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Short Report

Effects of hand disinfection with alcohol hand rub, ozonized water, or soap and water: time for reconsideration?

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SUMMARY

The effect of alcohol hand rub was tested in eradicating *Escherichia coli*, and compared with hand wash using ozonized tap water or soap and water. Alcohol eradicated all bacteria in 10 out of 35 participants, but with an average (SD) of 2330 (4227) cfu/mL left after disinfection, whereas ozonized water removed all bacteria in 10 out of 55 participants, with an average of only 538 (801) cfu/mL left ($P = 0.045$). Soap washing was the most effective with total removal of bacteria in six out of 20 participants, with an average of 98 (139) cfu/mL ($P = 0.048$ and 0.018 versus ozonized water and alcohol, respectively).

Introduction

Preventing the spread of virulent micro-organisms is an essential part of infection control programmes where hand disinfection plays a pivotal role [1]. The microbiological flora on the hands can be classified into resident and transient groups. Organisms in the former normal flora group are stable and reproduce locally, are generally non-virulent, and the concentrations can only be reduced with disinfection. By contrast, transient microbes do not reproduce while on the hands, and are normally only viable for a short time. However, they can be pathogenic, easily transmitted or colonize skin wounds or dermatitis [1,2].

Considering the necessity for frequent hand sanitization in hospitals, healthcare workers (HCWs) show a high prevalence of skin irritation [3]. Studies have found a high prevalence of

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bacteria on the hands of healthcare personnel, and the Centers for Disease Control and Prevention have reported that frequent hand washing among HCWs ranges from 5% to 81%, with an average of 40% [4].

The maintenance of healthy skin is crucial for HCWs. In particular, the preservation of lipids, fatty acids and resident microbial flora is important [5]. Hand disinfection with alcohol has become a standard procedure, and wall-mounted dispensers can be easily found in healthcare institutions. Soap-and-water hand wash is recommended more for hand cleaning, rather than for disinfection. However, World Health Organization (WHO) guidelines state that disinfection with alcohol is more effective for eradicating transient bacteria [1,5].

Ozone has been reported to have a broad spectrum of antimicrobial effects. It was first used for disinfection of water, and is now being used in food hygiene, fish farming, air purification, hot tubs, and in dentistry [6]. Appelgrein *et al*. reported that ozonized water was inferior to propanol-based hand rubs, whereas Nakamura *et al*. observed a 3 $\log_{10}$ reduction in colony-forming units (cfu) after hand washing with ozonized water, or with antimicrobial soap and water [7,8]. One concern is that ozone gas is toxic to humans at high concentrations and can substantially damage the lungs. However, the gas can be dissolved in tap water for hand washing, and most of the gas then passes in the water through the outlet of the sink. The Norwegian Labor Inspection Authority accepts an 8 h average exposure (time-weighted average) of 0.1 ppm in the working atmosphere [9]. Usually humans can also notice the characteristic smell of ozone gas at very low concentration.

Previously, we compared alcohol hand disinfection with ozonized water for eradicating or removing transient *Escherichia coli* from contaminated hands [10]. Alcohol hand disinfection, even under optimal conditions, failed in removing all transient *E. coli* from hands of participants and cfu/mL values were higher than for the ozone-water hand wash. Besides, several participants reported adverse skin irritation from frequent use of alcohol disinfection.

Against this background, a follow-up study was designed, this time including also a group using soap-and-water hand wash. The results from both crossover studies were merged, comparing standard alcohol disinfection, ozonized water wash, and soap-and-water wash.

Methods

A modified procedure from the standard EN 1500:2013 was used as described previously [10]. Briefly, a non-pathogenic *E. coli* strain (ATCC 25922) (American Type Culture Collection, Manassan, VA, USA) was used to contaminate hands followed by three hand disinfectant procedures under the supervision of a hygiene nurse. The disinfectant alcohol was Antibac with 85% ethanol and <5% propan-2-ol and glycerine (KiiltoClean AS, Asker, Norway). Non-alcoholic Antibac hand soap (3 mL liquid soap) (KiiltoClean AS) was used in a regular soap-and-water washing procedure, and 0.8 ppm ozone water was produced (Ozonator CYS300C; Cleanzone, Bergen, Norway) directly in tap water for a hand-wash procedure.

The participants were checked for any visible signs of dermatitis before being included in the tests. Twelve (22%) of 55 volunteers were men and 43 (78%) were women. Ages ranged from 20 to 66 years. Out of 40 participants, half were merged with 15 students from the earlier study [10]. Altogether this represented 35 participants. The other half washed their hands with ozone water or regular soap-and-water wash. The temperature of tap water was ~20°C and the water flow was ~8 L/min.

Table I
Post-disinfection tests of *Escherichia coli* cultivation

Hands	Ozonated tap water ($N = 55$)	Alcohol ($N = 35$)	Soap and water ($N = 20$)
Both hands			
Mean (SD)	538 (801)	2330 (4227)	98 (139)
Median (range)	250 (3450)	300 (14,000)	50 (450)
cfu/mL = 0 (no.)	10	10	6
Right hand			
Mean (SD)	281 (474)	974 (2477)	38 (54)
Median (range)	100 (2100)	50 (10,500)	25 (200)
Cfu/mL = 0 (no.)	15	16	10
Left hand			
Mean (SD)	257 (397)	1356 (2996)	60 (95)
Median (range)	100 (2000)	100 (12,500)	0 (300)
cfu/mL = 0 (no.)	17	13	11

SD, standard deviation.

The alcohol and ozone groups were included in a crossover design. This was not possible for the soap-and-water wash group due to the limited number of participants and laboratory facilities.

Besides descriptive statistics, differences in outcomes were investigated using a paired *t*-test with 1000 bootstrap samples, Wilcoxon's test, or Mann–Whitney *U*-test. Statistical analysis was performed using SPSS v24, and graphs were prepared using GraphPad Prism v8.

Ethics

Volunteers were informed in a separate lesson about the study, supplied with written information, and invited to participate. All participants supplied written consent. The study was approved by the Regional Committee for Medical Research Ethics and Norwegian Data Inspectorate (reference no.: 2017/943).

Results and discussion

The pre-test cfu/mL values exceeded the cut-off value of ≥30,000 in 52 out of 55 participants, and, for the samples from the remaining three participants, the cfu/mL values were 10,500, 10,600, and 2900, respectively. The post-test cfu/mL values are presented in Table I and Figure 1. Alcohol eradicated all bacteria in 10 out of 35 participants, but with an average (SD) of 2330 (4227) cfu/mL left after disinfection, whereas ozonized water removed all bacteria in 10 out of 55 participants, with an average of only 538 (801) cfu/mL left (paired *t*-

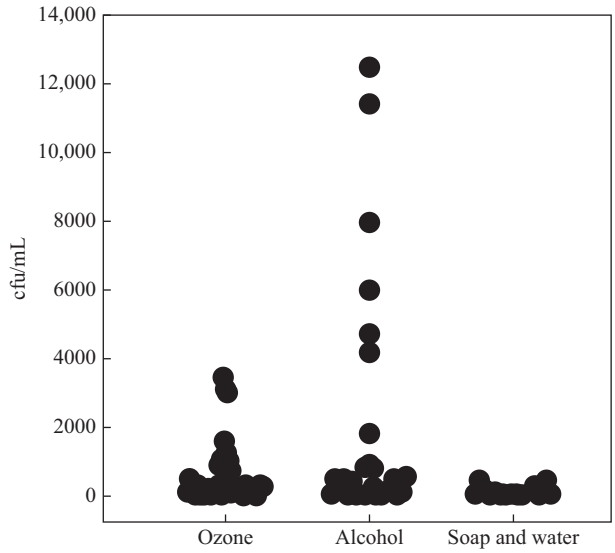

Figure 1. Individual distribution of post-disinfection cfu/mL from both hands after disinfection with 85% alcohol ($N = 55$), ozonated 0.8 ppm tap water ($N = 35$) or soap (3 mL) and water ($N = 20$).

test; $P = 0.045$). Soap and water was more effective than were ozone (Wilcoxon's test; $P = 0.048$) and alcohol (Mann–Whitey *U*-test; $P = 0.018$). However, the median values (ignoring the high values in the alcohol group) were quite similar (Wilcoxon's test; $P = 0.359$). Figure 1 illustrates the difference in outliers with a rather high dose of *E. coli* left after disinfection for some of the alcohol group.

For the alcohol hand-rub group, there was also a difference between the left and right hands (mean cfu/mL: 1356 vs 974), whereas the ozone and soap groups showed less left- or right-hand differences.

The measured ozone concentration in the surrounding air was $\leq$0.01 ppm, but nevertheless most of the participants had noticed a mild smell of ozone gas.

Based on self-reporting, one-third of participants reported adverse skin effects (burning/dryness) from regular alcohol disinfection, even if none had active dermatitis. None of the participants reported unpleasant skin symptoms with the ozone water or regular soap and water. The majority (66%) also stated that they would prefer using ozonized tap water if the disinfection effect was similar to that of other agents.

Based on the results, the best methods seem to be hand washing with soap and water, and alternatively ozonized water. However, the use of standard alcohol disinfection is more flexible for hand sanitization when no water supply or sinks are required, and dispensers can be easily installed in healthcare institutions or public places. This method will therefore continue to play a vital role in hand disinfection.

We think that some of the transient bacteria on hands may be removed by the running water alone, and this should be examined further. We also suspect that the disinfection procedure with 3 mL alcohol is more complicated to perform correctly, as it fails to disinfect the hands in a substantial number of participants. Also, a difference in bacterial counts for the left and right hands was observed for the alcohol disinfection group. In regular public use, the disinfection failure rate may be expected to be even higher, and this should be further scrutinized.

The WHO guidelines for hand hygiene in healthcare do not take into account ozonized water as an alternative method [1]. However, we found that it is a simple and skin-friendly method that leaves no residual chemicals in the environment. An environmental and economic incentive to use ozone in water could be that this device, once installed, requires no need for transportation or stocking of refill. Ethanol and soap distribution and packaging waste is huge, compared to none with ozone gas.

In conclusion, we observed that washing with a mild disinfectant such as ozonized water, besides a regular soap-and-water hand wash, may be more effective than only alcohol for the removal of transient *E. coli* from artificially contaminated hands. Based on this work, ozonized water might be an alternative to traditional hand disinfectants. However, larger studies in clinical settings would be required to validate its efficacy. The potential use of ozonized water against viruses should also be further examined, including a possible effect on the new COVID-19.

Conflict of interest statement

D.E.L. has a share in the ownership of Cleanzone in Bergen, Norway. No conflict of interest is declared for all other authors.

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References

[1] World Health Organization. WHO guidelines on hand hygiene in health care. A summary. Geneva: WHO; 2009. Available at: https://www.who.int/gpsc/5may/tools/9789241597906/en/ [last accessed March 2020].

[2] Fagernes M, Sorknes N. Hand hygiene, national recommendations. Oslo: Norwegian Institute of Public Health; 2017. Available at: https://fhi.no/nettpub/handhygiene/ [last accessed March 2020].

[3] Larson E, Girard R, Pessoa-Silva CL, Boyce J, Donaldson L, Pittet D. Skin reactions related to hand hygiene and selection of hand hygiene products. Am J Infect Control 2006;34:627–35.

[4] Pittet D, Simon A, Hugonnet S, Pessoa-Silva CL, Sauvan V, Perneger TV. Hand hygiene among physicians: performance, beliefs, and perceptions. Ann Intern Med 2004;141:1–8.

[5] Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin Microbiol Rev 2004;17:863–93.

[6] Scott DB, Lesher EC. Effect of ozone on survival and permeability of *Escherichia coli*. J Bacteriol 1963;85:567–76.

[7] Appelgrein C, Hosgood G, Dunn AL, Schaaf O. Ozonated water is inferior to propanol-based hand rubs for disinfecting hands. J Hosp Infect 2016;92:340–3.

[8] Nakamura K, Saito K, Kashiwazaki J, Aoyagi T, Arai K, Hara Y, et al. Evaluation of ozonated water using ASTM E1174 for standardized testing of handwash formulations for healthcare personnel. J Hosp Infect 2018;100:211–3.

[9] Norwegian Labour Inspection Authority. The Working Environment Act 704-ENG. Regulations concerning action and limit values. Oslo: NLIA; 2016.

[10] Breidablik HJ, Lysebo DE, Johannessen L, Skare A, Andersen JR, Kleiven OT. Ozonized water as an alternative to alcohol-based hand disinfection. J Hosp Infect 2019;102:419–24.