Aerosolized sodium hypochlorite inhibits viability and allergenicity of mold on building materials

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Background: Commercial and residential buildings can become contaminated with molds, which may trigger allergic disorders. Mold remediation efforts may require costly replacement of mold-contaminated building materials. Disinfectants that contain dilute sodium hypochlorite can kill mold and are practical to use. Whether they also inhibit mold allergy symptoms is unknown.

Objective: We tested the hypothesis that sodium hypochlorite–containing spray products kill *Aspergillus fumigatus* and inhibit *A fumigatus* allergens.

Methods: *A fumigatus* was grown on 3 common building construction materials, as well as in solution by conventional laboratory methods. Two sodium hypochlorite–containing household products (diluted bleach and Tilex) were sprayed on the mold-contaminated materials or added to mold in solution and compared with untreated controls. Surface mold and associated debris were mechanically removed from treated and untreated boards. Conidia in the extracted board materials were quantified by light microscopy, examined for morphologic changes by scanning electron microscopy, and cultured for viable mold. Extracts were tested for *A fumigatus* antigen by ELISA, and for *A fumigatus* allergen by skin prick testing using extracts prepared from both the boards and the cultured solutions.

Results: Both sodium hypochlorite disinfectants killed *A fumigatus* in solution and on mold-contaminated building materials. Light microscopy and scanning electron microscopy demonstrated changes to the conidial surface. Both dilute bleach and Tilex inhibited *A fumigatus* recognition by ELISA. Skin testing supported the results of the ELISAs and demonstrated loss of skin test reactivity to the sodium hypochlorite–treated mold solutions in most of the subjects. Of the 4 individuals who had a positive skin test result to mold grown on oriented strand board building material, 3 no longer reacted to extracts from bleach-treated boards.

Conclusion: Spray application of sodium hypochlorite–containing disinfectants onto mold-contaminated building material kills *A fumigatus*, modifies the surface characteristics of *A fumigatus* conidia, reduces recognition of *A fumigatus* mold by ELISA, and results in loss of skin test reactivity to the treated mold in individuals allergic to *A fumigatus*. (J Allergy Clin Immunol 2005;116:630-5.)

Key words: *Mold, bleach, Tilex,* Aspergillus fumigatus*, allergy, skin testing, building materials*

The ability of household bleach (sodium hypochlorite) to disinfect surfaces is well known. Dilutions of bleach have been used since the 1800s in various applications to disinfect hospitals, laboratories, schools, prisons, and homes. Its widespread use reflects the ability of bleach to kill a broad spectrum of microorganisms in a concentration that presents little toxicity to individuals.

Household bleach typically consists of an aqueous solution of sodium hypochlorite between 4% and 6%. For disinfection, the bleach concentration is diluted to 1:16 by adding ¼ to 1½ cups of bleach per gallon of water. The resulting solution contains about 5000 ppm of sodium hypochlorite,[1-5] which is sprayed or wiped onto the surface to be disinfected. Although the actual mode of action is unknown, the primary active agent is undissociated hypochlorous acid.[1]

Although sodium hypochlorite can kill molds *in vitro*,[1] less is known about the ability of bleach to denature mold allergens. The United States Environmental Protection Agency indicates that because most health complaints associated with exposure to mold are allergic, simply killing the mold may not reduce symptoms.[6] Other authors support this contention.[5] As such, the EPA and other authorities do not recommend biocides to treat mold-contaminated surfaces, because dead mold may still be allergenic.[6,7] However, to our knowledge, the ability of bleach to denature mold allergens has not been investigated.

Several studies have demonstrated that bleach can inactivate other household allergens. Chen and Eggleston[8] showed that typical household concentrations of sodium hypochlorite fragmented mouse, cockroach, and dust mite proteins. In solution, sodium hypochlorite also reduced the detection of protein antigens by ELISA. Antigen levels also fell when allergen-contaminated smooth surfaces were wiped with sodium hypochlorite solution. More porous household surfaces and building materials were not tested. A similar study conducted by

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Abbreviations used
CFU: Colony-forming unit
OSB: Oriented strand board
PBST: PBS containing 0.05% Tween 20
PDA: Potato dextrose agar

Matsui et al[9] found that low concentrations of sodium hypochlorite reduced detection of cat antigen in solution. Together, these studies demonstrate the ability of sodium hypochlorite to reduce household allergens. Periera et al[10] hypothesized that the mechanism for this effect was a result of chlorination of amide nitrogen followed by protein oxidation to an imine with further hydrolysis, thus denaturing allergenic proteins. To date, there are no published studies that show the inhibition of fungal allergens by sodium hypochlorite solution on environmental surfaces. This is a critical issue in light of the growing recognition of health hazards caused by mold contamination.[5]

To justify the use of a biocide such as sodium hypochlorite for the remediation of mold, the biocide should be effective in an environment heavily loaded with organic matter. It should also have an acceptable safety profile. The biocide should not only kill the mold but also reduce mold antigen load. The purpose of this project was to determine whether household bleach or a liquid sanitizer containing sodium hypochlorite could reduce both viable mold and detectable allergen on environmental surfaces.

METHODS

Growth of *Aspergillus fumigatus* and preparation of conidia

We obtained *Aspergillus fumigatus* from the American Type Culture Collection (#16913; Manasssas, Va) as a freeze-dried material. The fungus was reconstituted and inoculated onto potato dextrose agar (PDA) plates (Remel, Lenexa, Kan) and incubated at 30°C. Plates were inspected daily for growth and to ensure a pure culture. PDA slants were made of the initial pure culture and were stored at 4°C. The conidia were harvested by modification of the procedure described by Allen et al.[11] In brief, the conidia were harvested by gently scraping the fungal mat from a confluent culture of *A fumigatus* grown on PDA into sterile, distilled water. The number of conidia was determined by using a hemocytometer.[11]

Effect of bleach in aqueous solution

The ability of bleach and Tilex (Clorox Co, Oakland, Calif) to kill *A fumigatus* was first determined by using a liquid culture of the organism. We prepared three 10-mL suspensions of the stock *A fumigatus* solution at 10^6 colony-forming units (CFU)/mL. An additional 10 mL sterile distilled water was added to 1 *A fumigatus* suspension as the control solution. A 10-mL solution of household strength sodium hypochlorite bleach (Clorox Co), prepared at 1 cup per gallon of water, or 1:16, was added to the second mold suspension. A 10-mL solution of undiluted Tilex containing 2.4% sodium hypochlorite plus surfactants was added to the third suspension. We used similar concentrations of bleach solutions to treat mold-contaminated building materials. All of the suspensions were allowed to sit for 10 minutes and then were washed 3 times with 50 mL sterile distilled water. The suspensions were next diluted with sterile PBS containing 0.05% Tween 20 and 1% BSA (PBST-BSA) to obtain a series of *A fumigatus* concentrations—10, 5, 2.5, 1.25, 0.63, 0.31 $\times$ 10^6 CFU/mL—and verified by light microscopy. Aliquots from each concentration in each treatment group were evaluated by ELISA for levels of *A fumigatus* antigens.

Building materials

We next cultivated *A fumigatus* on building materials that commonly grow mold after water intrusion. Three types of building materials were inoculated with conidia: (1) oriented strand board (OSB), (2) gypsum drywall, and (3) plywood. All 3 of these materials were purchased at a local lumberyard and cut into 9-cm by 9-cm squares. The squares were packaged into autoclave bags, autoclaved in steam heat for a period of 30 minutes, removed from the bags by using sterile technique in a biological safety cabinet, and placed into sterile Petri dishes 150 mm in diameter and 25 mm deep (Nalge Nunc, Naperville, Ill). Thirty milliliters of sterile, distilled water was added over the top of each of the building material squares. The plates were then covered and incubated at 30°C for a minimum of 24 hours before inoculation.

Inoculation of building materials

The plates were next divided into 6 treatment groups, with each condition performed in triplicate. Groups 1, 2, and 3 were inoculated with a 1-mL solution of 1 $\times$ 10^5 *A fumigatus* conidia spread over the surface of the building material square by using a cell scraper. Groups 4 to 6 were control plates for the treatment conditions and not inoculated with mold.

All plates were further incubated for 14 days, at which time visible mold growth was evident on all of the mold-inoculated building material squares.

Treatment solutions on building materials

Groups 1 (with mold) and 4 (without mold) squares served as controls for the sodium hypochlorite treatment groups and were sprayed with 7.5 mL sterile water. Groups 2 (with mold) and 5 (without mold) building material squares were sprayed with a 1:16 solution of bleach to distilled water. To establish reliable and consistent application, each square was sprayed with 5 sprays at 1.5 mL/spray, for a total of 7.5 mL of each solution. Groups 3 (with mold) and 6 (without mold) were treated the same way with Tilex at the supplied commercial concentration. All building material squares were then incubated at 30°C for 24 hours. The mold on the building material squares (groups 1, 2, and 3) was removed by using a disposable cell lifter and placed into solutions of sterile distilled water. The negative control plates (groups 4, 5, and 6) were similarly scraped into solution and washed with sterile distilled water. All solutions were centrifuged and washed several times with sterile distilled water to stop any continuing action of the bleach and Tilex solutions.

Assessment of mold counts, culturability, and morphology

Mold culture. A portion of the solution obtained from each group of building material squares was serially diluted and plated on PDA plates to determine the culturable colony count (CFU) for each treatment.

Mold count. We counted the number of visible conidia in an undiluted aliquot of each treatment group by using a hemocytometer and a light microscope. We recorded the presence and extent of mold spore clumping and the presence of building material square debris for each of the treatment conditions.