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Surface Disinfection Using a Hydrogen Peroxide Aerosol

Summary

Background: As a consequence of its instability and lack of efficacy in the presence of an organic bioburden hydrogen peroxide is contained in very few surface disinfectants only, although it offers a broad antimicrobial activity. The current investigation examines the application of a hydrogen peroxide aerosol to disinfect surfaces.

Methods: Test items with test organisms were distributed in various places throughout an experiment room. The hydrogen peroxide aerosol was released and subsequently the number of surviving test organisms was determined.

Results: The results indicate a distinct microbiocidal effect on artificially contaminated surfaces. The standard test organisms *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* and *Candida albicans* were reduced by > 5 lg even in the presence of a high organic burden consisting of 0.3% albumin and 0.3% sheep erythrocytes. The optimization of the procedure, particularly with regard to the initially low efficacy against *Staphylococcus aureus*, is presented and discussed.

Discussion: In view of the nearly complete ban on the application of formaldehyde the procedure described here may entail perspectives for future developments.

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Introduction

Hydrogen peroxide is one of the best-known antimicrobial substances with a broad spectrum of action. It is contained in products used for scrub/wipe surface disinfection methods, for disinfection of drinking water piping systems and water and even of dialysis equipment. Apart from being used for scrub/wipe surface disinfection methods, its use as an aerosol using an aerosol generator has also been widely discussed. In the meantime, data on initial observations for specific hygiene-related issues are now available [1,2,3]. The prime focus of the application studies was on efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), which had been selected as an indicator of disinfectant efficacy.

The present study investigated under what application conditions a disinfectant efficacy level comparable with that of a scrub/wipe disinfection method could be achieved on using an aerosolised aqueous hydrogen peroxide solution. Here it was endeavoured to comply with the tests conditions specified in the standard methods of the German Society for Hospital Hygiene (DGKH) regulating testing of chemical disinfection processes [4] (status: 1 September 2001) as well as in the catalogue of requirements for inclusion of chemical disinfection processes in the official list of disinfectants drawn by the German Society of Hygiene and Microbiology (DGHM) [5] (status 4 February 2002).

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Materials and Methods

Pursuant to the standard DGHM methods for testing chemical disinfection processes (1 September 2001) [4] as well as the catalogue of requirements for inclusion of chemical disinfection processes in the official list of disinfectants drawn by the DGHM) (4 February 2002) [5], frosted-glass operating room (OR) tiles were contaminated with the following test organisms: *Staphylococcus (S.) aureus* (DSM 799 and ATCC 25923), *Enterococcus (E.) hirae* (ATCC 10541), *Pseudomonas (P.) aeruginosa* (ATCC 15442) and *Candida (C.) albicans*, ATCC 10231) using a low organic challenge (0.03 g albumin/l) or high organic challenge (0.3 % albumin + 0.3 % sheep erythrocytes). In order to be able to furnish proof that this method was as effective as the conventional methods usually recommended, compliance with the conditions featured in the catalogue of requirements, mentioned above, was stipulated for the in vitro germ carrier tests.

As neutralisation agent, 1 ml catalase (15, 000,000 UNITS, Merck) in a 1 litre diluent was used. The efficacy of neutralisation and non-toxicity of the method used for neutralisation was demonstrated by means of appropriate control tests based on the DGHM standard methods.

A building dryer was used for some of the tests (see results) in order to reduce the relative atmospheric humidity in the experimental room, which had a volume of 56 m3. The building dryer was placed in operation to that effect for 12 hours non-stop with the doors and windows of the experimental room closed.

Operating principle

The aerosol generator used (manufacturer: Infection Control, NL) is able to generate a finely dispersed aerosol, while distributing this throughout the room. This application is microprocessor controlled and the only requirement is that the appliance be programmed in line with the degree to which the relative atmospheric humidity is to be increased and with the duration of exposure. For example, one can specify that the relative atmospheric humidity be increased by 20 % by means of the aerosol generated. In such a case enough aerosol is generated until the corresponding difference in the relative atmospheric humidity is recorded for the room. Then the likewise already specified exposuretime is activated, with the relative atmospheric humidity being kept to a constant value. If for example an exposure time of three hours is programmed, this means that once the relative atmospheric humidity has been increased to the desired value, this value is maintained throughout the entire exposure time (e.g. three hours). In general what happens is that, if the relative atmospheric humidity drops by more than 2 %, the aerosol generator automatically produces an aerosol once again until the desired relative atmospheric humidity is restored to the required range. The exposure times selected were three or four hours, and relative atmospheric humidity increase was by between 30 and 53 %. The relative atmospheric humidity was continuously monitored by the aerosol generator so that, in the event of the programmed values being undershot, aerosol could be generated once again without delay. Once the exposure time had expired, the room was aired and microbial recovery begun.

The room must be sealed during aerosol generation and no one must be allowed to enter it. This phase is indicated by placing a signalling device before the room. A red light is seen on the signalling device to draw attention to this state. Once the programmed exposure time has expired, a green light shows that the room may be entered again.

For the various experiments, at least 10 OR tiles per test organism were positioned at different distances from the aerosol generator, and in particular at different heights within the room. Figure 1 shows the aerosol generator in operation; arrows are used to indicate the test surfaces seen from this angle.

Once aerosol release was completed and after expiry of the exposure time, the OR tiles were collected and, as described in the DGHM methodology, any viable test organisms, i.e. those capable of forming colonies, were recovered. OR tiles that had been stored in a different room from the aerosol-treated room were used for control purposes. These germ carriers, i.e. control tiles, were used to determine the baseline microbial count, from which then, to calculate the disinfectant performance, the organisms remaining on the germcarriers are subtracted.

In concrete terms, the logarithms were subtracted from the baseline microbial counts of the test organisms remaining on the germ carriers (= reduction factor - RF).

Based on the DGHM catalogue of requirements [5], a disinfectant is deemed to be effective if, under the specified test conditions, it reduces the test bacteria by at least 5 log levels and *C. albicans* by at least 4 log levels.

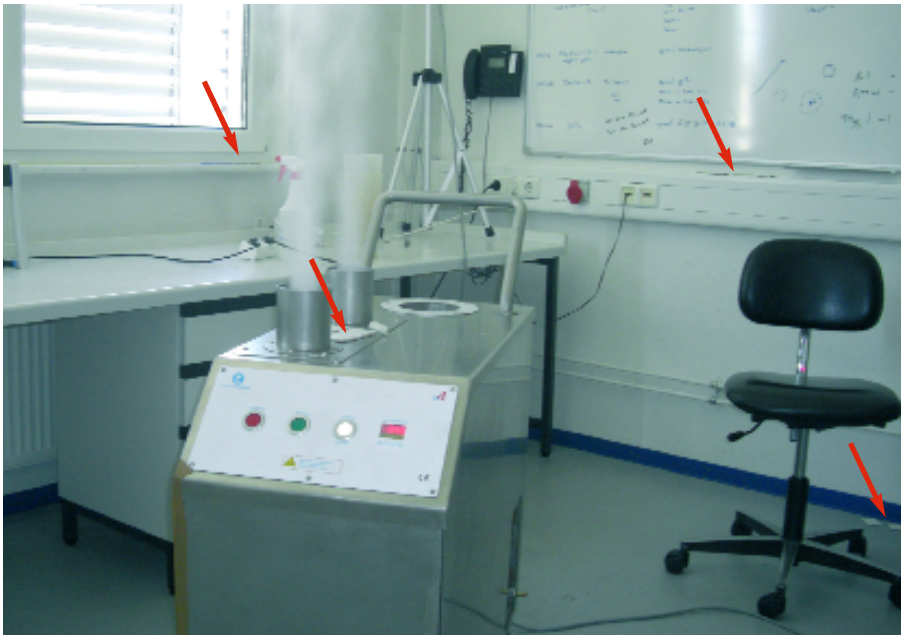

Figure 1: Aerosol generator in operation; the arrows point to the locations at which the exposed germ carriers are visible from this angle within the experimental room.

Table 1: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 30 %; experiment without organic challenge, exposure time 3 h, temperature 20 °C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923	2.38	0.68 – 2.38	2.38	1.92
	4.81	1.14 – 3.25	1.43	1.76
E. hirae ATCC 10541	3.57	1.97 – 3.57	2.09	2.52
	4.95	1.23 – 4.95	2.55	2.71
P. aeruginosa ATCC 15442	2.30	1.70 – 2.30	2.30	2.18
	4.23	2.38 – 4.23	2.97	3.09
C. albicans ATCC 10231	3.56	2.66 – 3.56	3.56	3.26
	4.92	2.84 – 4.92	3.92	3.79

Table 2: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 40 %; experiment without organic challenge, exposure time 3 h, temperature 25.3°C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923	6.26	< 1.78 – 6.26	< 1.78	< 2.91
E. hirae ATCC 10541	7.10	7.10	7.10	7.10
P. aeruginosa ATCC 15442	4.65	4.65	4.65	4.65
C. albicans ATCC 10231	6.82	6.82	6.82	6.41

Table 3: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 40 %; experiment without organic challenge, use of different S. aureus strains, exposure time 3 h, temperature 25.7 °C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923	7.10	< 2.62	< 2.62	< 2.62
S. aureus DSM 799	6.82	< 2.34	< 2.34	< 2.34

Table 4: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 30 %; experiment without organic challenge and with homogenisation / suspension of the S. aureus test organisms by suspending in 0.05 % Tween 80 and filtering, exposure time 3 h, temperature 24.6 °C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. epidermidis ATCC 12225	6.7	2.88 – 6.7	6.7	6.31
S. aureus ATCC 25923	7.08	< 2.6 – 7.08	< 2.86	2.81
E. hirae ATCC 10541	6.99	6.99	6.99	6.99

Contact plate tests

In addition to the test steps conducted as per the DGHM methodology, contact plate tests were carried out at marked locations before and after room treatment with the hydrogen peroxide aerosol generator.

Disinfectant substance and application conditions

A pure hydrogen peroxide (H_2O_2) solution was not used since this would be too unstable. The first investigations were conducted with the product "Sanosil SO 10", which contains 5 % H_2O_2 plus 0.01 % silver.

For further tests, the product "Sanosil SO 15" was used, containing 7.5 % H_2O_2 plus 0.0075 % silver.

Results

The initial tests conducted using 5 % H_2O_2 solution plus 0.01 % silver (Sanosil SO 10) aimed at increasing the relative atmospheric humidity by 30 % to a final value of 80 %. The results observed here are summarised in Table 1. Each mean value or median is based on 10 germ carriers (frosted-glass OR tiles), which had been positioned at different locations within the room. For all experiments, the germ carriers with different test organisms were positioned at the same locations. The positions chosen were locations close to the floor, on work surface as well as in the region of walls/ceilings. From the results obtained from the first series of experiments could be inferred that a sufficient reduction level could not be expected. The method used proved to be completely ineffective, in particular, against *S. aureus*.

In the next test series the relative atmospheric humidity was raised by 40 % to a final value of 90 %. An impressive effect was generated against *E. hirae* and *C. albicans* with a 7.1 and 6.82, respectively, log level reduction of the arithmetic mean (Table 2). However, against *S. aureus*, it was possible to achieve a reduction of only < 2.91 log levels of the arithmetic mean.

To try to explain this gap in efficacy against *S. aureus*, a further experiment was conducted with various *S. aureus* strains. Furthermore, for this experiment the relative atmospheric humidity was raised again by 40 % to a final value of 92 %. However, after a disinfection time of 3 hours, only a

reduction of < 3 log levels could be achieved against *S. aureus* (Table 3).

Since it was suspected that the poor efficacy against *S. aureus* was possibly to be imputed to the fact that the bacteria were not present as single bacteria but rather as aggregates (clumps), a further test series was conducted with the *S. aureus* bacteria now present as separate test organisms. To that effect, 0.05 % Tween 80 was added to the test organisms to obtain a suspension, and the bacteria were then filtered out using glass wool. Coagulase-negative Staphylococcus (S.) epidermidis and *E. hirae*, being other Gram-positive bacteria, were used for control purposes. The results proved that adequate efficacy against S. epidermidis and *E. hirae* was assured but major fluctuations and only overall inadequate results were obtained for *S. aureus* (Table 4).

As part of the experiments, contact plate tests were carried out at marked sites before and after aerosol treatment of the room. Following treatment, no viable microbes were recovered, whereas microbial counts of between 18 and 45 colony forming units (cfus) per area measuring 25 cm2 were recovered from untreated surfaces (Table 5). Interestingly, there was no evidence either of the spore-forming organisms that had been detected before room treatment.

In a further test a newly received formulation (active substances: 7.5 % H_2O_2 and 0.0075 % silver nitrate, product name: Sanosil SO 15) was used. In addition, all microorganisms were placed on the OR tiles under the conditions of low organic challenge (= 0.03 % albumin), as well as two different *S. aureus* strains (ATCC 25923 and DSM 799) under low-organic challenge conditions as well as unchallenged. These tests showed better efficacy against *S. aureus* (Table 6).

From the data obtained, it is hard to explain the fact that *S. aureus* was either completely eradicated or a state of virtually no e fficacy at all was documented. All other test organisms were almost completely eliminated. As far as *S. aureus* was concerned, a relevant diff e rence was noted between the unchallenged and low-organic test challenge conditions. A good disinfectant result was achieved in particular against *S. aureus* DSM 799 on using a low organic challenge. These results suggest that it is possible that the disinfectant substance had not been able to gain access equally well to

Table 5: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 30 %; contact plate tests

	Contact plate test Sampling site (distance from generator [cm]/height above floor [cm])	cfu/25 cm² before disinfection	cfu/25 cm² after disinfection
1	Work surface TM (120 / 80)	18	0
2	Laminar air flow box, top (100 / 200)	34	0
3	IC4, top (0 / 80)	14	0
4	Floor (20 / 0)	31	0
5	Incubator (160 / 200)	37	0
6	Floor (200 / 0)	45	0
7	Work surface (180 / 80)	36	0
8	Laboratory trolley (200 / 90)	25	0
9	Windowsill (180 / 100)	37	0
10	Floor (220 / 0)	40	0

Table 6: Results obtained on using H_2O_2 7.5 % and increasing the relative atmospheric humidity by 30 %; experiment without challenge (wc) and with low organic challenge (lc), exposure time 3 h, temperature 23.4°C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923 (oB)	6.93	< 1.45 – 6.93	4.39	4.34
S. aureus DSM 799 (oB)	6.86	< 1.38 – 6.86	< 1.38	2.25
S. aureus ATCC 25923 (gB)	6.86	<1.38 – 6.86	6.86	4.80
S. aureus DSM 799 (gB)	6.7	<1.22 – 6.7	6.7	4.02
P. aeruginosa ATCC 15442 (gB)	5.2	5.2	5.2	5.2
E. hirae ATCC 10541 (gB)	6.85	<1.37 – 6.85	6.85	6.17
C. albicans ATCC 10231 (gB)	4.36	2.23 – 4.36	4.36	4.15

Table 7: Results obtained on using H_2O_2 7.5 % and increasing the relative atmospheric humidity by 35 %; experiment with low organic challenge (lc); enhanced aerosol distribution using ventilators, 20 germ carriers for S. aureus, exposure time 3 h, temperature 22.4 °C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923 (gB)	6.86	< 2.38 – 6.86	6.86	5.28
P. aeruginosa ATCC 15442 (gB)	5.3	5.3	5.3	5.3
E. hirae ATCC 10541 (gB)	6.58	6.58	6.58	6.58
C. albicans ATCC 10231 (gB)	6.45	6.45	6.45	6.45

Table 8: Results obtained on using H202 7.5 % and increasing the relative atmospheric humidity by 53 %; experiment with low and high challenge (lc/hc); enhanced aerosol distribution using ventilators, exposure time 4 h, temperature 28.2 °C.

For each 10 germ carriers with	Log prevalue	RF (range)	RF (median)	RF (mean)
S. aureus ATCC 25923 (gB)	7,04	< 1,56 – 7,04	7,04	5,98
S. aureus ATCC 25923 (hB)	7,08	1,8 – 7,08	7,08	6,08

Table 9: Results obtained on using H_2O_2 5 % and increasing the relative atmospheric humidity by 30 %; contact plate tests.

	Contact plate test Sampling site (distance from generator [cm]/height above floor [cm])	cfu/25 cm² before disinfection	cfu/25 cm² after disinfection
1	Work surface (250 / 80)	8	0
2	Work surface (200 / 80)	40	0
3	Work surface at window (110 / 80)	18	8
4	Windowsill (180 / 100)	29	0
5	Work surface (220 / 80)	15	0
6	Laminar air flow, front (0 / 40)	15	1
7	Floor beneath laminar air flow (0 / 0)	29	0
8	Blackboard, right (200 / 180)	0	1
9	Paper towel dispenser (160 / 170)	4	0
10	Waterbath (140 / 100)	9	0
11	Cabinet (140 / 70)	18	0
12	Floor (140 / 0)	31	3
13	Door of incubator (200 / 120)	7	0
14	Incubator surface (220 / 160)	30	0
15	Blackboard, right (200 / 180)	10	0
16	Floor (200 / 0)	48	1
17	Floor (240 / 0)	31	0
18	Left side panel of lower cabinet (200 / 60)	3	0
19	Window frame ((200 / 180)	2	0
20	Front of laminar flow box (100 / 150)	12	0

all germ carriers. This was to be clarified in a new test using in addition stand ventilators to assure better distribution of the aerosol within the experimental room.

The efficacy values obtained under these conditions attest to the virtually complete inactivation of all test organisms. However, here too, a number of outliers were detected in the case of *S. aureus*, where virtually no efficacy was detected (Table 7). The mean value (5.28) and median (6.86) demonstrate the efficacy of this procedure. To provide for better evaluation of the effects exerted on *S. aureus*, duplicate germ carriers were used (i.e. now 20 instead of the previous 10).

The data given above show that with better distribution of the aerosol within the room, better efficacy can be achieved against *S. aureus*. At that point it was pondered how a greater quantity of the disinfectant could be released into the room. Since the relative atmospheric humidity in the room with values between 50 and 65 % did not permit an increase in this relative atmospheric humidity by markedly more than 35 %, further experiments were conducted using a building dryer. To that effect, on using a normal commercially available building dryer over a period of 12 hours, the relative atmospheric humidity was reduced to 30 %. In doing so, it was possible to increase the relative atmospheric humidity by 53 % during the experiments. This necessitated a consumption of 4.35 litres of H_2O_2 over a total period of 4 hours for a room volume of 56 m3. Under these application conditions, 20 frosted-glass OR tiles contaminated with *S. aureus* and with a low-organic challenge (0.03 % albumin) as well as high organic challenge (0.3 % albumin + 0.3 % sheep erythrocytes) were distributed throughout the room. The results (Table 8) confirm the pronounced efficacy of this method.

The contact plate tests carried out for these series of experiments also confirm the sustained efficacy of this disinfection process (Table 9).

Discussion

The experiments carried out attest to the fact that under the initially selected test conditions, an adequate disinfectant efficacy, as specified in the catalogue of requirements for inclusion of chemical disinfection processes in the official list of disinfectants drawn by the German Society of Hygiene and Microbiology (DGHM) [5], could not be achieved for the germ carriers exposed to the disinfectant. A conspicuous finding was the major fluctuations in efficacy against *S. aureus*, something that could possibly be imputed to clumping of this test organism or to the well-documented catalase activity of *S. aureus*. To rule out the former, homogenisation measures were taken, but they did not produce the hoped-for successful outcome. Hence it is believed that because of the catalase produced by *S. aureus*, this bacterium is able to deactivate the H_2O_2 molecules before oxidative damage is caused to the bacterial cells. If that were true, then only a much higher concentration of the disinfectant would result in damage and, ultimately, inactivation, of the bacterial cells. Bearing in mind this aspect, three measures were taken to increase the disinfectant concentration on the surface of the bacterial cells:

– Enhanced distribution of the aerosol within the room by installation of rotating stand ventilators
– Use of a product containing 7.5 % H_2O_2 rather than 5 % H_2O_2
– Reduction of the relative atmospheric humidity by first using a building dryer so that a much greater quantity of the disinfectant could be distributed throughout the room (increase of the relative atmospheric humidity by 53 %)

Under these application conditions, it was possible to adequately reduce all test organisms exposed to the disinfectant on the OR tiles, both on using a low challenge (0.03 % albumin) and in the presence of a high organic challenge (0.3 % albumin + 0.3 % sheep erythrocytes). In concrete terms this means that the test organisms *S. aureus, E. hirae, P. aeruginosa* were reduced by > 5 log levels and *C. albicans* by > 4 log levels.

While it was not possible to obtain data to support the original belief that, with the release of a hydrogen peroxide aerosol in a patient's room without prior conditioning (reduction of the relative atmospheric humidity), it would be possible to achieve widespread disinfection of all surfaces and of the fittings within the room, even at this stage there are very promising application possibilities in sight.

Further tests must now be carried out to clarify whether the application conditions can be simplified and whether the spectrum of action can include other microorganisms. In particular tests with mycobacteria and bacterial spores are needed. It is precisely against the background of a virtual ban on the use of formaldehyde that further prospects could be opened up for the disinfection method described in this study.

Conflict of Interest

The authors declare that there is no conflict of interest as understood by the International Committee of Medical Journal Editors.

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