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Failure of dry mist of hydrogen peroxide 5% to kill *Mycobacterium tuberculosis*

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SUMMARY

A dry mist of hydrogen peroxide (DMHP; Sterinis), was used to test for surface decontamination of air-dried samples of *Mycobacterium tuberculosis*, 3×10^5 cfu/mL in open plastic trays. No significant decontamination effect of DMHP could be observed after three ordinary cycles with hydrogen peroxide or after doubling the effect with six repeated cycles.

Introduction

Infections with *Mycobacterium tuberculosis* continue to be a global problem.[1–3] Multidrug-resistant (MDR) or extensive drug-resistant (XDR) strains of *M. tuberculosis* are increasing, resulting in the spread of infections that are extremely difficult to treat.[4–8] The infectious dose in humans may be very low (1–10 bacilli), whereas the sputum from a patient with tuberculosis can contain millions of bacilli per millilitre.[9] The lethality rates are high both in human immunodeficiency virus (HIV) and non-HIV patients; and especially for XDR-infected patients.[4–8]

M. tuberculosis is resistant to drying, and may remain alive and virulent in the environment for a long time; 90–120 days on dust, 45 days on manure, 105 days on paper, 45 days on clothing, 3 months in polluted water kept in the dark at room temperature, and 6–8 months in sputum (cool, dark location), if not exposed to sunshine (or ultraviolet light).[10,11] Therefore, it is very important to clean and disinfect hospital environments exposed to *M. tuberculosis* to control the spread of infection. Disinfectants such as chlorine and 5% chloramine have been used for surface disinfection purposes during isolation, and for terminal decontamination of isolation rooms and furniture after discharge of patients with contagious tuberculosis.[12–15]

During the last few years, a dry mist of hydrogen peroxide (DMHP) disinfectant has been used for decontamination of rooms, medical equipment and ambulances.[16,17] The DMHP has also been used against *M. tuberculosis* and as a routine decontamination of biosafety level 3 laboratories.[18–20] In this study, the effect of the DMHP on *M. tuberculosis* was examined in a biosafety level 3 laboratory at the Department of Medical Microbiology, Ullevål University Hospital.

Methods

Tuberculosis cases in Norway and in Oslo County

In Norway (4.5 million inhabitants), and especially in the Oslo County (0.55 million inhabitants), tuberculosis has been increasing since 1995, mostly associated with immigration from countries with high tuberculosis prevalence, as shown in the summary of yearly statistics from the Norwegian National Health Institute (Figure 1).

Study localisation

The study was performed in a tuberculosis laboratory, biosafety level 3, at the Department of Medical Microbiology, Ullevål University Hospital.

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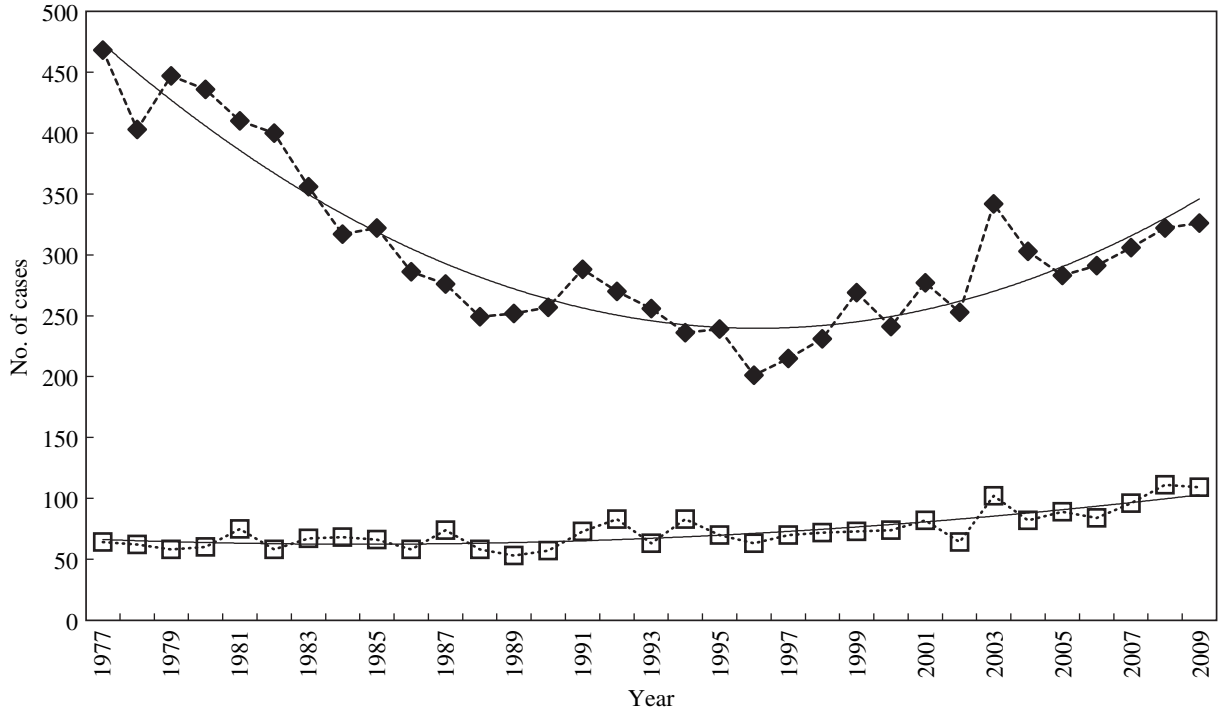

Figure 1. Development of *Mycobacterium tuberculosis* in Norway (yearly statistics from the National Health Institute – MSIS statistik 2010). ◆, Norway; □, Oslo County. Dashed line, Norway; dotted line, Oslo County; complete line, polynome trend curve.

Preparation of biological indicator

M. tuberculosis strain CCUG 37357 was subcultured in Løwenstein Jensen medium (Bio-Rad, Marnes la Coquette, France). After 15 days of subculture, a bacterial suspension was performed in sterile saline adjusted to one McFarland standard: 3×10^7 colony-forming units (cfu)/mL. The suspension was mixed for 2 min with a vortex, 100 μL was then added to 900 μL sterile saline, and further mixed to a final dilution of 3×10^5 cfu/mL. It was difficult to completely homogenise the suspensions. Of the final suspension, 100 μL was added to each of the 20 plastic wells and dried at room temperature for 2 h.

Dry mist hydrogen peroxide decontamination

Four separate, repeated experiments were performed during a period of 60 days, using three decontamination cycles in two experiments and six cycles in two. The decontamination room B was closed with all openings taped, and not ventilated during each disinfection period. The mist-producing apparatus (Sterinis apparatus, Gloster Sante Europe, Toulose, France) was placed in a corner and pre-programmed to the required concentration of H_2O_2 dry aerosol, depending on the exact volume of the enclosed room (30 m^3). The duration of each cycle (diffusion of the dry mist) is dependent on the volume of the room.[17] The whole disinfection process, including the number of cycles (three and six cycles, respectively), and the contact time, was preset. The contact time after each cycle of diffusion was 60 min. Each cycle was followed by the contact time, which was followed by the next cycle, and so on. The misting fluid (Sterusil, Gloster Sante Europe, Toulouse, France) contains H_2O_2 5%, phosphoric acid <50 parts per million (ppm), silver cations <50 ppm, gum Arabica <1 ppm and bio-osmotic water 95%.[17] The airborne concentration of H_2O_2 was monitored during the experimental period with a Polytron 7000 meter (Draeger Gas Visio).

Position of the test wells with dried M. tuberculosis during the test period

The 20 well samples with the dried *M. tuberculosis* were divided in two. Ten samples were placed in an open box in the tuberculosis cabinet at room temperature in the tuberculosis laboratory A. The other 10 were placed in an open box (lid off) on an open bench at room temperature in a separate decontamination room B. This room was treated with three to six cycles with DMHP (H_2O_2 5%).

Spore control

Spores of *Bacillus atrophaeus* Raven 1162282, ATCC No. 9372 (Raven Biological Laboratories Inc., NB, USA) were used to control the effect of decontamination in room B. The spore strips were removed after 24 h, blinded and sent to the Department for Sterility Control, Oslo University Hospital – Rikshospitalet. The culture of the spores has been described earlier.[17] On each occasion eight spore strips were placed as single test strips on preset places in the decontamination room B: over the entrance door, on the ceiling, behind a refrigerator, two strips on the floor under the laboratory bench, one on the bench plate, and two in the box which contained the dried culture samples.

Culture of decontaminated and control wells

The 10 well samples of dried *M. tuberculosis* bacteria exposed to the DMHP were removed from the decontamination room B after 24 h. Treated and control wells were separately studied for growth in (i) Løwenstein Jensen (LJ) medium with glycerol, (ii) LJ medium with pyruvate, and (iii) Bactec TB medium (MGIT; Becton Dickinson, Sparks, MD, USA). Each well was washed with 100 μL saline × 3, and 100 μL of the suspension was then transferred to each of the two LJ media and to the tuberculosis broth medium

Table I
Decontamination of *Mycobacterium tuberculosis* using a dry mist of hydrogen peroxide (DMHP) 5%

Experiment	No. of cycles DMHP	Growth on LJM control	Growth on LJM after decontamination	Read after	Spore tests (no. 6)	H_2O_2 top level per cycle (ppm)	Contact time per cycle (min)
20–9	3	19/20	20/20	6 weeks	Negative	43, 44, 59	60
25–9	3	19/20	16/20	5 weeks	Negative	82, 85, 88	60
7–11	6	13/20	10/20	4 weeks	Negative	65, 75, 88, 97, 98, 100	60
14–11	6	17/20	10/20	3 weeks	Negative	70, 80, 85, 110, 114, 113	60
Total		68/80	56/80				
% positive		85%	70%				

LJM, Løwenstein Jensen medium.

(Bactec), respectively. The samples were incubated at 37 °C for 4 weeks, and read after 1, 2, 3, and 4 weeks.

Growth was defined as growth or no growth on the LJ media and in the broth culture.

Results

There was growth of *M. tuberculosis* in all tuberculosis broth media (Bactec) after 10–21 days; both in controls and in DMHP-treated samples (not shown). Growth was present on both types of Løwenstein Jensen media after 2–4 weeks, both in controls and in treated samples (Table I). Since it was difficult to obtain a homogeneous dilution of bacteria, the results were only read as growth or no growth. All the spore strips gave no growth. The ppm level reached during each cycle is shown in Table I. No significant decontamination effect could be observed on bacteria grown on LJ media, neither after three nor six decontamination cycles with DMHP 5%.

Discussion

Hydrogen peroxide in liquid form (3–6%) is an effective disinfectant for most microbes, by exerting its effect by hydroxyl-free radicals that may attack lipid membranes, DNA and other cell components. DMHP may kill vegetative bacteria, spores and some viruses.[16,17,21–24] There are also reports concerning a 3–5 $\log_{10}$ reduction of *M. tuberculosis* after treatment with hydrogen peroxide aerosol.[18–20]

In our study, DMHP was used to test for surface decontamination of *M. tuberculosis*, 3×10^5 cfu/mL in dried samples in open plastic trays. Earlier studies have demonstrated that at least three cycles of DMHP is necessary to kill spores.[17] Therefore, we used three and six cycles, respectively, to study the decontamination effect of the DMHP on *M. tuberculosis*. No significant decontamination effect of DMHP was observed after three ordinary cycles with hydrogen peroxide or after doubling the effect with six repeated cycles. This result was in contrast to findings in other studies.[18–20]

Grare *et al.* showed that viable bacteria of *M. tuberculosis* were reduced by values of >5 $\log_{10}$, and no colony grew from any biological indicators of cotton tissue on which 10^7 cfu/mL *M. tuberculosis* H37Ra were dried.[20] One important difference in their work was in the preparation of the suspension, which was dried from distilled water, rather than from saline as in our study. Distilled water may perhaps weaken the cell membrane and make it more vulnerable to disinfectants.

The reason why DMHP in our experiments had nearly no effect on dried bacteria is not understood. There may have been other factors to explain the differences between our and other studies, such as temperature and humidity both for drying, and for the exposure to the mist. It may be that the mist did not penetrate the bacilli dried directly to the plastic well, or into aggregates of the dried bacilli. In the study of Grare *et al.*, the mist could penetrate the cotton samples from all sides.[20]

Environmental contamination of *M. tuberculosis* may occur directly from patients with tuberculosis or from splashes and aerosol during laboratory work. Bacterial contamination may vary in quantity and is often accompanied by other organic materials that can shield against, or inactivate, disinfectants.[15] Few, if any, other vegetative bacteria are as difficult to kill as *M. tuberculosis*.[15,25] Therefore, even if the DMHP system seems to be effective against other bacteria, it has no significant killing effect on environmental space contamination with *M. tuberculosis*. Other gaseous disinfectants, such as formaldehyde, are most often impaired by the presence of protein matter and may not be relied on for the killing of acid-fast bacilli.[26,27] The penetration of gaseous formaldehyde into sputum and fabrics is poor.[27] Formaldehyde fumigation on environmental space contamination is therefore 'often unpredictable'.[27] Thus, as far as we know, there is no 100% effective gaseous or mist disinfectant against environmental contamination with *M. tuberculosis*.

Conflict of interest statement
None declared.

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