Brief report: CLINICAL OBSERVATIONS, INTERVENTION, AND THERAPEUTIC TRIALS

EXPERIMENTAL ASSESSMENT OF DISINFECTION PROCEDURES FOR ERADICATION OF ASPERGILLUS FUMIGATUS IN FOOD.

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ABSTRACT

Aspergillus fumigatus spores in food may represent an infectious risk for neutropenic patients. We examined the efficiency of disinfection procedures applicable to foods for A. fumigatus eradication. Boiling and microwave (MW) treatment fully decontaminated an experimental spore suspension and naturally contaminated liquid foods (reconstituted dried food, herbal tea). Full decontamination of experimentally contaminated surfaces was only obtained with 70% ethanol or heating at 220°C for 15'. Pepper was decontaminated when heated for 15' at 220°C but not by MW. Fruit skin was partially decontaminated by 70% ethanol. We conclude that A. fumigatus spores can be eradicated from food by heating to a temperature of at least 100°C. When foods cannot be exposed to high temperature or microwaves, ethanol only partially reduces the level of surface contamination.
INTRODUCTION

Invasive aspergillosis (IA) is a major opportunistic infection among patients with hematological malignancies\(^1\). Spore inhalation is the usual route of infection, but other routes may exist\(^2\). Anaissie et al.\(^3\) recently reported the presence of pathogenic molds in hospital water distribution systems, leading to aerosolization of fungal spores and potential patient exposure. Several foods can also be colonized by *Aspergillus* and other molds, and can lead to primary gastrointestinal colonization and subsequent systemic infection in animals\(^4\). Massive *Aspergillus* contamination of foods was first reported in pepper\(^5\)\(^-\)\(^8\), and subsequently in regular and herbal tea, corn, coconut, cashew nuts, coffee, beans, soy, cheese and smoked meat\(^9\)\(^-\)\(^12\). Regarding meals served in hematology wards, we recently confirmed that pepper and tea were contaminated by *Aspergillus* and non *Aspergillus* molds, as were also downy-skinned fruits (apricots, kiwis and peaches), smooth-skinned fruits (apples, bananas, lemons and oranges), freeze-dried soups and even individual food wrappings\(^13\). It has been previously recommended that immunocompromised patients should avoid such contaminated foods, and granulocytopenic patients should receive sterile or low-microbial-content diets\(^14\). However, such restrictions may affect these patients’ well-being.

One alternative is to disinfect potentially contaminated foods. However, little is known of the efficacy of food-disinfection procedures, especially with regard to *A. fumigatus*. The aim of this study was to examine the efficacy of several physical and chemical procedures that can be applied to foods and wrappings in order to eradicate *A. fumigatus*. 
MATERIALS AND METHODS

Disinfection procedures

(i) Spore suspensions and contaminated Petri dishes. Spores were collected from a culture of A. fumigatus that was allowed to grow for 8 days on Sabouraud-chloramphenicol agar (Bio-Rad Laboratories, Marnes-la-Coquette, France), by rinsing the culture surface with 10 ml of sterile water containing 0.05% Tween 80. Ten milliliters of spore suspension adjusted to 3x10^8 spores/ml was exposed to the following physical disinfectant procedures: oven heating, microwave irradiation (MW), freezing at −20°C, and heating to 60°C and 100°C (Table 1). Sterile empty Petri dishes that had been seeded with 3x10^8 spores of A. fumigatus then air-dried were submitted to the same conditions and also to 70% ethanol solution.

(ii) Naturally and experimentally contaminated foods. Food samples were then subjected to the disinfection procedures that proved effective on spore suspensions and contaminated Petri dishes. Naturally contaminated foods consisted of commercial black-pepper sachets and tea bags. Black-pepper sachets were directly exposed to MW or oven heating (8 bags for each treatment). Tea bags were boiled in 250 ml of sterile water, and the infusions were stored at 4°C for analysis. Tea from bags of the same batches was cultured on Sabouraud-chloramphenicol agar for 5 days, and infusions of bags from batches that were culture-positive for A. fumigatus were treated by oven heating or microwaving. Freeze-dried soup and fruits were experimentally contaminated. Ten milliliters of freeze-dried soup reconstituted with boiling sterile water was seeded with A. fumigatus (3x10^8 spores/ml) then exposed to MW irradiation or oven heating. The skins of oranges and apples were contaminated by immersion in a spore suspension of A. fumigatus (3x10^8/ml). The efficacy of washing the contaminated fruits with water, soap and ethanol, alone and in combination, was then examined.
Assessment of the disinfection procedures

After treatment, 100-μl samples of each spore suspension, reconstituted freeze-dried soup and tea infusion were cultured in Sabouraud-chloramphenicol broth (Bio-Rad Laboratories) for 72 h at 32°C, then titrated by a limiting dilution method (ten-fold dilutions) in 96-well plates.

Moist swabs were applied to the entire inner surface of contaminated Petri dishes and fruit skins, then agitated in 1 ml of sterile distilled water; spores were counted in 100-μl samples, as described above for spore suspensions.

Spore titers were expressed as log10 spores/ml. The mean spore titer of 8-10 replicate experiments was calculated for each treatment and control.

The contents of black-pepper sachets were suspended in 1 ml of sterile distilled water and seeded on Sabouraud-chloramphenicol agar. The rate of culture positivity after treatment was expressed as a percentage of untreated controls.

ANOVA analysis was used to compare results between treated samples and untreated controls. *P* values <0.05 were considered significant.
RESULTS

Spore suspensions and contaminated Petri dishes (Table 1)

Full decontamination of spore suspensions was obtained after at least 5' at 150°C in a conventional oven and 2' of exposure to 800-watt MW irradiation ($P < 0.05$). Freezing at –20°C for 24 hours, and addition of hot water (60 and 100°C) had no significant effect on spore titers. MW irradiation and hot water had no significant effect on Petri dish surface contamination. Heating at 150°C for 5-15' resulted in significant but incomplete decontamination. Fungal load was reduced by 90% after 5-10 min at 220°C. Complete eradication was only obtained after heating at 220°C for 15 min or with 70% ethanol ($P < 0.05$).

Naturally and experimentally contaminated foods (Table 2)

*A. fumigatus* was cultured from all untreated pepper sachets. Complete decontamination was only obtained when bags were heated for 15' at 220°C in a conventional oven. Tea from 30 bags was culture-positive in 24 cases, and eight (33%) of the corresponding infusions were also culture-positive. Complete decontamination of tea infusions was obtained by exposure to MW for 2 min (which resulted in boiling). Oven heating at 220°C for 2 min was partially effective (data not shown). All eight samples of reconstituted freeze-dried soup were decontaminated by boiling.

Washing with water or soap was ineffective on experimentally contaminated apples, but had a small significant effect on oranges. Exposure to 70% ethanol, used alone or preceded by washing with soap, significantly reduced the fungal load.

DISCUSSION
Fungal decontamination of food served to at-risk patients mainly relies on thorough cooking. However, no consensus approach has been agreed on and little is known of the susceptibility of filamentous fungi to biocides. Most disinfectants with fungicidal activity are designed for soil and surface treatment (not food decontamination), and their activity has mainly been tested against *Aspergillus niger*. We chose to study *A. fumigatus*, one of the most prevalent and life-threatening pathogenic molds, that has been isolated from several types of food.

After experimental contamination, we found that prolonged exposure to temperatures of at least 100°C is necessary to eradicate *A. fumigatus* spores in liquid suspension. Freezing was ineffective, as previously reported. Experimentally contaminated dry surfaces (Petri dishes) were partly decontaminated by lengthy heating in an oven, but not by microwave irradiation. We confirmed that 70% ethanol is highly effective for decontaminating dry surfaces and also found that washing with soap and water before ethanol treatment has no added benefit.

These results were confirmed on naturally and experimentally contaminated foods. Oven heating of reconstituted dried foods at boiling point was the only effective method. Exposure of black pepper to high temperatures was effective but resulted in a marked loss of flavor. With regard to surfaces that can be naturally contaminated, such as fruits and individual food wrappings, washing with water followed by 70% ethanol treatment was the most effective procedure, although complete eradication was never obtained.

Various non-*Aspergillus* molds have emerged as significant pathogens in immunosuppressed patients. Our findings can probably be extrapolated to other potentially pathogenic yeasts and molds, as *A. fumigatus* spores are highly resistant to physical and chemical disinfectants. Few data are available on non-*Aspergillus* molds. *Fusarium* is sensitive to alcoholic solution and to high water temperature, and...
microwave heating to boiling point is a rapid and convenient method for eradicating *Candida albicans* from milk. 26 These are also the conditions that we found most effective on *Aspergillus fumigatus* spores.

Our results provide a basis for guidelines on *A. fumigatus* decontamination of foods, and supplement existing recommendations for the prevention of fungal contamination during food storage and meal preparation. These recommendations should probably be extended to other naturopathic products and herbal medicines, that are frequently contaminated by various *Aspergillus* species and can also contain *Aspergillus*-derived mycotoxins 27-30.

ACKNOWLEDGMENTS

We thank David Young for editing the manuscript.
REFERENCES


Table 1: Experimental assessment of disinfection procedures on 10-ml suspensions containing $3 \times 10^8$ spores of *A. fumigatus*/ml, and Petri dishes contaminated with $3 \times 10^8$ spores of *A. fumigatus* (Mean log value ± standard deviation of 10 determinations. * Significantly different from the untreated control group: $P<0.05$).

<table>
<thead>
<tr>
<th>Spore suspension</th>
<th>Inoculated Petri dishes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>5.75 ± 0.49</td>
</tr>
<tr>
<td>Freezing –20°C; 24h</td>
<td>5.15 ± 0.34</td>
</tr>
<tr>
<td>Microwave irradiation, 800 watts; 1 min</td>
<td>3.45 ± 1.40</td>
</tr>
<tr>
<td>Microwave irradiation, 800 watts; 2 min</td>
<td>0*</td>
</tr>
<tr>
<td>Oven heating, 150°C; 5 min</td>
<td>0.05 ± 0.16*</td>
</tr>
<tr>
<td>Oven heating, 150°C; 10 min</td>
<td>0*</td>
</tr>
<tr>
<td>Oven heating, 150°C; 15 min</td>
<td>0*</td>
</tr>
<tr>
<td>Oven heating, 220°C; 5 min</td>
<td>0.10 ± 0.32*</td>
</tr>
<tr>
<td>Oven heating, 220°C; 10 min</td>
<td>0*</td>
</tr>
<tr>
<td>Oven heating, 220°C; 15 min</td>
<td>0*</td>
</tr>
<tr>
<td>Hot water; 60°C</td>
<td>5.75 ± 0.54</td>
</tr>
<tr>
<td>Hot water; 100°C</td>
<td>5.15 ± 0.63</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Efficacy of disinfection procedures on food
Fungal load after decontamination of pepper naturally contaminated by *A. fumigatus* and on foods (oranges, apples and freeze-dried soups) experimentally contaminated with a suspension containing $3 \times 10^8$ spores of *A. fumigatus/ml
(Mean log value ± standard deviation of 8 determinations. * Significantly different from the untreated control group: $P < 0.05$)

<table>
<thead>
<tr>
<th></th>
<th>Oranges</th>
<th>Apples</th>
<th>Black pepper</th>
<th>Freeze-dried soup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.19 ± 0.37</td>
<td>4.44 ± 0.32</td>
<td>7.00 ± 0.82</td>
<td>6.63 ± 0.25</td>
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<tr>
<td>Microwave irradiation, 800 watts; 2 min</td>
<td>-</td>
<td>-</td>
<td>1.13 ± 1.13*</td>
<td>0*</td>
</tr>
<tr>
<td>Oven heating, 220°C; 5 min</td>
<td>-</td>
<td>-</td>
<td>0.31 ± 0.53*</td>
<td></td>
</tr>
<tr>
<td>Oven heating, 220°C; 15 min</td>
<td>-</td>
<td>-</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>0.56 ± 0.50</td>
<td>0.75 ± 0.89</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water washing; 1 min</td>
<td>2.69 ± 0.53*</td>
<td>4.00 ± 0.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water + soap washing; 1 min</td>
<td>2.38 ± 0.58*</td>
<td>3.63 ± 1.27*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water + soap + 70% ethanol washing; 1 min</td>
<td>0.75 ± 0.53*</td>
<td>1.38 ± 0.52*</td>
<td>-</td>
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</tr>
</tbody>
</table>
Experimental assessment of disinfection procedures for eradication of Aspergillus fumigatus in food

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