative Ranks	4	3.50	14.00	-1.753	0.080
	Positive Ranks	1	1.00	1.00		
	Ties	0				
	Total	5				
Baby shampoo	Negative Ranks	5	3.00	15.00	-2.023	0.043*
	Positive Ranks	0	0.00	0.00		
	Ties	0				

Figure 1

A flowchart of methodology from sampling to in-vitro tests

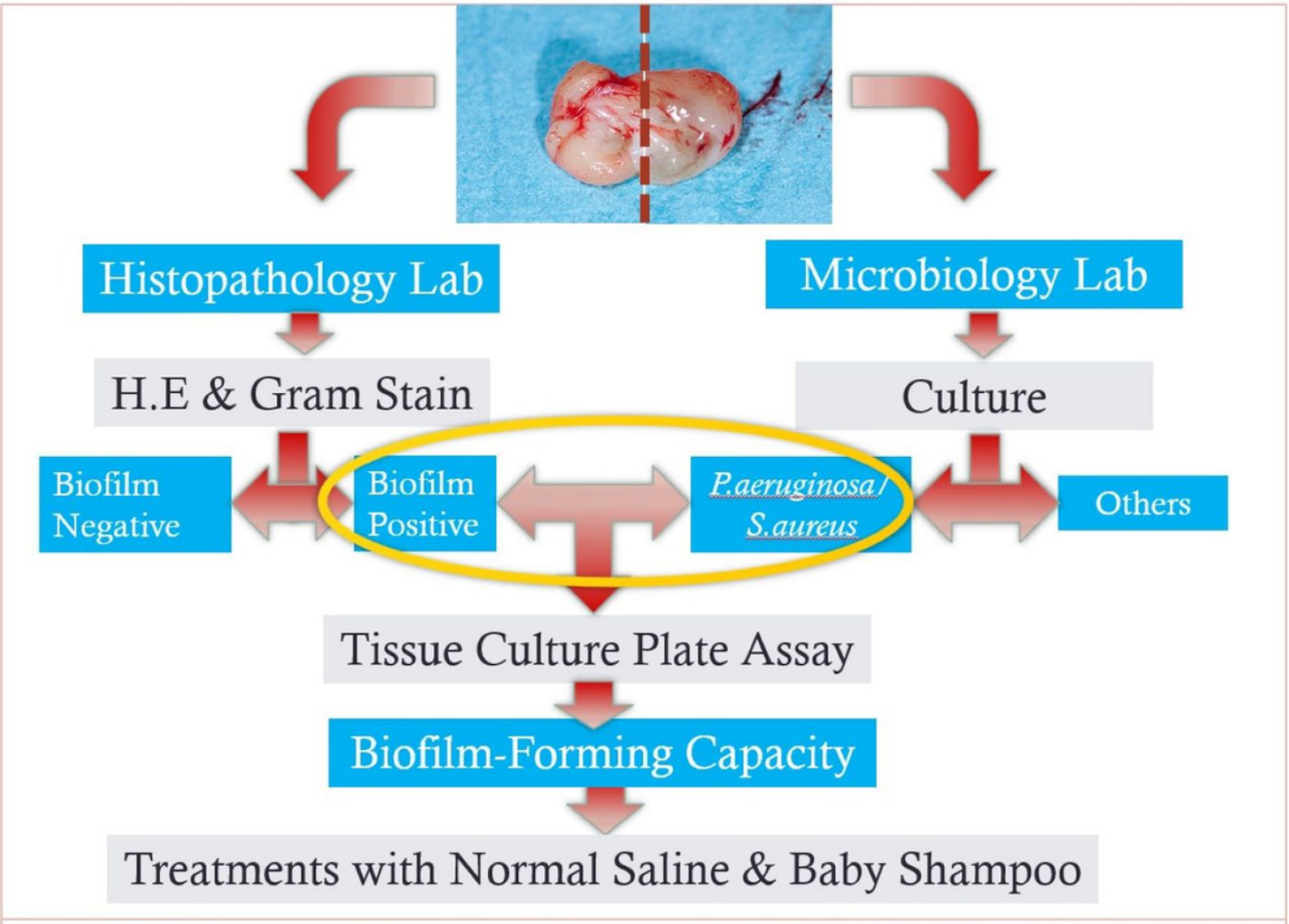

Figure 2

Nasal polyp histopathology specimen

(A) Nasal polyp specimen with H&E staining, under light microscopy 20x magnification. (B) Nasal polyp specimen with Gram staining, under light microscopy 40x magnification

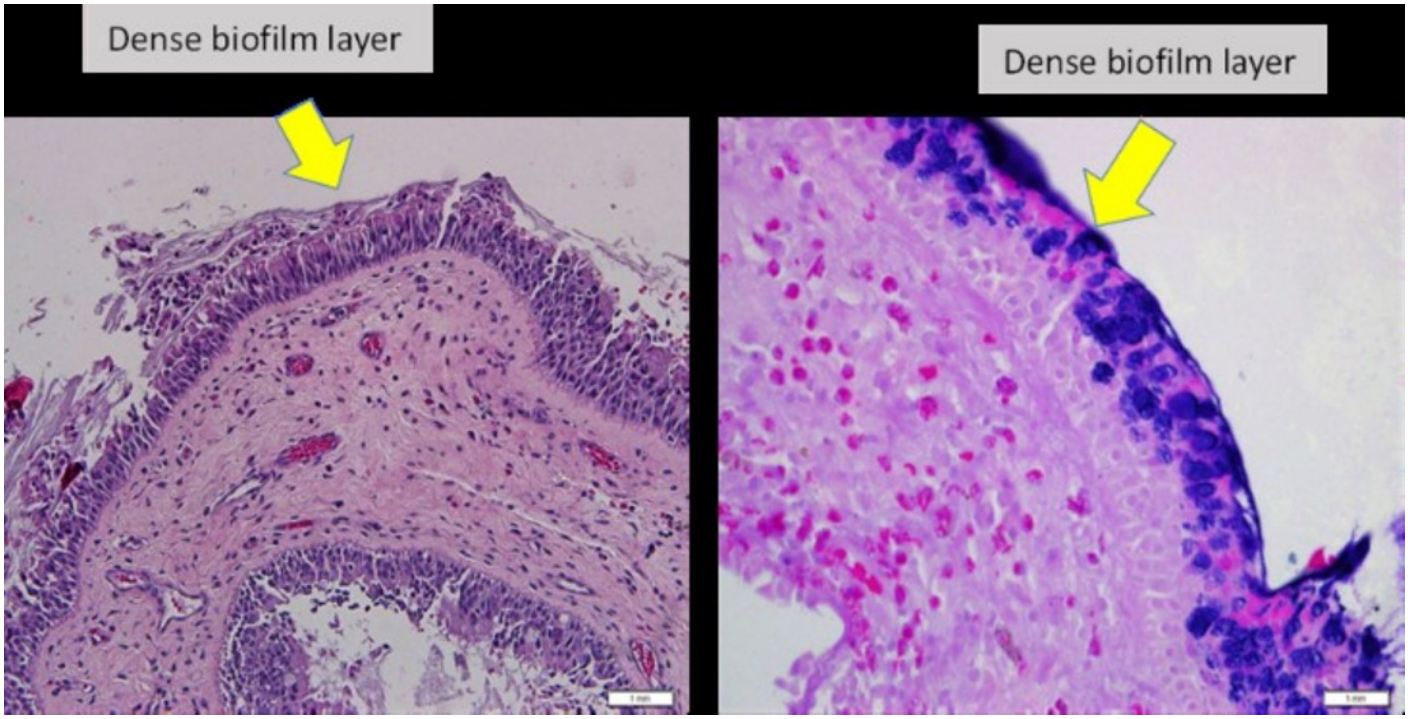

Figure 3

Comparison of the effects of normal saline and diluted 1% baby shampoo treatment in biofilm mass reduction