REBREATHER CLEANING EFFICACY

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ABSTRACT

Titan® rebreathers were used for 30-minute dives in normal operation. The rebreathers were then treated using a variety of protocols, including no postdive cleaning, postdive drying only, freshwater rinse, and application of RelyOn®, Listerine®, Betadine®, or Steramine® disinfectants. Based on procedures currently used in closed-circuit diving operations, disinfectants were applied using one or more of three methods: spray application, rinse through, or flooding. They were then swabbed in four locations (mouthpiece, exhalation hose, exhalation counterlung, and inhalation hose), the swabs cultured for 48 hours at 35-37°C (95-99°F), and individual colonies of bacteria either counted or estimated. Outcomes indicate that failing to wash rebreathers after use resulted in confluent (>100,000 colonies) colonial growths. Growth resulted from swabs in all four locations, including post-scrubber sampling points. Growth was seen even after allowing rebreathers to dry for two weeks prior to sampling. Freshwater rinsing reduced colonial growth to an approximate 30,000 colonies per culture. All disinfectant applications reduced colonial growth from 0-3,500 colonies. Application procedures varied in efficacy, with the following methods ranked in increasing efficacy: spray, rinse, flooding. The most effective combination tested was the use of Steramine® floods, with zero postincubation colonial growths seen. Based on these results, the authors conclude that rebreathers should be cleaned after use and that some type of disinfecting agent used.

Keywords: bacteria, closed-circuit, contamination, disease, disinfect, illness, sterilize

INTRODUCTION

Closed-circuit rebreather (CCR) use is becoming increasingly common in the recreational-diving community. Since the mid-1990s a wide variety of models and types of rebreathers have been utilized. All of these differ from more commonly available open-circuit (OC) scuba equipment in that exhaled gas is circulated through a closed system, called the breathing loop. Carbon dioxide is removed using a soda lime (calcium hydroxide) absorbent, oxygen is added to replace what was metabolized, and the gas is reused by the diver. One equipment factor that is different between the two types of equipment is that the internal surface area of CCRs is much greater than OC equipment and much less prone to rinsing by water in which the diver is submerged. Contamination of these internal surfaces by the diver is consequently greater in CCRs than OC.

When the diver exhales into a rebreather, germs from the mouth and respiratory system, such as *Streptococcus salivarius*, Spirochetes, and *Lactobacillus* sp. (Todar, 2011) are distributed throughout the unit. This biological contamination of the breathing apparatus may lead to infections during subsequent use (Anon, 2010). Another concern is that a single rebreather may be used by more than one diver in a given day or period of time. This leads to a risk of bacterial cross contamination between individuals (Bozanic, 2010).

Anecdotally, many rebreather users have complained of low-grade respiratory ailments (Howard, 2013), including coughs, colds, chest congestion, and similar problems. It is uncertain whether these problems are related to rebreather usage. In at least one case, a severe fungal infection of the lungs was attributed to rebreather use (N. Bailey, May 2012, pers comm). The course of treatment took more than nine months.

In a parallel case, a professional musician contracted a similar fungal infection after playing a set of bagpipes that had not been disinfected after prior usage (T. Howard, March 2013, pers comm). The pipes in question had been stored for several months since the previous use.

After using a rebreather, experts recommend some type of cleaning regimen. Different manufacturers recommend chemicals such as Betadine®, RelyOn®, Listerine®, and bleach. However, these regimens differ significantly. No studies have tested or validated standards for cleaning rebreathers used for underwater breathing.

The closest example to a rebreather in common usage is an anesthesia machine. Though research on cleaning rebreathers is lacking, many studies have done on anesthesia machines (Baillie et al., 2007; Browne and Chernesky, 2011). Anesthesia machines and rebreathers are similar in that both are closed-circuit systems and both use soda lime to remove carbon dioxide. The absorption of carbon dioxide by the soda lime is an exothermic reaction that causes the air in the loop of the machine to become warm and moist, producing an environment conducive to bacterial and viral growth. A variety of organisms have been found

in previous studies involving anesthesia machines, such as *Candida* sp., *Dermatophytus* sp., Penicillium sp., *Staphylococcus, Pseudomonas*, and *Mycobacterium tuberculosis* (Maslyk et al., 2002; Arai and Azevedo, 2011). It used to be thought that the soda lime in the carbon-dioxide absorbent bed would kill the bacteria in the breathing loop, but Leijten et al. (2011) found that soda lime had no demonstrable bactericidal action. Procedures for cleaning anesthesia machines have included disinfection, steam sterilization, disposable parts, scrubbing and the incorporation the hydrophobic membrane heat and moisture exchanging bacterial/viral filter (HMEF) (Rathgeber et al., 1997; Hogarth, 2011). Many of these cleaners are not available to the general public nor is it possible to have autoclaves in one's home. Last, the incorporation of an HMEF has not been possible in rebreathers used as an underwater breathing apparatus (UBA) due to the structure of the rebreather itself.

The purpose of this project is to find a chemical cleaner and procedure that is effective at preventing bacterial growth in closed-circuit rebreather UBAs.

CURRENT DISINFECTING PROCEDURES

Rebreather divers utilize a variety of methods and agents to clean their rebreathers. A brief summary of the more common of these follows.

Nothing. Some divers do not clean their CCR. The rationale is that the scrubber material (calcium hydroxide) is extremely alkaline and kills any germs, fungi, bacteria, or viruses that pass through it. They further postulate that adequate internal drying of the rebreather components between usages will kill organisms that might cause infection.

Freshwater rinses. A step beyond doing nothing utilizes a daily freshwater rinse. Freshwater rinses are done by flushing clean water through the hoses of the unit and filling and draining the counterlungs one or more times. The belief is that a thorough freshwater rinse is adequate to flush any contaminants or biological organisms from the equipment.

End-of-trip wash. Some utilize their rebreathers during multiple consecutive days (up to six days of use is commonly reported) and then clean them at the end of the dive sequence. Their opinion is that any growth is not harmful, because insufficient growth occurs during short intervals when the equipment is used during consecutive days. They further argue that since they are the only person using the rebreather it is only their personal flora and fauna to which they are exposed and thus is not a safety issue.

Daily disinfection. Some divers maintain their units by cleansing them daily, usually with some form of disinfectant. Many methods are used, generally falling into two main procedures: spraying internal parts of the components with a disinfecting agent followed by a freshwater rinse or soaking the components to varying degrees with a disinfecting agent.

Cleaning Agents. Different disinfectants have been used in the recreational CCR community, including RelyOn®, Virkon®, Betadine®, Listerine®, Steramine®, ORF chemicals, alcohol, ozone gas, and many others. Disinfecting agents are used in different manners: liquid solutions, spray applications, rinses or sloshing through the system, soaks, and aerosols.

RelyOn® and Virkon® are generally used by spraying hose and counterlung internal surfaces with a solution from a spray bottle and then allowing them to sit for 10 minutes. The active ingredient in both RelyOn® and Virkon® is potassium peroxyumonosulfate.

Betadine® and Steramine® are typically applied by rinsing or sloshing appropriately diluted solutions through the breathing loop components or flooding the internal volumes completely. Generally a 10-minute contact time is utilized when Betadine® is used, while immediate contact is considered adequate with Steramine®. The active ingredient in Betadine® is povidone-iodine (10 percent) and in Steramine® are alkyl dimethyl benzyl ammonium chloride (5 percent) and alkyl dimethyl ethylbenzyl ammonium chloride (5 percent) (Anon 2006).

Listerine® is used the same way as Betadine®. It has been used full strength and also in varying dilutions. The active ingredients are menthol, thymol, methyl salicylate, eucalyptol and alcohol.

Ozone gas is blown through the breathing loop of the rebreather to control growth of biological organisms.

HYPOTHESES

A CCR that is not cleaned will have more growth of biological contaminants than the CCR that is cleaned. CCRs cleaned with the disinfecting agents will have less growth than those solely rinsed with fresh water.

METHODS

Each Titan® rebreather was used for 30 minutes underwater during ocean dives. A minimum of two divers using two CCRs were used for each trial. After the dives the rebreathers were swabbed in four locations: mouthpiece, breathing hoses, counterlungs, and the inhale connection hose directly after the scrubber. The swabs were then used to inoculate sheep blood agar plates divided into four quadrants labeled with the numbers 1 (mouthpiece), 2 (exhalation hose), 3 (exhalation counterlung), and 4 (inhalation hose). Swabs were taken after an initial freshwater rinse and then again after cleaning with a disinfection agent.

The plates were placed inside sealed containers with a lit candle so that oxygen would be depleted, leaving the plates in a high carbon-dioxide environment for optimal culture growth. All plates were then placed into an incubator for 48 hours at 35-37°C (95-99°F). At that time the number of bacteria colonies was counted. If there were too many colonies to count,

an estimate was made. The plates were photographed for documentation.

Staff at the pathology laboratory at Long Beach Memorial Medical Center grossly identified organisms and verified counting estimates. When there was a delay between incubation and counting, photography, or identification, the plates were maintained in a refrigerated condition at approximately 34°F (1°C) to stabilize the plates and prevent further growth.

On the first rebreather trial, three dives were conducted on a single day. Rebreathers were not cleaned (Experimental Condition #1) so that it could be determined if there was bacterial growth after sitting for two weeks or if bacteria were killed by the time period and internal component drying. Swabs using both dry cotton swabs and swabs dampened with de-ionized water were taken two and 14 days after the dives were concluded.

On the other dives, when the divers surfaced the rebreathers were transported to the test location, where they were given a freshwater rinse (Experimental Condition #2) and initial swabs taken. Sampling was done within one hour of the dives being concluded.

During two trials each, a variety of disinfecting agents and procedures were utilized to clean the CCR. These experimental conditions (#3-9) included the following.

RelyOn® using spray application. Disinfectant solution was made using five grams of RelyOn® mixed with 500 mL water. The resultant solution was sprayed into every breathing-loop opening (mouthpiece, inhale- and exhale-hose openings, and the two counterlung openings). The components were allowed to sit for 10 minutes and then were rinsed. Subsequent swabs were then taken.

RelyOn® using flood application. Disinfectant solution was made using five grams of RelyOn® mixed with 500 mL water. Breathing-loop components were then completely filled with the resultant solution and allowed to sit for 10 minutes. Swabs were taken after a final freshwater rinse.

Betadine® using flood application. Disinfectant solution was made using 4.0 mL of Betadine® liquid to one liter of water (U.S. Navy, 2008). Breathing-loop components were then completely filled with the resultant solution and allowed to sit for 10 minutes. Swabs were taken after a final freshwater rinse.

Listerine® using rinse application. Breathing-loop components were partially filled with undiluted Listerine®, which was then flushed through the system and poured out. Components were allowed to sit for 10 minutes and then rinsed with fresh water before swabbing.

Listerine® using flood application. Breathing-loop components were completely filled with undiluted Listerine® and allowed to sit for 10 minutes. Swabs were taken after a final freshwater rinse.

Steramine® using rinse application. Disinfectant solution was made using two tablets of Steramine® to four liters of water. Breathing-loop components were partially filled with the resulting solution, which was then flushed through the system and poured out. Components were immediately rinsed with fresh water before swabbing.

Steramine® using flood application. Disinfectant solution was made using two tablets of Steramine® to four liters of water. Breathing-loop components were completely filled with the resulting solution and poured out. Components were immediately rinsed with fresh water before swabbing.

Controls. De-ionized water used to produce the disinfectant solutions and tap water used to rinse the rebreathers were both cultured to rule out the water as a source of bacteria. The swabs were put under the running water, agar medium inoculated, and then the plates incubated for 48 hours.

Data are presented as mean ± standard deviation or median, as appropriate.

RESULTS

Colonial growth results for each experimental condition are tabulated in Table 1. Because the sample size was small (n=2-6 per experimental condition), the results from the swabs in different locations were treated as individual data points for each condition. Likewise, when comparing swabs from different sites on the rebreathers, all of the test conditions for an individual swab site were utilized to compare against other swab sites. These results are in Table 2. Finally, because clean laboratory conditions were limited, a single value outlier from each data series was removed to reduce extreme variability, making result interpretation more consistent.

Table 1. Average counts of colonial growth after incubation per experimental condition.

Experimental Condition	Colonies (mean)	Colonies (sd)	Samples (n)
Nothing	100,000	0	7
Freshwater Rinse	30,807	34,618	23
RelyOn® Spray	3,333	4,714	7
RelyOn® Flood	23	23	7
Betadine® Flood	10	8	7
Listerine® Rinse	11	7	7
Listerine® Flood	3	6	7
Steramine® Rinse	10	25	7
Steramine® Flood	0	0	7

Table 2. Average counts of colonial growth after incubation per swab site.

Swab Site	Colonies (median)	Samples (n)
Mouthpiece	5,280	8
Exhalation Hose	375	8
Exhalation Counterlung	135	8
Inhalation Hose	34	8

The rebreathers that sat for two weeks after cleaning had relatively little growth when dry swabs were taken. The dry swab showed confluent growth (>100,000 colonies) in the mouthpiece and minimal growth in the other swab sites. After being swabbed with dampened swabs, results showed confluent growth in all locations. These values are different from those presented in Table 1, which includes only the swabs taken immediately after diving but before rinses. The prevalent organism was *Pseudomonas* sp. (T. Chen, February 2012, pers comm).

The swabs taken from the divers' noses were variable. One diver's culture grew *Streptococcus* sp. and *Staphylococcus* sp. (T. Chen, February 2012, pers comm). The other diver's culture grew nothing.

Cultures from the de-ionized water and tap water used for mixing disinfectants and rinsing the rebreathers grew nothing.

DISCUSSION

Our data suggest that the most contaminated parts of the rebreather system are the mouthpiece and the exhalation hose. The mean for each component was utilized as a variable for trend analysis; however, only those cleaning methods that might have left significant contamination were included, i.e., experimental conditions 4-9 (disinfectant rinses and floods) were excluded. Because this trend analysis included multiple cleaning modes, standard deviations are not provided because the conditions are too dissimilar for the values to be meaningful.

However, all parts of the rebreather cultured bacterial growth after use, including those on the inhalation side of the rebreather after the breathing gas had passed through the absorbent bed. It is apparent that having breathing gas merely pass through the scrubber bed does not kill all organisms. Bacterial growth included Coliform bacteria and *Pseudomonas* sp. (T. Chen, February 2012, pers comm). This indicates the need for some type of cleaning regimen for rebreathers.

To test if prolonged storage is effective in preventing bacterial growth, two rebreathers were not cleaned after use but were allowed to dry completely for two weeks as the sole disinfection protocol. When dry swabbing was performed, there was relatively little growth. However, when a wet swab was used to sample the internal components, confluent growth resulted. Wet swabs may have been more effective in sampling because water is slightly polar-negative and thus may have picked up more bacteria. It is important to note that the wet-swab results more likely mimic real diving scenarios, as use in water environments and the moisture generated by exhaled breaths probably also facilitate bacterial transfer. It is possible that airborne contamination may have occurred during storage; however, this is unlikely because the cultured bacteria were identical between the stored units and the rebreathers sampled immediately postdive. This suggests that contamination occurred during the dives.

Using freshwater rinses reduced bacterial growth. Average growths of approximately 30,000 colonies is roughly one-third of that seen when no cleaning was performed. However, these growth results indicate that this is an inadequate disinfection protocol.

Several disinfecting agents were tested using multiple application procedures. When compared to the protocols discussed above, all of the procedures involving the use of disinfectants were much more effective at preventing bacterial growth (Figure 1).

However, when the procedures using disinfecting agents were examined separately, it was apparent that the practice of using spray applications was less effective than rinse or flood protocols (Figure 2). When disinfectant solution is applied using a

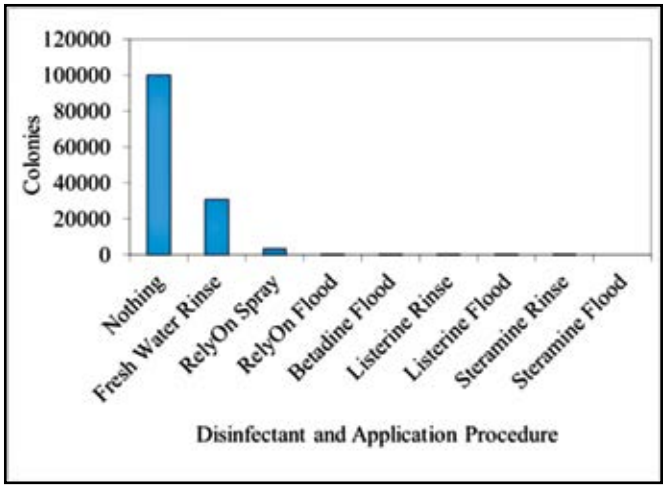

Figure 1. Average bacterial growth colonies by cleaning procedure.

spray application, the practice is to use a spray bottle like those used to apply window cleaners or typical kitchen cleaning solutions. Disinfectant solution is sprayed into all of the orifices of the breathing loop (mouthpiece, inhalation and exhalation hose ends, and tops of both counterlungs). Ten squirts are made into each opening with the spray nozzle set to spray.

There are difficulties in coating all internal surfaces in this manner. The breathing hoses are corrugated and thus are

shielded to some extent from a spray applied from only one direction. In addition, the breathing hoses flex, further increasing the probability that internal surfaces will not be coated with disinfectant. Some of the openings to the internal surfaces are a significant distance (30 cm or more) from the distal portions of the component. This would include both breathing hoses and both counterlungs. This compounds the problem of adequately coating internal surfaces. It is hypothesized that these barriers to disinfectant application impairs the efficacy of spray application procedures.

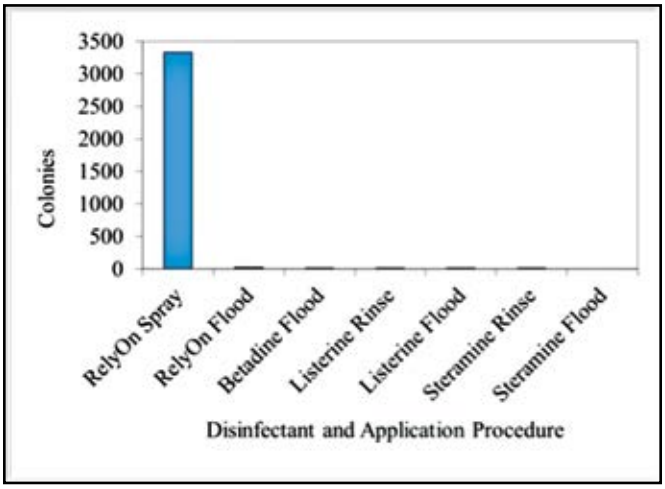

Figure 2. Average bacterial count by disinfectant application protocol.

When spray application protocols are removed from consideration, it can be seen that both rinse and flood applications exhibit similar efficacy, with flooding protocols better than rinsing practices (Figure 3) in preventing bacterial growth. In this study Steramine® and Listerine® provided slightly better results than the Betadine® and Virkon®. While all of the

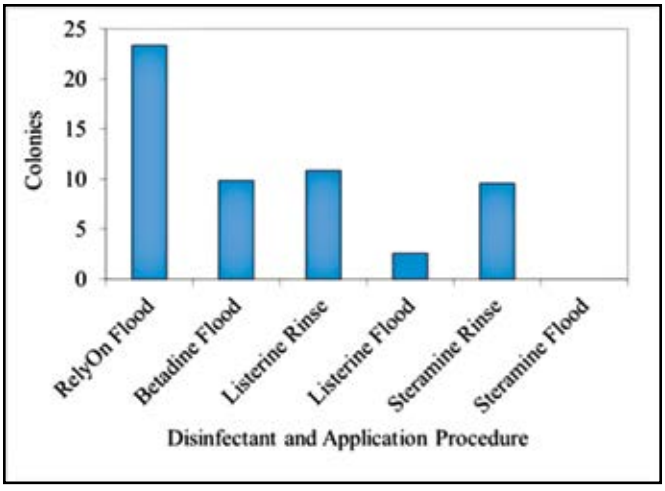

Figure 3. Average bacterial count with rinse- and flood-application protocols.

tested cleaners dramatically reduced bacterial growth when used with rinse or flood applications, only flood application of Steramine® completely eliminated bacterial growth.

Our results were somewhat variable within each category. This can be attributed to the actual procedures used, which mirror how divers clean their equipment. With RelyOn® we used a spraying method and let it sit for 10 minutes before rinsing. The amount applied per spray probably was inconsistent, as was the angle of application. With the rinse-application procedure, the fluid was poured into and out of the hoses and counterlungs, and then the breathing-loop components sat for 10 minutes. There was never an exact volume of liquid cleaner poured into the hoses and counterlungs nor could the breathing-loop components be laid in consistent positions during the sit time. The most consistent disinfection protocol was the flooding procedure, in which the components cleaned were completely filled with solution.

Further variability might be explained based on the sites where the dives occurred. The dives took place in two different sites. We are not sure what the bacterial load was in the water at either site.

We also have insufficient repetitions within each category to make a statistical evaluation between the cleaners. This in part was due to time limitations, location, weather, and diver health issues (common cold).

CONCLUSIONS

Our data show that rebreathers do need cleaning after diving. Neither calcium hydroxide in the absorbent nor complete drying and extended durations between dive activities kills all of the bacteria in the loop. It can also be concluded that freshwater rinses are inadequate to kill off the bacteria in the breathing loop. Spray applications of disinfectants were less effective than rinse or flood applications, presumably due to inadequate coating of all internal surfaces. Disinfectant rinse applications provided better results, with the best results seen when the breathing loop was completely filled with disinfectant solutions. RelyOn®, full-strength Listerine®, Betadine®, and Steramine® all provided substantially identical efficacy when flooding application procedures were utilized, with Steramine® providing marginally better results.

FURTHER WORK

There are numerous areas in which this work can be expanded. The first is the number of replicates. Simply repeating this study to expand the number of trials may produce statistically valid results. There are many other cleaning or disinfecting agents that could be included in a similar study. These include, in part, ozone gas protocols, Enviroguard 64®, other dilutions commonly used in the field (such as 50 percent dilution of Listerine®), CaviCide®, Envirocide®, and MetriGuard®. We did not attempt to ascertain the adverse impacts of any of the

cleaning agents utilized in this study on either human health or rebreather component damage. Finally, utilization of other models of rebreathers may not present identical findings, based on differing component design and structure.

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