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Ozonized water as an alternative to alcohol-based hand disinfection

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SUMMARY

Background: Hand hygiene plays a vital role in the prevention of transmission of microorganisms. Ozone (O_3) is a highly reactive gas with a broad spectrum of antimicrobial effects on bacteria, viruses, and protozoa. It can easily be produced locally in small generators, and dissolved in tap water, and quickly transmits into ordinary O_2 in the surrounding air.

Aim: To compare ozonized tap water and alcohol rub in decontamination of bacterially contaminated hands.

Methods: A cross-over study among 30 nursing students. Hands were artificially contaminated with *Escherichia coli* (ATCC 25922), then sanitized with ozonized tap water (0.8 or 4 ppm) or 3 mL standard alcohol-based rub (Antibac 85%). The transient microbes from fingers were cultivated and colony-forming units (cfu)/mL were counted. The test procedure was modified from European Standard EN 1500:2013.

Findings: All contaminated hands before disinfection showed cfu >30,000/mL. The mean (SD) bacterial counts in (cfu/mL) on both hands combined were 1017 (1391) after using ozonized water, and 2337 (4664) after alcohol hand disinfection. The median (range) values were 500 (0–6700) and 250 (0–16,000) respectively (non-significant difference). Twenty per cent of participants reported adverse skin effects (burning/dryness) from alcohol disinfection compared with no adverse sensations with ozone.

Conclusion: Ozonized tap water is an effective decontaminant of *E. coli*, and it could be an alternative to traditional alcohol-fluid hand disinfectants both in healthcare institutions and public places. Ozonized water may be especially valuable for individuals with skin problems.

Introduction

Our interest in ozone as an alternative hand disinfectant started several years ago when two eye surgeons in our hospitals developed hand dermatitis and had to stop performing

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operative procedures. Both obtained permission to use ozone gas in tap water in their preoperative hand hygiene, and this solved their skin problems. In the same period, there was an outbreak of *Giardia lamblia* infection in the city of Bergen, Norway, originating from the domestic water supply, and many kindergartens were advised to start using ozonized tap water in hand hygiene for children, as it was known that ozone could eradicate protozoans [1]. Some kindergartens continued using ozone as hand disinfection for several years after, and the staff reported a clear impression that both the children and staff had fewer infectious diseases during this period (C. Andreassen, personal communication). We are also experiencing a global threat from multidrug-resistant bacteria, and the need to reduce the use of broad-spectrum antibiotics [2]. Hand disinfection has thus become even more important as a preventive procedure.

Ozone gas (O_3) was probably first detected by a Dutch chemist (Martin van Marum), but the first systematic studies were done by Christian Friedrich Schönbein in around 1840 [3]. He noted the characteristic smell around an equipment for electrolysis of water, and named the gas based on the Greek word ‘ozein’ (scent) [4]. Ozone is produced from electric generators when an electrical discharge (a spark) splits an oxygen molecule into two oxygen atoms, and then the unstable ozone molecule is formed according to the reaction $O + O_2 \rightarrow O_3$. According to the balanced equation $2O_3 \leftrightarrows 3O_2$, where ozone quickly decomposes into O_2 ($t½ = 20–30$ min), it must be produced in the location where it is used. When it decomposes, O_3 acts as an oxidant with release of free radicals. Being an oxidant, ozone has antimicrobial properties against bacteria, viruses, and protozoa, and it was first used for general disinfection of water. Later, ozone was used in food hygiene, fish farming, air purification, hot tubs, and in some areas of medicine, especially dentistry [5,6].

Ozone leads to the destruction of both bacteria and viruses by interfering with metabolism, most likely by inhibiting enzymes. Some ozone breaks through the bacterial cell membrane, and this leads to cell death [7,8]. Ozone destroys viruses by diffusing through the protein coat into the nucleic acid core, resulting in damage to the viral RNA. At higher concentrations, ozone destroys the capsid by oxidation [9].

Ozone is toxic to humans at higher concentrations, especially to the lungs. The Norwegian Labour Inspection Authority accepts an 8 h working day average exposure of 0.1 ppm, and this may be exceeded by 200% for periods of 15 min [10]. This limits the use of ozone gas as a disinfection agent in surroundings with human activity. However, the gas can be dissolved in tap water for handwashing, and most of the gas then passes in the water through the outlet of the sink.

Hand hygiene is one of the most important tools available for reducing the spread of transient microbial pathogens in healthcare and community settings. In the era of multidrug-resistant bacteria and frequent outbreaks of viral gastroenteritis, hand disinfection has become an even more essential tool in reducing the spread of micro-organisms both in health institutions and in wider society [11]. The main disinfection methods used are handwashing with soap and alcohol-based solutions [12]. However, both of these are associated with adverse skin reactions such as dryness and dermatitis among sensitive individuals, and compliance with hygiene recommendations is also variable among health professionals [13,14]. Among nurses, 25–55% report adverse skin reactions related to hand hygiene procedures [15]. Meanwhile, the effect of alcohol disinfection on viruses such as norovirus and rotavirus is unclear, and it has little effect on spores of the bacterium *Clostridium difficile* [14]. Ozone gas in higher concentration has also been shown to be an effective alternative for sanitizing rooms [16,17].

There have been few studies on the potential of using ozonized water as a simple, cheap and skin-friendly alternative to standard hand disinfection with alcohol. Appelgrein *et al.* found ozone to be inferior in effect to propanolol-based hand rubs [18]. Isosu *et al.* found ozonized water combined with benzalkonium chloride and alcohol to be an effective alternative to traditional surgical washing procedures [19]. In a unpublished study where ATP was used as an indication of the efficacy of hand disinfection, Liceaga *et al.* found that ozone combined with soap removed 97.3% of ATP from hands, and noted the lack of studies regarding ozone and hand sanitation [20]. In a recent study, Nakamura *et al.* found that a 3 $\log_{10}$ cfu reduction was achieved by washing hands with ozonated water or antimicrobial soap and water. However, ozonated water was not significantly superior to non-antimicrobial soap and water [21].

The aim of this study was to compare the effect on *Escherichia coli*-contaminated hands of ozonized water with standard alcohol-based hand disinfection using a modified European Standard procedure (EN 1500:2013) [22].

Methods

The test procedure was modified from European Standard EN 1500:2013, which specifies a method for simulating practical conditions for establishing whether a product for hygienic hand sanitation reduces the release of transient microbes after use on the artificially contaminated hands of volunteers. For testing one product at a time, a crossover design was used. The 30 test persons were all nursing students (26 women/four men) at the end of their second year of study (average age 23 years). None of the students had visible signs of dermatitis on their hands during the study days. On day 1, the 30 students were divided randomly into two equal groups of 15, one using ozonized water 0.8 ppm and the other 4.0 ppm. Six days later the same students used the reference disinfectant, ethanol supplemented with propan-2-ol (Antibac, 85% with glycerol, http://www.antibac.no). The test organism was non-pathogenic *E. coli* (ATCC 25922), as recommended in the standard procedure. This bacterial strain is often used as a reference strain in microbial research.

E. coli was cultured for 18–24 h at 36°C on Tryptic Soy Agar plates (Thermo Fisher Scientific, Waltham, MA, USA). A single colony was then inoculated into 10 mL Tryptic Soy Broth (TSB), and cultivated for 18–24 h. The 10 mL bacterial solution was then used to inoculate 1 L TSB before further cultivation for 18–24 h. The final concentration of cultured *E. coli* was estimated as $>10^9$ colony-forming units (cfu)/mL. The 1 L solution was divided into two 500 mL glass containers.

The test subjects prepared their hands by washing for 1 min with soft soap and lukewarm tap water to remove natural transients. The hands were then dried with paper towels and immersed up to the mid-metacarpals for 5 s, one hand in each of the glass vessels containing bacterial solution. Hands were air-dried in a horizontal position with the fingers spread and rotating for 3 min. Immediately after drying, the fingertips were rubbed on the base of a Petri dish containing 10 mL of TSB using separate Petri dishes for each hand. Without further delay, the test subject then performed the hygienic hand-rub procedure, using ozonized water or the reference hand rub (3 mL of 85% Antibac) according to information provided by the manufacturer. The hygienic hand-rubbing time for both ozone water and Antibac was 30 s. After cleaning, the fingertips were dried and rubbed on the base of new Petri dishes containing 10 mL of TSB. Immediately after performing the tests, 1 mL of pre- or post-value sampling fluid was transferred into Eppendorf tubes (Thermo Fisher Scientific) and brought to the laboratory in a cooling bag. Within 24 h, 10 μL of the pre- or post-value samples were spread on two parallel MacConkey agar plates (Mac3; Thermo Fisher Scientific) and incubated for 18–24 h at 36 ± 1°C before counting the number of cfu. The pre-test value was the number of cfu sampled from the left or right hand prior to hand sanitization treatment, and the post-test value was the number of cfu after treatment. Calculation of cfu/mL was performed by multiplying the arithmetic mean of the plate cfu counts for each hand by the dilution factor of 100. For both reference and test procedures, the counts from the right or left hand of each subject were averaged separately to obtain pre- and post-values. Colony-forming unit estimates of >300 per 10 μL were not counted and were noted as >30,000 cfu/mL.

Ozonized water was produced by two separate generators, a Water Ozonator CYS 300C from Cleanzone (Bergen, Norway) delivering 0.8 ppm in tap water, and a BioSure CSS from Ozone Scandinavia (Oslo, Norway) AS delivering 4.0 ppm in tap water (Figure 1). For measuring the ozone gas concentration in surrounding air, three different sensors were used (EZ-1X, EcoZone, Newark, CA, USA; A-21ZX, Eco Sensors, Inc., Newark, CA, USA; Portable Ozone Detector, Murco, Dublin, Ireland), and the level of ozone in the tap water was also measured. The tap water was regular domestic water of about 20°C from the local water reservoir in Førde, Norway. Water here is filtered through a layer of marble sand, and then disinfected with chlorine and ultraviolet light. It has a pH of 7.8–8.3 [23]. The tap water-flow was different. For the ozone 0.8 ppm generator the flow was about 8 L/min, whereas the 4.0 ppm BioSure CSS delivered only 2 L/min.

Ethics

The students were informed in a lesson about the study and invited to participate. The resulting participants gave their written consent. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate (reference number: 2017/943).

Statistical analysis

The Wilcoxon signed-rank test was used to test for differences in cfu/mL values after using ozone water or alcohol disinfection. The Mann–Whitney *U*-test was used to determine whether cfu/mL was different using ozone water with 4.0 versus 0.8 ppm. $P < 0.05$ was considered significant. The analysis was conducted using SPSS software version 24.

Results

All 30 participants completed the study. Eight of the students (27%) reported varying degrees of previous skin problems connected to handwashing and disinfection.

In connection with the experiment, 20% of participants reported some adverse skin effects (burning/dryness) from

Model:	BioSure CSS
Ozone concentration in water:	2–8 ppm
Voltage:	100–240 V, 50/60 Hz
Energy usage:	60 W
Size (in.):	17.1 × 12.9 × 6.9
Weight:	7.5 kg

Model:	CYS 300C
Ozone output:	0.5 g/h
Ozone concentration in water:	0.4–0.8 ppm
Ozone concentration in air:	0.04–0.08 ppm
Voltage:	110–250 V, 50/60 Hz
Energy usage:	9 W
Size (W×H×D):	5.3 in. × 7.4 in. × 2.3 in. 135 mm × 188 mm × 58 mm
Weight:	0.62 kg/1.4 lb

Figure 1. Equipment (generators) used for ozonization of tap water: BioSure CSS and CYS 300C.

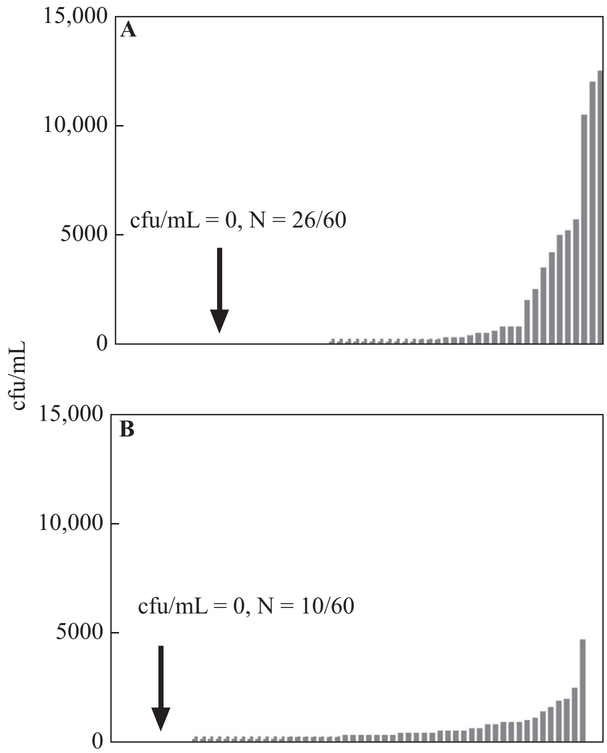

Figure 2. (a) Individual distribution of cfu/mL after disinfection of hands with 3 mL of 85% alcohol for 30 s. (b) Individual distribution of cfu/mL after disinfection of hands with ozonized water for 30 s.

using the alcohol disinfection method, but they continued the procedure as planned. Their post-cfu counts showed no special pattern. None reported adverse sensations using ozonized water. Half of the participants reported that their hands felt smoother/softer after ozone use, and after the experiment the majority (77%) said that they would prefer ozonized water disinfection if the two methods were equal in antimicrobial effect.

The raw data (cfu/mL) after using ozonized water and alcohol disinfection are shown in Figure 2.

Table I summarizes the bacteriological results. From all 60 hands (30 participants, each with left and right hand) the pre-tests yielded cfu >30,000/mL. The mean (SD) post-test value for both hands combined in cfu/mL after using ozonized water was 1017 (1391), and for regular alcohol hand disinfection was 2337 (4664). The median (range) post-test value (in cfu/mL) after using ozonized water was 500 (0–6700), and for regular alcohol hand disinfection was 250 (0–16,000), $P = 0.713$. Where disinfection used the higher ozone concentration (4.0 ppm), values were not statistically different in cfu/mL compared to those for the lower concentration (0.8 ppm) ($P = 0.142$).

More hands ($N = 26$) showed zero post-value cfu counts from fingers using alcohol versus ozone ($N = 10$) (Figure 2). There was greater individual variation in the cfu counts for alcohol (0–12,500) compared with ozone (0–4800), especially for the left hand. Skin symptoms from alcohol disinfection earlier or during the study were not correlated with higher cfu counts in post-tests.

The measured level of ozone gas in the air around the face-level of the students never exceeded 0.01 ppm (one-tenth of the exposure limit of 0.1 ppm for 8 h). However, when asked afterwards, 77% of the students reported that they had noticed the characteristic smell of ozone gas, which also represented an extra security factor in connection with ozone use.

Discussion

This study indicated that ozonized water on average eradicates *E. coli* from artificially contaminated fingers as effectively as 85% alcohol. The variation in results between individuals, and between the two hands of the same individual, was higher for alcohol disinfection than disinfection using ozonized water. The concentration of ozone gas in the surrounding air was low, and there were no adverse skin reactions with the ozone method. The ozone method was preferred by most of the nursing students. The ozone hand disinfection method is simple, cheap, and leaves no residual waste, and when dissolved in water ozone also seems to be safe.

Our conclusion is that ozonized water could be an alternative to traditional fluid hand disinfectants with alcohol, for example in institutions. Appelgrein *et al.* also found an effect of ozone, but this was inferior to alcohol [19]. In their study with 4.0 ppm ozone, they used a lower concentration of alcohol

Table I
Data for *Escherichia coli* cultivation

Hands	Ozone (cfu/mL)			Alcohol (85%)
	0.8 and 4.0 ppm combined	0.8 ppm	4.0 ppm	
Both hands				
Mean (SD)	1017 (1391)	600 (643)	1433 (1795)	2337 (4664)
Median (range)	500 (0–6700)	300 (0–2500)	700 (0–6600)	250 (0–16,000)
Right hand				
Mean (SD)	550 (942)	240 (232)	860 (1256)	797 (2147)
Median (range)	300 (0–4800)	200 (0–800)	400 (0–4800)	100 (0–10,500)
Left hand				
Mean (SD)	467 (574)	360 (526)	573 (617)	1540 (3294)
Median (range)	250 (0–2000)	200 (0–2000)	300 (0–1800)	100 (0–12,500)

$N = 30$ in all analyses.
Samples were taken from left and right hands treated with a standard solution of non-pathogenic *E. coli* (ATCC 25922), then disinfected with ozonized tap water (ozone) or alcohol rub (alcohol; Antibac, 85% v/v). All pre-tests had cfu >30,000/mL.

(60%), but the amount was double (3 + 3 mL) and the exposure time was longer (3 min), and on one of the hands they used a delayed 3 h method. It is therefore not comparable with our study in every detail.

The seemingly better effect of 0.8 ppm ozone compared to 4.0 ppm was surprising, but both the different technical design of the generators and especially the different water flow could be possible explanations for this observation. Under ideal circumstances we should have used the same generator producing different concentrations, but CYS 300C can only deliver 0.8 ppm at maximum output.

Other studies have shown that the presence of organic carbon in water can severely reduce ozone effectiveness when used as a method to eradicate the bacterial load [24].

In the present study, we found differences in effect between the left hand and right hand in cfu counts post disinfection with alcohol, but less difference with ozone. Fierera *et al*. found significant heterogeneity in bacterial community composition between left and right hands from the same individual depending on handedness, gender, and time since last handwashing [25]. We do not have data on the handedness of our participants. However, the spreading of a sparse amount of alcohol disinfectant (3 mL) to all parts of both hands may be more difficult, even under ideal circumstances, than the simpler procedure of just holding and rubbing both hands under a greater volume of running tap water (8 L/min) containing ozone. The inferior results for ozone 4.0 ppm when delivered in only 2 L/min of water could also point in this direction.

Adverse skin reactions connected to hand hygiene are prevalent among health professionals [12]. Among the participants in this study, 20% of the participants reported adverse skin effects (burning/dryness) from using the alcohol disinfection method whereas none reported these problems when using ozonized water. We find this to be an interesting observation. The standard method for comparing hand disinfectants (EN 1500:2013) has been criticized for not being in line with clinical practice, and for not testing micro-organisms other than *E. coli* [26]. To be more in line with the present advised practice in healthcare institutions, we modified the Standard by using only 3 mL/30 s for alcohol hand disinfection, and we used a higher concentration of alcohol (Antibac 85%) than the 60% in the Standard.

This study was performed in optimal circumstances with well-informed and professional healthcare students knowing the background and correct technique for hand disinfection. The hand disinfection with alcohol was administered and observed by a nurse specializing in hygiene. The effect of hand wash/hand disinfection on contaminated hands can therefore be expected to be poorer in general public health circumstances, and especially among children. The broad-spectrum antimicrobial effect of ozone, including on naked viruses and protozoa, could represent an important potential in public health efforts aimed at communicable diseases. We have found no publication regarding the potential for developing bacterial resistance to ozone. This study confirms that ozonized water possesses antimicrobial properties in line with alcohol, and that it could have a place in effective hand hygiene protocols, especially targeted at individuals with skin problems. For some, this becomes a major problem resulting in sick leave, and, eventually, working disability. This was the situation for one of the authors of this study (D.E.L.) before he obtained permission to use ozone in his preoperative hand disinfection procedure.

There are few studies about the effect of ozone as an antimicrobial agent in human medicine. We need further studies comparing regular hand washing with soap versus ozonized water, and on the use of ozonized water as primary prevention of prevalent infections in schools and kindergartens. Research should also focus on the possibility of using ozone as an alternative to soap washing and alcohol disinfection for persons with contact dermatitis.

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Conflict of interest statement

D.E.L. has a small ownership in a firm that manufactures ozone generators. The other authors have nothing to declare.

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