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Microbiological alterations in the conjunctiva of hot tub-soaking ophthalmologists (MACHO): a randomized double-blind clinical trial

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Abstract

Background: To determine if there is a difference in the quantity of microbial flora of the conjunctiva in individuals practicing head submersion (“dunk”) versus no head submersion (“no-dunk”) during hot tub use.

Methods: In this double-blind randomized clinical trial, healthy volunteers aged ≥ 18 years were recruited. Participants were randomized to head submersion versus no head submersion during a 15-minute hot tub soak. Study personnel, masked to the dunk or no-dunk group assignment, obtained conjunctival cultures before and immediately after hot tub use. De-identified specimens were submitted to the clinical microbiology laboratory for culture and analysis. The main outcome measure was the difference in the quantity of organisms cultured from the conjunctiva before and after hot tub exposure, as determined using a defined ordinal scale. A two-tailed Student’s t-test was performed to compare the total microbial colony counts between the two arms. Simpson’s diversity was used to measure the changes in organism diversity between the arms.

Results: Of 36 enrolled subjects, 19 were randomly assigned to the dunk and 17 were assigned to the no-dunk groups. Water samples obtained from all hot tubs were culture negative. Eleven of 19 eyes (58%) from the dunk group and eight of 17 eyes (47%) from the no-dunk group had negative conjunctival bacterial cultures before and after hot tub exposure. However, six of 19 eyes (32%) and four of 17 eyes (24%) of the dunk and no-dunk groups, respectively, were culture-positive after, but not before hot tub exposure. The quantity of organisms before and after hot tub exposure was not significantly different between the two arms ($P = 0.12$). However, the

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Ethical approval: The study adhered to the Declaration of Helsinki and was compliant with the Health Insurance Portability and Accountability Act. Approval from the University of California San Francisco’s Institutional Review Board/Ethics Committee was obtained before the study began. The trial was registered at clinicaltrials.gov (Identifier: NCT03987178). Subjects were recruited from the Department of Ophthalmology at the University of California San Francisco (UCSF) and informed of the study with a verbal announcement and subsequent follow-up emails (approved by the UCSF Institutional Review Board).

Conflict of interests: None.

dunk group only showed a small increase in the quantity of organisms after as compared to before hot tub use ($P = 0.03$). None of the samples from subjects or hot tubs were culture-positive for *Acanthamoeba*.

Conclusions: Head submersion in a public hot tubs during a 15-minute soak does not appear to change conjunctival flora, as determined by culture plate yield, this does not eliminate the association between hot tub use and devastating and painful corneal blindness. Therefore, our recommendation is to remove contact lenses prior to hot tub use, avoid head submersion in a hot tub, and urgently seek ophthalmological help if any eye pain and/or decrease in vision is experienced after hot tub use.

Keywords

keratitis; corneal ulcer; cornea; conjunctiva; hot tub; conjunctivitis; bacterial; conjunctival diseases; eye disease

INTRODUCTION

Hot tub immersion is associated with relaxation and stress relief. Unfortunately, hot tub use is also a risk factor for vision-threatening eye infections [1–3]. Infectious keratitis resulting from *Acanthamoeba spp*. is a cause of corneal blindness and is specifically associated with hot tub exposure. Contact lens wear is a risk factor for acanthamoeba keratitis, and wearing contact lenses in a hot tub increases this risk [4, 5]. In the United States, the Federal Drug Administration and the Centers for Disease Control recommend against wearing contact lenses in hot tubs [6, 7].

The mechanism underlying infectious keratitis typically requires disruption of the corneal epithelium, followed by exposure to an infectious organism. Contact lens wear can cause chronic recurrent corneal epithelial trauma. Subsequent exposure to contaminated water sources is likely to provide a source of corneal infection. More than 21% of random cultures obtained from hot tubs demonstrate growth of *Pseudomonas aeruginosa*, an obligate aerobic, motile, gram-negative bacillus associated with rapid and destructive corneal infections [8]. In addition, *Acanthamoeba* spp. have been isolated from 50% of hot tub samples by polymerase chain reaction (PCR) in random sampling of various indoor recreational water centers, and 21.2% of hot spring water samples [9, 10]. It would be reasonable to postulate that keratitis from hot tub use may be caused by direct exposure of the cornea to organisms from the hot tub. Alternatively, hot tub use may cause changes in the conjunctival flora or even the biofilm coating the contact lenses, and these changes may increase the risk of subsequent corneal infections. However, most people who use hot tubs, even those who elect to wear their contact lenses in the hot tubs, do not seek emergency care for corneal infections. Factors that increase the susceptibility to hot tub-related infectious keratitis are unknown.

Here, we investigated whether the microbial flora of the conjunctiva changed after soaking in a hot tub. We hypothesized that more colonies of microorganisms would be identified from the conjunctiva of subjects who submerged their heads while soaking in a hot tub than from those who did not.

METHODS

The study adhered to the Declaration of Helsinki and was compliant with the Health Insurance Portability and Accountability Act. Approval from the University of California San Francisco's Institutional Review Board/Ethics Committee was obtained before the study began. The trial was registered at clinicaltrials.gov (Identifier: NCT03987178). This study was performed from May 27, 2019 to March 26, 2020.

In this randomized controlled trial, subjects were involved in the design and conduct of this research and were willing to participate. Subjects were recruited from the Department of Ophthalmology at the University of California San Francisco (UCSF) and were informed of the study by a verbal announcement and subsequent follow-up emails (approved by the UCSF Institutional Review Board). In the announcement and emails, potential subjects were informed about the priority of the research question, treatment arms, and choice of outcome measures. A contact email was provided for follow-up questions or discussion.

Potential subjects were excluded if they were under 18 years of age, were pregnant or possibly pregnant, had active diarrhea, or had a diagnosis of high or low blood pressure, lymphedema, heart disease, and/or a seizure disorder. If a subject wore contact lenses, they were asked to remove the lenses 24 h prior, during, and 24 h after hot tub exposure. As we recruited study subjects from the Department of Ophthalmology, their healthy ocular status was verified by relying on self- disclosure. Prior to participation, a self-administered screening questionnaire was administered to confirm that the participants did not meet any of the exclusion criteria. The subjects were made aware that all collected information was anonymous and confidential. After recruitment, each subject provided a written informed consent. Each participant was given a timer set to 15 min, which was started immediately upon entrance into the hot tub.

Each enrolled subject was randomly assigned to the head submersion ("dunk") or no head submersion ("no-dunk") group, assigned a study eye ("right eye" or "left eye"), and four culture plate sample numbers. Randomization was performed by block randomization using Microsoft Excel 2010 (Version 14.0; Microsoft Corporation, Redmond, Washington, USA) to randomize participants to the dunk versus no-dunk and right eye versus left eye group. Each identifier was assigned to an exposure arm, study eye, and four culture plate sample numbers prior to the study date. Each subject was assigned an identifier in order of enrollment. Allocation was not concealed by the investigator assigning the treatment. All other members of the study team were masked to the exposure type. We calculated that 17 subjects per arm would provide at least 80% power to detect one standard deviation (SD) unit difference in organism quantity (two-sided alpha of 5%). If a subject was randomized to the right eye group, only the right conjunctiva was swabbed before and after hot tub use. If the participant was randomized to the dunk group, they were asked to submerge their head at least up to brow level at least once during hot tub use (Figure 1). There was no upper limit to the specified dunks. Subjects who were randomized to the no-dunk group were asked to maintain their chin above water for the entire 15 minutes and were asked to refrain from submerging their head in the hot tub.

A member of the study group, who was completely masked to the treatment assignment, performed four minimally invasive conjunctival cultures for each subject, two prior to hot tub use (“pre”) and two after hot tub use (“post”) on each subject’s assigned study eye. Masking of the dunk or no-dunk designation was achieved by asking study subjects to rinse off, including wetting their hair, prior to hot tub use. At each time point, the conjunctival swabs were plated on sheep’s blood agar (Remel Products; Lenexa, KS, USA) and non-nutrient agar (Hardy Diagnostics; Santa Maria, CA, USA). Each sample was de-identified, randomized, and then sent to the UCSF Clinical Microbiology Department for identification. Blood agar culture plates were incubated for 48 h and analyzed using matrix-assisted laser desorption/ionization (MALDI; MALDI Biotyper CA System; Bruker Daltonics Inc., Billerica, MA) to identify bacteria. *Escherichia coli* overlay was performed on all non-nutrient agar plates, which were then incubated for 7 days prior to a final read for *Acanthamoeba spp*.

The primary outcome measure was comparing the quantity of microbial colonies between the dunk and no-dunk arms of the study. To measure the total number of colonies for each person per time-point, genus and species were identified for each sample and quantified by using an ordinal scale of 0–4 (Table 1). The total score was the microbial colony quantity for that person and time-point. A two-tailed Student’s *t*-test was performed to compare the total microbial colony counts between the two arms. A pre-specified secondary measure was used to assess whether the organism diversity changed before and after hot tub use in all groups. Analysis was performed with a paired *t*-test using Microsoft Excel 2010. Simpson’s diversity was used to measure the changes in organism diversity between the arms (post-test comparison) and longitudinally (pre- versus post-test comparison), expressed in units of effective number. This analysis was performed using Stata version 11 (StataCorp LP, College Station, TX).

RESULTS

Thirty-six subjects were recruited within a 2-month period from May to June 2019. Nineteen subjects were randomized to the dunk arm and 17 subjects were randomized to the no-dunk arm (Figure 2). The baseline demographics and characteristics are displayed in Table 2. The mean age of participants was 35.7 years, and 48.6% of participants were female. There was a higher percentage of contact lens wearers randomized to the dunk group than to the no-dunk group, although the average time since contact lenses were last worn was longer in the dunk group (38.1 days for the dunk group compared to 3.5 days for the no-dunk group). The majority of participants were ophthalmologists (77.8%); the remaining participants were optometrists (5.3%), other physicians (5.3%), medical students (2.8%), and eye clinic staff (8.3%).

Three hot tubs from commercial facilities were used as study sites; these hot tubs were not selected randomly and were located in Burlingame, CA; Foster City, CA; and San Francisco, CA. During the study, water from each hot tub as well as a soft contact lens soaked in hot tub water for 15 min were swabbed and plated on blood and non-nutrient agar plates. All hot tubs were completely culture-negative on both blood agar (Remel Products) and non-nutrient agar (Hardy Diagnostics). Two contact lenses yielded no microbial growth and

one contact lens soaked in water from hot tub #3 grew *Neisseria spp.*, which was identified by MALDI-TOF mass spectrometry as *Neisseria sicca*, a commensal organism (Figure 3). None of the participants' samples were culture-positive for *Acanthamoeba*, and the majority of bacterial culture plates did not show any growth before or after hot tub use (Tables 3–4, Figure 4).

There was no significant difference in the quantity of microbial colonies when comparing the eyes of subjects randomized to the dunk versus no-dunk groups (Table 5). Interestingly, there was an increase in the number of organisms after hot tub use compared to before hot tub use in patients who were in the dunk group ($P = 0.03$) (Table 6). Using Simpson's diversity to measure and compare organism diversity within each population, there was no statistically significant difference in organism diversity between the two arms or time-points (Tables 7, 8).

DISCUSSION

Ophthalmologists routinely ask patients who present with infectious corneal ulcers whether they had had any recent hot tub exposure, to determine the relative risk of acanthamoeba keratitis, as diagnostic strategies to detect this organism are unique [11–13]. Acanthamoeba keratitis is also resistant to traditional antimicrobial therapy and requires a completely different therapeutic algorithm [11–14]. With the rising popularity of hot tubs and whirlpools, hot tub-related keratitis is increasingly becoming a public health issue [2, 9]. It would be ethically inappropriate to construct a prospective randomized clinical trial in which we scraped the corneal epithelium of a healthy ophthalmologist for culture before and after hot tub use, and we were also uncomfortable allowing our contact lens-wearing colleagues to wear their lenses in a hot tub. Therefore, we decided to investigate the role of conjunctival flora and hot tub use to elucidate the mechanism of increased risk of keratitis in this setting.

In this study, we performed a randomized controlled trial to evaluate the role of hot tub use in changes in the conjunctival flora using the diagnostic gold standard of conjunctival cultures [15]. The public hot tub samples used in this study did not show any microbial growth. Only one contact lens soaked in tub water grew a single organism, which was a commensal skin flora (*Neisseria sicca*). Additionally, we found no significant change in the quantity of conjunctival organisms 1) before or after hot tub use, or 2) with or without head submersion. We found that a 15-minute soak in a public hot tub, with or without head submersion, did not significantly alter the microbial quantity count for obtained conjunctival cultures . These results suggest that keratitis caused by hot tub use may not be related to microbiological alterations of the conjunctiva. There may be other mechanisms that contribute to an individual's risk of hot tub-associated infectious keratitis.

This study had some limitations. While culture plates remain the diagnostic gold standard, the sensitivity of conjunctival cultures (and hot tub water culture) is low, especially when compared to PCR and novel diagnostic modalities, such as metagenomic deep sequencing [16–18].We also used hot tubs found in commercial facilities, which are regularly chlorinated, frequently monitored for chemical composition, and possibly cleaner

than hot tubs in private homes (the use of which was strictly forbidden by our institutional review board for this particular study). Therefore, these results do not apply to all hot tubs, as it is possible that our study was conducted in three particularly clean hot tubs and facilities.

To simulate a real-world hot tub experience, we did not specifically instruct the subjects on how long or the number of times to submerge their head in the hot tub. Therefore, we cannot comment on whether the number and/or the duration of head submersions had any influence on these results. Additionally, all participants remained in the hot tub for 15 min. It is unknown if longer soaks would yield different results. Another limitation is that the study conjunctival cultures was masked only to the dunk versus no-dunk, and not to pre- versus post-hot tub use cultures.

Despite these limitations, no previous randomized controlled trial had sought to determine the relationship between hot tub use and potentially blinding keratitis. This study was designed and powered to detect any significant differences in the conjunctival microbiome, and the study members were blinded whenever possible. Future studies may investigate the use of more sensitive diagnostic modalities and, perhaps, with appropriate institutional review board approval, the use of less regulated real-world hot tubs.

CONCLUSION

In conclusion, although the mechanism of the increased risk of corneal infections from hot tub exposure did not appear to be secondary to hot tub-induced conjunctival changes in our study, this does not eliminate the association between hot tub use and devastating and painful corneal blindness. Therefore, our official recommendation is to remove contact lenses prior to hot tub use, avoid head submersion in a hot tub, and urgently seek ophthalmological help if any eye pain and/or decrease in vision is experienced after hot tub use.

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Figure 1.
Representative image of an ophthalmologist randomized to the head submersion (“dunk”) group during hot tub exposure. This image was captured during a pilot hot tub experiment. Upon careful scrutiny of the technique, for the formal study, all participants were instructed to lower their eyebrows into the water to complete the “dunking” maneuver successfully.

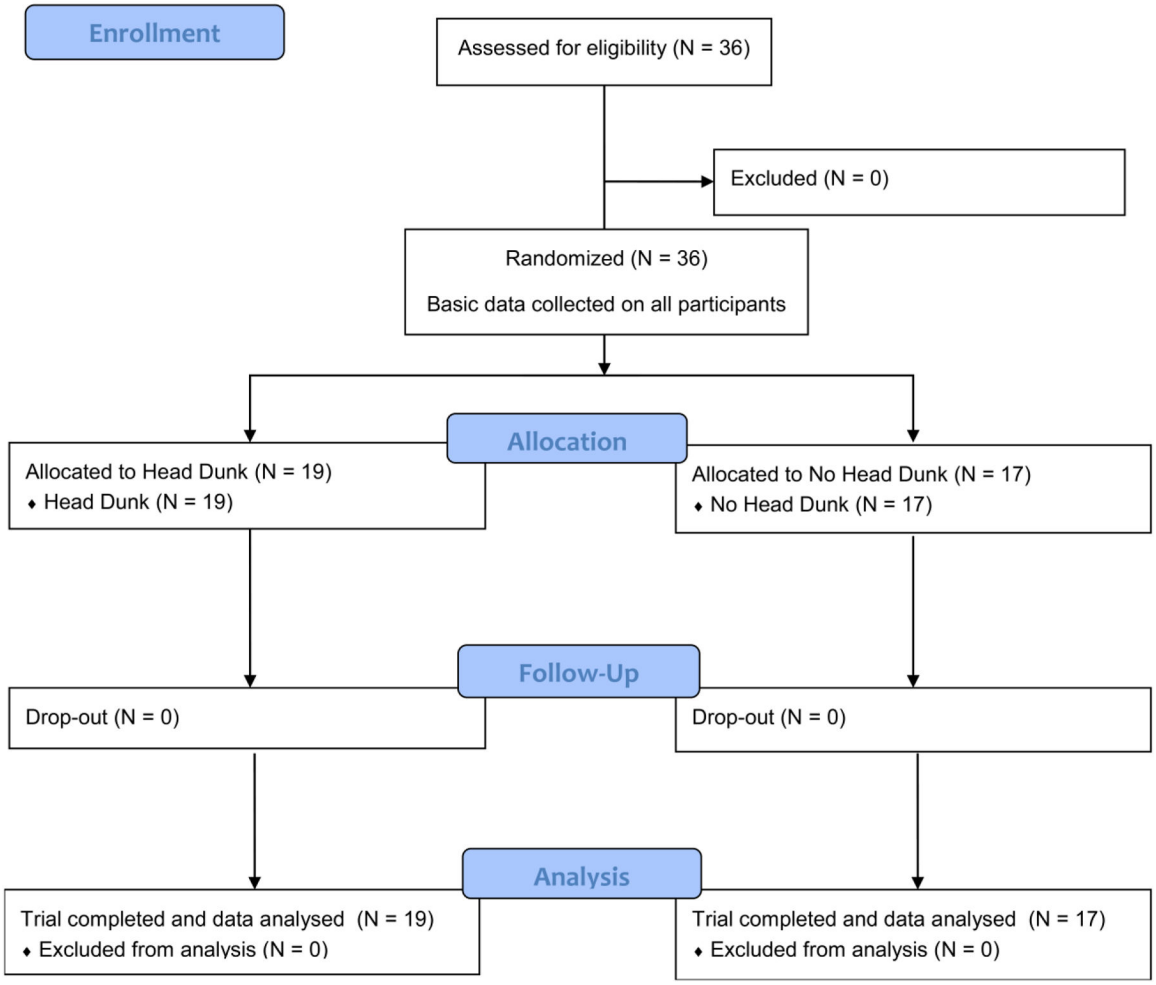

Figure 2.

The study flow diagram: Partcipants' distribution in the microbiological alterations in the conjunctiva of hot tub-soaking ophthalmologists trial.

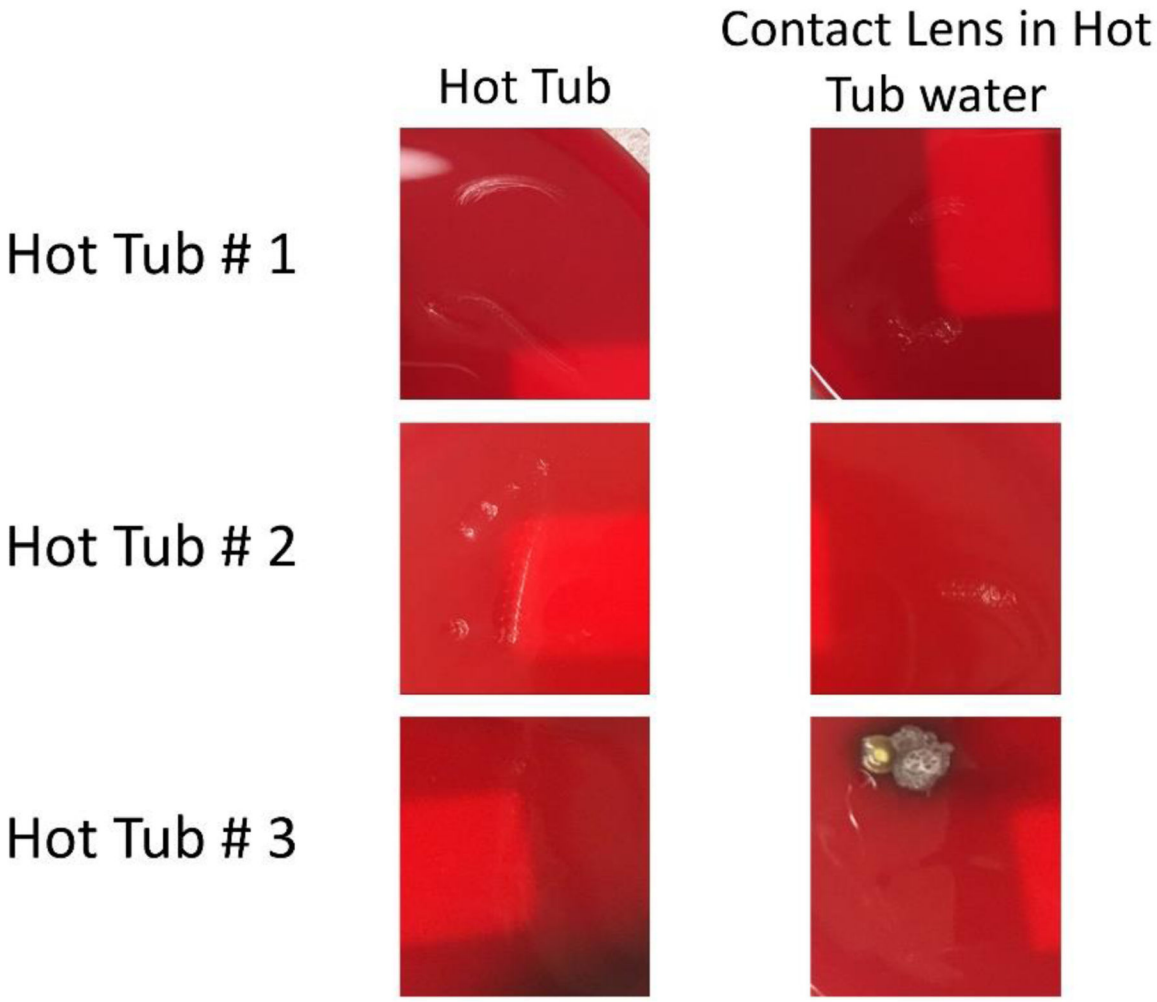

Figure 3.

All hot tubs were culture negative. A soft contact lens (representative biofilm) was soaked for 15 minutes in hot tub water and grew *Neisseria sicca*, a commensal organism, in hot tub #3. None of the samples from subjects using hot tub #3 grew this particular organism before or after hot tub exposure.

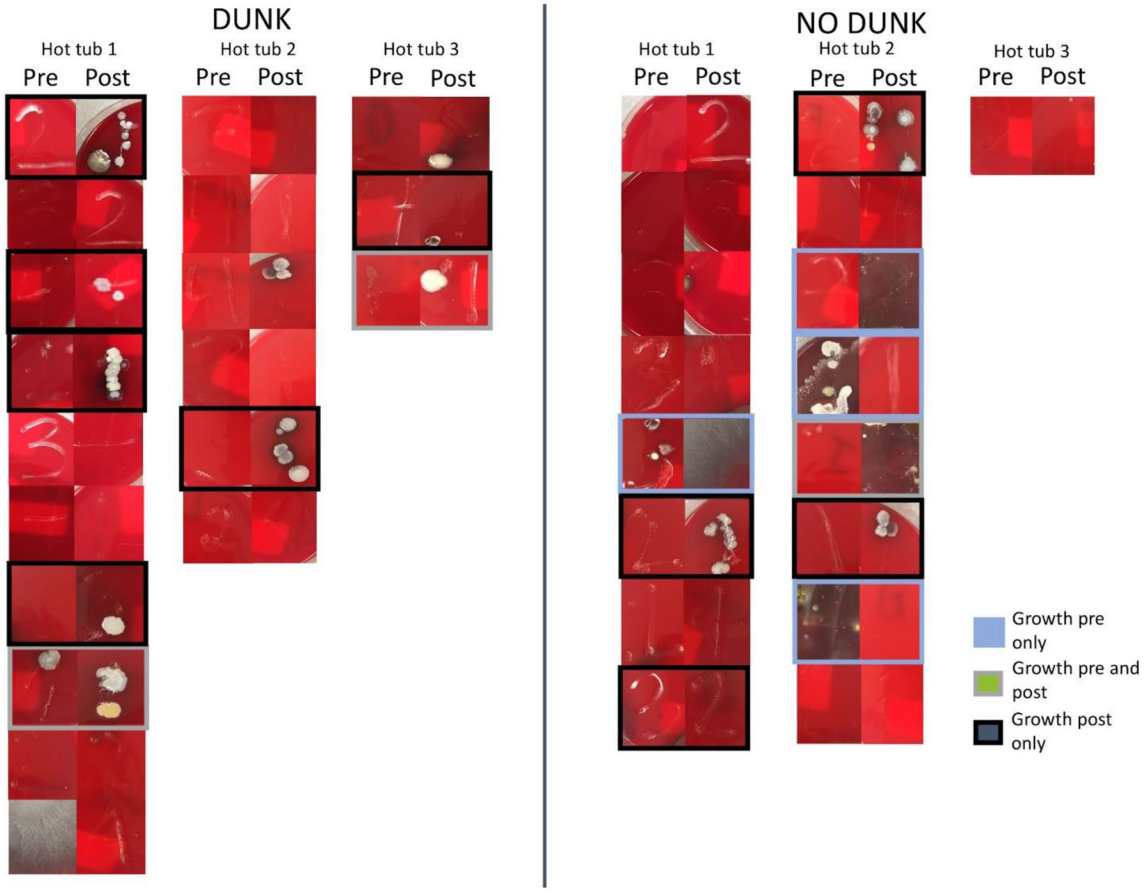

Figure 4.

Visual representation of bacterial culture results. The majority of blood agar plates did not grow any organism before or after hot tub use. All plates were officially analyzed for growth at 48 hours. Plates may have been photographed for colony representation at later time-points which is why some blood plates appear darker than others.

Table 1.

Ordinal scale used to quantify the number of organisms.

Assigned Number	Report (# colonies)
0	“None”
1	“Rare”(1)
2	“Few” (2–4)
3	“Moderate” (5+)
4	“Numerous” (confluent)

Each genus/species reported in a sample was assigned an ordinal number based on the culture results report. These ordinal numbers were summed in order to assign a total quantity of colonies for each person and time-point.

Table 2.

Demographics of subjects randomized to head submersion (dunk) and no head submersion (“no-dunk”) during hot tub exposure.

	DUNK (*n* = 19)	NO-DUNK (*n* = 17)	ALL (*n* = 36)
Age (years), mean	36.7	34.2	35.7
Sex (% female)	57.9	52.9	54.3
Contact lens wearers (%)	57.9	35.3	48.6
Time since contact lenses were last worn (days)	38.1	3.5	25.9
Occupation			
Ophthalmologist, n (%)	16 (84.2)	12 (70.6)	28 (77.8)
Optometrist, n (%)	1 (5.3)	2 (11.8)	3 (8.3)
Other Physician, n (%)	1 (5.3)	0 (0)	1 (2.8)
Medical Student, n (%)	0 (0)	1 (5.9)	1 (2.8)
Eye Clinic Staff, n (%)	1 (5.3)	2 (11.8)	3 (8.3)

Table 3.

Number and percentage of plates that showed microbial growth before (“pre”) and after (“post”) hot tub exposure, separated by exposure type (no-dunk and dunk).

	NO-DUNK (*n* = 17)	DUNK (*n* = 19)	*P*-value
No growth, n (%)	8 (47.1)	11 (57.9)	0.53
Growth pre- and post-hot tub exposure, n (%)	1 (5.9)	2 (10.5)	0.62
Growth pre- but not post-hot tub exposure, n (%)	4 (23.5)	0 (0)	**0.04**
Growth post- but not pre-hot tub exposure, n (%)	4 (23.5)	6 (31.6)	0.53

***P*-value less than 0.05 is shown in bold**.

Table 4.

Summary of cultured micro-organisms in the head submersion (dunk) and no head submersion (no-dunk) groups before (pre) and after (post) hot tub use.

ID #	Group	PRE	post
1	NO-DUNK		
2	DUNK		
3	DUNK		
4	DUNK		
5	DUNK		
6	NO-DUNK		
7	DUNK		*Staphylococcus epidermidis*
8	NO-DUNK		
9	DUNK		*Staphylococcus epidermidis*, Corynebacterium sp.
10	NO-DUNK		
11	NO-DUNK		
12	DUNK		
13	NO-DUNK	Coryneform gram-positive rods	
14	DUNK		
15	NO-DUNK	*Staphylococcus epidermidis*	
16	NO-DUNK	Coryneform gram-positive rods	Coryneform gram-positive rods, Proteus sp.
17	NO-DUNK		
18	DUNK		
19	NO-DUNK	*Staphylococcus epidermidis*, Proteus sp.	
20	DUNK		
21	NO-DUNK		
22	DUNK		*Staphylococcus epidermidis*
23	DUNK		
24	DUNK		*Staphylococcus epidermidis*
25	DUNK	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
26	DUNK		
27	NO-DUNK		

ID #	Group	PRE	post
28	NO-DUNK	*Moraxella* sp.	
29	DUNK		
30	NO-DUNK		*Staphylococcus epidermidis*
31	NO-DUNK		
32	NO-DUNK		
33	DUNK		
34	DUNK		Coryneform gram-positive rods
35	DUNK	*Corynebacterium* sp.	Corynebacterium sp.
36	NO-DUNK		

Empty cell indicates no growth on blood agar plates after 48 hours of incubation.

Table 5.

Primary analysis comparing the difference in quantity of microbial colonies before and after hot tub exposure in the two arms: head submersion (dunk) and no head submersion (no-dunk).

	DUNK (*n* = 19)	NO-DUNK (*n* = 17)	Mean difference (95% CI)	*P*-value
Mean quantity pre-hot tub	0.21	0.94	0.73 (−0.12 to 1.58)	0.10
Mean quantity post-hot tub	1.05	0.71	0.35 (−0.58 to 1.27)	0.46
Difference in quantity (post-pre)	0.84	−0.23	1.08 (−0.17 to 2.32)	0.12

Total microbial quantity count for each sample was calculated using an assigned ordinal scale for each genus-species and summed for each sample (refer to Table 1).

Abbreviations: CI: confidence interval. Statistical analysis was performed using a two-sided, paired Student's *t*-test.

Table 6.

Pre-specified secondary analysis comparing quantity of microbial flora before hot tub use with that after hot tub use, for all groups.

	Pre-hot tub	Post hot-tub	Mean difference (95% CI)	P-Value
Mean quantity (ALL)	0.89	0.56	0.33 (−0.57 to 1.24)	0.31
Mean quantity (NO-DUNK)	0.94	0.71	0.23 (−0.76 to 1.24)	0.67
Mean quantity (DUNK)	0.21	1.05	0.84 (0.05 to 1.64)	**0.03**

Abbreviations: CI: confidence interval; n: number; %: percentage. P-value less than 0.05 in bold. Statistical analysis performed using a two-sided paired students t-test.

Table 7.

Simpson's Diversity Within Each Population ("pre" and "post" Refer to Culture Results Before and After hot tub Exposure, Respectively).

	Mean (units of effective number)	95% Confidence Interval
PRE/NO-DUNK	2.22	1.48 to 3.18
POST/NO-DUNK	1.36	1.00 to 2.07
PRE/DUNK	1.00	1.00 to 1.00
POST/DUNK	1.25	1.00 to 1.75

Table 8.

Simpson’s diversity between arms (dunk versus no-dunk) or time-points (pre- versus post-hot tub exposure).

	P-Value
PRE/NO-DUNK versus POST/NO-DUNK	0.18
PRE/DUNK versus POST/DUNK	0.35
PRE/NO-DUNK versus PRE/DUNK	0.06
POST/NO-DUNK versus POST/DUNK	0.81
All PRE versus all POST	0.22