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Quantitative evaluation of bacteria released by bathers in a marine water

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Abstract

Enterococci, a common fecal indicator, and *Staphylococcus aureus*, a common skin pathogen, can be shed by bathers affecting the quality of recreational waters and resulting in possible human health impacts. Due to limited information available concerning human shedding of these microbes, this study focused on estimating the amounts of *enterococci* and *S. aureus* shed by bathers directly off their skin and indirectly via sand adhered to skin. Two sets of experiments were conducted at a marine beach located in Miami-Dade County, Florida. The first study, referred to as the “large pool” study, involved 10 volunteers who immersed their bodies in 4700 L during four 15 min cycles with exposure to beach sand in cycles 3 and 4. The “small pool” study involved 10 volunteers who were exposed to beach sand for 30 min before they individually entered a small tub. After each individual was rinsed with off-shore marine water, sand and rinse water were collected and analyzed for *enterococci*. Results from the “large pool” study showed that bathers shed concentrations of *enterococci* and *S. aureus* on the order of 6×10^5 and 6×10^6 colony forming units (CFU) per person in the first 15 min exposure period, respectively. Significant reductions in the bacteria shed per bather (50% reductions for *S. aureus* and 40% for *enterococci*) were observed in the subsequent bathing cycles. The “small pool” study results indicated that the *enterococci* contribution from sand adhered to skin was small (about 2% of the total) in comparison with the amount shed directly from the bodies of the volunteers. Results indicated that bathers transport significant amounts of *enterococci* and *S. aureus* to the water column, and thus human microbial bathing load should be considered as a non-point source when designing recreational water quality models.

Keywords

Recreational water; Bacteria indicators; *Enterococci*; *Staphylococcus aureus*; Bathers; Sediments; Beach sand; Non-point pollution sources; Water quality models

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1. Introduction

Beaches serve an important role in the US economy. Coastal recreation is estimated to contribute approximately 85% of all US tourist revenues (NRDC, 2005). However, this revenue depends upon the availability of coastal areas that are safe for recreational purposes. According to the latest surveillance of the US Centers for Disease Control (CDC) (Yoder et al., 2004), the largest number of recreational water-associated outbreaks (65 outbreaks causing illness among an estimated 2536 persons) occurred between 2001 and 2002. The National Resources Defense Council (NRDC, 2005) indicates that during 2004, US beaches had 24,853 beach closing and advisory days, the highest in 15 years since the NRDC started reporting this data, a 9% increase from 2003. In 2004, 85% of the total closings and advisories were issued because water quality exceeded the recommended bacterial indicator standards for which the sources of contamination were not identified. The inability to identify sources, in particular when point sources of pollution are not obvious and/or not present, has made it difficult to remediate and prevent the impacts to beaches.

Bathers are considered a potential non-point source of contamination impacting recreational waters. Studies have found that bathers shed appreciable amounts of microbes via their skin into the water column, and swimming related illnesses appear to be associated with the microbial water quality, even in the absence of point sources of fecal contamination. Mallman (1962) and Favero et al. (1964), suggested that large numbers of cocci are washed off the skin of bathers into freshwater swimming pools, and thus concluded that cocci are a valid indicator to measure the recreational quality. Calderon et al. (1991) found that gastrointestinal illnesses observed in swimmers were associated with higher numbers of bathers per day and high densities of *S. aureus*. Robinton and Mood (1966), Hanes and Fossa (1970), and Smith and Dufour (1993) concluded that high bacterial densities were shed by bathers into the water column, especially *S. aureus*. Finally, Gerba (2000) and Stewart et al. (2002) found that bathers shed pathogenic organisms via body contact and fecal accidents in drinking water reservoirs, and thus bathers increased the risk of water borne illnesses among drinking water consumers. Of note is that all of these studies were conducted in fresh waters and evaluated the effects of single washing events. Studies are lacking in marine waters and no studies to the authors' knowledge evaluated the effects from sequential bathing events.

Enterococci and *S. aureus* were the bacteria chosen for the current study. *Enterococci* are commonly found in the feces of humans and other warm-blooded animals. Although some *Enterococcus* species are also found naturally in the environment, the US Environmental Protection Agency recommends the use of *enterococci* to measure potential fecal contamination in marine waters (US EPA, 1983, 1984, 2002). *S. aureus* is a Gram-positive coccus that commonly inhabits the anterior nares of humans and *S. aureus* is considered one of the common causes of skin infections in the US. *S. aureus* can survive outside human hosts, and studies have shown correlations between *S. aureus* skin infections and swimming. Charoenca and Fujioka (1995), and Gabutti et al. (2000) suggested that recreational waters characterized by high *S. aureus* densities may increase the risk of contracting skin, eye, and ear infections among bathers.

For over four decades, beach sands and sediments in tropical and subtropical environments have been documented to contain high concentrations of the bacterial indicators, *E. coli* and *enterococci*, and sand is one of the non-point sources of those indicators. Studies conducted in Hawaii and Guam (Fujioka, 1988; Fujioka and Roll, 1997; Fujioka et al., 1999), and in Puerto Rico (Toranzos and Marcos, 2000) have shown that in the absence of any known sources of human/animal waste, *enterococci* and *E. coli* are consistently present and recovered in high concentrations in the subtropical environment. Specifically in South Florida, river bank soils and beach sands have been implicated as the source of indicator microbes to the water column

(Desmarais et al., 2002; Rogerson et al., 2003; Shibata et al., 2004). Recent evidence indicated that the significance of beach sands and other environmental sources is not necessarily limited to the sub/tropics. For example, sands have been implicated as a bacterial source in the freshwater beaches of Lake Michigan (Whitman and Nevers, 2003) and Lake Huron (Alm et al., 2006), both in Michigan. Given the high concentrations of indicator bacteria found in beach sands and sediments, bathers can contribute microbes to the water column by carrying sand on their bodies and washing it into the water column as they bath. Note, bacteria densities released from sand adhered to the bodies of bathers represent the total indigenous bacteria attached to sand particulates and those from body contact. No studies have evaluated the contribution of sand carried by bathers as part of the bacterial load to a recreational water body.

In order to fill some of the gaps in human shedding studies, the current study focused on evaluating bacterial shedding in a marine water. Specifically, *enterococci* and *Staphylococcus aureus* were quantified by measuring the amount of bacteria shed by bathers directly off their skin and indirectly via sand adhered to skin. Experiments were conducted under controlled conditions where bathers were either washed or immersed in marine waters characterized by low indicator levels. Mass balance considerations were used to calculate the average colony forming units (CFU) of *enterococci* and *Staphylococcus aureus* per bather or per bather group.

2. Materials and methods

This study was separated into two major efforts which are termed here as the “large pool” study and the “small pool” study. In the large pool study, microbial shedding from 10 volunteers as a group was evaluated. In the small pool study, microbial releases from individuals were evaluated, with a particular emphasis on measuring contributions from sand adhered to skin. Photographs illustrating the experimental set up are provided within the On-line Supplemental Information.

Work with the volunteers was approved by both the Miami Dade Department of Health Internal Review Board (IRB 1491) and by the University of Miami Internal Review Board (IRB 20057223). Consistent with IRB approval, consent forms were signed by each volunteer and volunteer identity was kept confidential. All volunteers were from either Miami Dade County Health Department (MDCHD) staff or from the University of Miami (UM). Statistical differences between groups of data were computed using the *t*-tests (two-sample assuming equal variances at 95% confidence level, $p = 0.05$).

2.1. Large pool field study

This experiment was designed to estimate the amount of *enterococci* and *S. aureus* released from the bodies of bathers into the water column. Demographic characteristics of the 10 participants (seven males and three females) included age ranging from 18 to 50 years, and weight ranging from 52 to 91 kg. The experiment took place a few feet from the water line at the subtropical marine study beach, Hobie Cat Beach, located on Virginia Key, Florida.

An inflatable pool (4700 L) was first sanitized by wiping the pool with alcohol then the pool was filled with off-shore water using a gas powered pump from a point where water quality was consistently characterized by low concentrations of *S. aureus* and *enterococci*. Before each cycle, the complete water volume of the pool was emptied, the pool was resanitized, and then refilled with off-shore water. Volunteers wearing bathing suits went into the pool for four 15 min cycles, and immersed themselves in the water up to their chest by sitting in the water. During each 15 min cycle, the volunteers were asked to immerse their heads three times. During the first 2 cycles, volunteers were not exposed to beach sand. Volunteers used beach shoes prior to entering the pool and were asked to remain on a paved area between cycles 1 and 2 to prevent sand from touching their bodies. Volunteers entered the pool via a plastic walkway

between the paved area and the pool at which time they removed their shoes before entering the pool. In the last 2 cycles, volunteers were exposed to beach sand for 15min before they entered the pool. Showers were not available at the beach site and so volunteers did not shower immediately before entering the pool or between cycles. During each cycle, pH, temperature, and salinity were measured using a field portable meter (YSI 600R series sonde, YSI Inc., Yellow Springs, OH) and water depth was measured using a ruler.

Before each cycle started, six 100 mL water samples (three for *enterococci* and three for *S. aureus* analysis) were collected from the pool after it was filled with off-shore water, and another two water samples (one for *enterococci* and one *S. aureus* analysis) were collected off-shore near the pumping location which served as a representative sample of source water quality. At the end of each cycle, the water in the pool was mixed by the volunteers walking around the inside of the pool and then six 100 mL water samples were collected (three for *enterococci* and three for *S. aureus* analysis). Sample volumes used during analysis for *enterococci* and *S. aureus* were 50 mL for the samples collected before volunteers entered the pool and 10 mL for the samples collected after the volunteers exited the pool.

2.2. Small pool field study

This experiment was designed to estimate the amount of sand transported on the bodies of bathers into the water column, and to estimate the *enterococci* concentration found in the transported sand. Two groups (Group I and Group II) of volunteers participated in this experiment which took place at the beach site. Each group consisted of five volunteers for a total of 10 volunteers. The field study for Groups I and II was conducted during two different dates in July and August of 2005, respectively.

During this “small pool” study, each volunteer wearing a bathing suit spent 15–30 min on the beach sand (i.e. sitting, lying, playing, walking, etc.). Thereafter, each volunteer was individually asked to enter a 190 L tub that had been sanitized by wiping with alcohol followed by air drying. Each volunteer was rinsed with off-shore marine water using three presanitized plant water containers (approximately 3 L each). Initial water samples were collected prior to rinsing each subject.

After rinsing, water was collected for subsequent *enterococci* analysis and the total volume of water collected was measured. During water collection, the sand in the pool was directed towards one edge, and this sand was then placed via sterilized spoon into a pre-weighed and pre-sterilized Whirl-Pak bag. This procedure was repeated until all volunteers were rinsed. In the laboratory, the total weight, water content, and the *enterococci* concentrations of the sand were measured. Sample volumes of 25, 50, and 75 mL were used for water analysis and five pre-determined volumes of liquid extract (2, 6, 12, 20, and 50 mL) were used for sediment analysis.

2.3. Laboratory analysis

Analyses of all samples were conducted the same day of delivery, no longer than 6 h after collection.

2.3.1. Water analysis—All water samples from the large pool experiment were analyzed for *enterococci* and *S. aureus* by the Florida Department of Health Bureau of Laboratories Miami Branch, and all sediment and water samples collected from the small pool experiment were analyzed for *enterococci* only at the UM laboratories. Use of two laboratories was necessary to address limitations in laboratory capacity.

A standard membrane filtration (MF) method was used for the analysis of *enterococci* (Method 1600, US EPA 1997) and *S. aureus* (Fowler et al., 2004). *Enterococci* were analyzed by placing the filter membrane on a selective medium (mEI agar, Becton Dickinson, Sparks, MD) and incubating the filters at 41 °C for 24 h. All colonies that were blue or characterized by a blue halo were recorded as *enterococci* colonies. *S. aureus* was analyzed using a chrome agar method. The MF containing bacteria was placed on a selective medium (BD BBL™ CHROMagar™), and the agar plates were incubated aerobically at 35±2 °C for a minimum of 24 h (the incubator limited the amount of light that the agar was exposed to in order to preserve the chromogens as recommended by BD BBL™). After incubation, the plates were read against a white background with the aid of a magnifying glass. All mauve colonies were counted as positive for *S. aureus*. Additional tests were performed to confirm that the mauve colonies were *S. aureus*. A representative few were sub cultured onto sheep blood agar and incubated for 24 h. The isolates were pure, cream colored, beta hemolytic, and coagulase positive. A Gram stain of the isolates showed Gram positive cocci in clusters.

2.3.2. Sediment analysis—For sand analysis, the “washable” *enterococci* were extracted from the soil using a modified version of the procedure outlined by Van Elsas and Smalla (1997). To enumerate the organisms in the sand samples, two preliminary steps were performed. The first step was to measure the water content of sand. Water content was determined by measuring the weight (Mettler, AG245) difference of sand before and after drying (110 °C for 24 h) approximately 18 g of sample on pre-weighed weighing dishes.

The second step was to extract the organisms from the sand particles to a predefined volume of sterile water. To accomplish this, approximately 7 g of un-dried sand were aseptically removed from the sampling bags and placed into sterile pre-weighed jars. Approximately 30 to 50 mL of sterile phosphate buffer dilution water (PBS) were then added to each jar. The jars were manually shaken for 30 s and the liquid samples were filtered using pre-sterilized 30 µm pore size nylon net filters (Type NY30, Millipore, Bedford, MA). An additional 50–70 mL of PBS was used to remove the sand from the jar. All of the additional liquid and sand were also filtered through the same 30 µm pore size nylon net filters. The final volume of filtrate was recorded, and this filtrate was analyzed for *enterococci* using the mEI agar method (Method 1600, US EPA 1997).

3. Results and discussion

3.1. Large pool study

The water depth ranged from 17 to 26 cm for all four cycles. Water temperature ranged from 30.3 to 31.3 °C and showed a consistent increase after each cycle. Water pH readings ranged from 7.95 to 6.82 and consistently decreased after each cycle.

The mean concentrations for source and initial water for *enterococci* were 170 CFU/100 mL and 9 CFU/100 mL, and for *S. aureus* were 17 CFU/100 mL and 10 CFU/100 mL, respectively (Table 1). Relatively high concentrations of bacteria were measured in the source water just before the first cycle, with a significant decrease in source water bacteria in subsequent cycles. The higher bacteria levels in the source water measured before cycle 1 was likely due to a rain event that occurred immediately before the first sampling event. Although no point sources of bacteria have been found at the beach, the increase in bacteria levels immediately after a rain event could be due to the wash-in of non-point sources of bacteria from the shoreline carried by water runoff. The first source water sample was collected before the initial pool water sample, and so the effect of the rainfall event had diminished by the time the first initial pool sample was collected.

The concentrations of *enterococci* and *S. aureus* in the initial pool samples for all four cycles were relatively low and the *enterococci* concentrations were well below the recommended guidelines for marine recreational waters (US EPA, 2002). This finding is important in that future human shedding studies can use marine water as source water as oppose to treated freshwater. All previous human shedding studies used filtered freshwater as source water (Table 2).

After immersion of the 10 individuals in the large pool, the bacteria concentrations in the pool increased by 1–2 orders-of-magnitude (Table 1). The range for mean *S. aureus* and *enterococci* for all four cycles were from 520 to 4200 CFU/100 mL and from 80 to 400 CFU/100 mL, respectively. Between cycles, the bacteria detected in the water column decreased after each subsequent cycle. The decrease was faster for *S. aureus* (50%) relative to *enterococci* (42%), on average (Fig. 1). This observation may be due to a washing effect leaving less bacteria on the body for shedding in the subsequent cycle.

Significant difference analysis was conducted to compare the means of six possible combinations of any two different cycles (1–2, 1–3, 1–4, 2–3, 2–4, 3–4). The concentrations of bacteria were significantly different for all combinations, except for cycle 2–3 for *S. aureus* and cycle 2–3 and 2–4 for *enterococci*. The lack of significant difference during these cycles may be due to the combined effects of variations in the replicate analyses observed in cycle 2, and the exposure to sand during cycles 3 and 4. It is possible that the introduction of sand to the body during cycle 3 may have served as an added source of *enterococci* and *S. aureus*, and this added source resulted in the decrease in the washing effect with additional cycles.

From mass balance computations which take into account the volume of water in the pool, the results indicated that *S. aureus* levels shed from the bathers (CFU/person) during the first three cycles were consistently greater by one order of magnitude than the *enterococci* numbers shed (Fig. 2). During the first three cycles, *S. aureus* densities ranged from 6.1×10^6 to 1.3×10^6 CFU per person, and *enterococci* densities ranged from 550 000 to 165 000 CFU per person. In cycle 4, the total average *S. aureus* shed per bather was approximately six times greater than *enterococci*, 670 000CFU versus 110 000CFU. This observation may be due to the effects of washing which may reduce *S. aureus* by a greater factor than *enterococci*. Between cycles 3 and 4, bathers were exposed to beach sand thus increasing available *enterococci* levels from sand for shedding. *Enterococci* are found in the sand at Hobie Cat Beach at typical concentrations of 380 CFU/g-dry sand, on average, immediately above mean high tide (Durbin et al., 2005).

The mean concentration of *enterococci* shed per subject in this study (3×10^5 CFU) was consistent, within an order of magnitude, with the amount typically released from feces during bathing (2.3×10^5 CFU; Gerba, 2000), as determined by comparing ratios of fecal indicator bacteria in sewage and feces (Rose et al., 1991). However, fecal releases cannot explain the *S. aureus* shed per subject as the mean concentration observed in this study (average for all 4 cycles, 3×10^6 CFU) was four orders of magnitude higher than would be expected from a fecal release (4.1×10^2 CFU). These findings support that the main source of *enterococci* is from the release of fecal matter, while *S. aureus* is from non-fecal sources predominantly shed from the skin and possibly anterior nares of bathers.

The results from the large pool study are consistent with the results from three other bather shedding studies (Table 2), even though the design of the studies was different. They differed in terms of the number of subjects, demographics, targeted bacteria indicators, type of water (marine versus fresh) and exposure (individual exposure as opposed to group exposure). Hanes and Fossa (1970) used a mix of 64 subjects including children with group exposures. Smith

and Dufour (1993) used eight demographically mixed adult subjects with individual exposures. Robinton and Mood (1966) used five adult females with individual exposures. Taken together, the results suggest that most bacteria shedding occurs within the first 15 min of swimming activities or exposure. Despite the differences in design, this study, Smith and Dufour (1993), and Robinton and Mood (1966) reported that *S. aureus* shed in significant densities per bather at 6.1×10^6 , 7.5×10^6 , and 1.3×10^6 , respectively. The type of water apparently did not impact the degree of shedding as the results from the current study using a marine water were consistent with prior studies which exclusively used freshwater.

The results suggest that *S. aureus* can serve as an indicator of bathing load. Studies that evaluated the risk of swimming related illnesses associated with exposure to waters contaminated with non-point sources, indicated that gastrointestinal illnesses observed in swimmers were correlated with high numbers of bathers and high densities of *S. aureus* (Calderon et al., 1991; Charoenca and Fujioka 1995). Since *S. aureus* is isolated from human waste in relatively low numbers, on the order of 10^3 CFU/100 mL (Gerba, 2000), it can be used as an indicator to predict human bather impacts which would include the combined effects of bather density, mixing, and dilution.

3.2. Small pool study

The amount of sand released per subject ranged from 24 to 70 g/subject (mean = 51) for Group I and from 7 to 65 g/subject (mean = 28) for Group II (Fig. 3a). The average *enterococci* density released from sand adhered to the bodies of the subjects ranged from 210 to 870 CFU/g-dry sand (mean = 390) for Group I and from 4 to 55 CFU/g-dry sand (mean = 24) for Group II (Fig. 3a). These concentrations may be due to *enterococci* naturally present in the sand or from contact of sand with the skin of the volunteers. The total numbers of *enterococci* shed per subject ranged from 5020 to 44 500 CFU/subject (mean = 20 300) and from 42 to 2150 CFU/subject (mean = 840) for Group I and II, respectively (Fig. 3b). The data generated from Groups I and II showed considerable variation. Differences in weather conditions and differences in participant behavior within each group may be part of the reason for this variation. The field study that included Group I participants was conducted shortly after a rain event whereas no rain occurred during the day of the Group II field study. The rain event may have affected the microbial quality of the sand and may have increased the degree to which the wet sand may have adhered to the participants. Furthermore, all of the Group I participants actively assisted in the set-up of the experiment whereas the majority of the Group II participants did not assist.

The total average *enterococci* density released from sand adhered to the bodies of bathers per subject for Groups I and II combined was 10 600CFU, assuming no desorption of the *enterococci* from the sand. This value represents approximately 16%, 1.9%, and 1.8% of the total average *enterococci* shed per bather per 15 min exposure estimated from Smith and Dufour (1993) (6.6×10^4 CFU), the current “large pool” study (5.5×10^5 CFU), and Hanes and Fossa (1970) (6×10^5 CFU), respectively. The relatively small contribution from sand in this study may be impacted by the characteristics of the beach sand which was relatively coarse (mean size of 620 μ m with less than 2% finer than 30 μ m).

4. Conclusions

This study demonstrated that bathers shed significant concentrations of *enterococci* and *S. aureus* into the water column and that *S. aureus* was shed at concentrations at least one order of magnitude greater than *enterococci*. This study also showed that total *enterococci* and *S. aureus* released by bathers decreased significantly between bathing episodes, in particular after the first wash cycle. This conclusion agrees with the long standing universal requirement that bathers should shower before entering recreational waters to reduce the microbial load in particular at swimming pools since the water volume is limited. It is concluded from this study

that the *enterococci* contribution from sand adhered to skin, was small relative to the amount shed directly from the skin and represented less than 5% of the total *enterococci* shed by bathers.

Future studies should be designed to evaluate the potential use of *S. aureus* as a measure of possible health effects from bather to bather transmission of illness, as *S. aureus* is shed in quantities one order of magnitude higher than *enterococci*. This study recommends additional targeted studies to confirm the results of this effort and to estimate how much *S. aureus* bathers carry into the water column via sand. Furthermore, given the significance of bathing load, water quality models of recreational beach waters impacted by non-point sources of microbes should include bathing load as one of the significant pollution sources. The contribution from sand adhered to skin can be potentially ignored in models which simulate non-point sources of *enterococci* as the quantities from sand on skin is small, on average, in comparison to the total body burden.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Total average *enterococci* and *S. aureus* densities (CFU/100mL) in the water column per cycle as observed during the large pool experiment. Error bars correspond to the standard deviation of replicate analyses.

Fig. 2. Mean total bacteria shed per person per 15 min exposure. Error bars correspond to the standard deviation of three replicate samples collected per cycle. Considering all 4 cycles, the overall average shedding of microbes shed per bather were 3×10^6 CFU for *S. aureus* and 3×10^5 CFU for *enterococci*.

Fig. 3. Results for group I and group II for the “Small Pool” experiment. (A) Average amount of sand released per subject and, average *enterococci* density in sand. (B) Average total *enterococci* from sand, CFU per subject.

Table 1
Concentrations of *enterococci* and *S. aureus* in the source water and in the pool water before and after immersion by 10 volunteers

Cycle #	<i>Enterococci</i> CFU/100 mL			<i>S. aureus</i> CFU/100 mL		
	Source ^a	Pool initial ^b	Pool final ^c	Source ^a	Pool initial ^b	Pool final ^c
1	672	21 (8)	400 (44)	64	13 (7)	4187 (439)
2	8	3 (1)	153 (64)	2	7 (3)	2080 (859)
3	<1	7 (2)	140 (10)	<1	11 (6)	1027 (189)
4	<1	3 (2)	87 (25)	<1	9 (2)	523 (81)
Average	170	9	195	17	10	1954

^aSingle ocean water sample collected at the pump intake.
^bAverage of three pool initial water samples before bathers entered pool. Value in parenthesis corresponds to the standard deviation of the three measurements.
^cAverage of three pool final water samples after bathers exited pool. Value in parenthesis corresponds to the standard deviation of the three measurements.

Table 2
Densities of *enterococci* and *S. aureus* Shed per bath for the current study and comparisons with three additional studies

Study	<i>Enterococci</i> , CFU	<i>S. aureus</i> , CFU	Summary of study design				
			No. Subjects	Gender and ages	Exposure period	Individual or group	Water type
Current Study ^a	5.5×10^5	6.1×10^6	10	Males and Females. Ages 18–50	15 min	Group	Marine
Smith and Dufour (1993)	6.6×10^4	7.5×10^6	8	Males and Females. Ages 4–59, mostly adults	15 min	Individual	Fresh
Hanes and Fossa (1970)	6×10^5	Not Measured	64	Males and Females. Ages 6–38	10–30 min	Group	Fresh
Robinton and Mood (1966)	Not Measured	1.3×10^6	5	All Females. Ages 25–45	15 min	Individual	Fresh

^a Average of 4, 15-minute exposure periods was 3×10^5 for *enterococci* and 3×10^6 for *S. aureus*.