Evaluation of the contaminant organisms of humidifier reservoir water and investigation of the source of contamination in a university hospital in Turkey

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This report describes the distribution of different contaminants (fungi, free-living amoebae, bacteria including *Legionella*) in the water of in-use oxygen humidifier reservoirs. We investigated reasons of contamination and also gave recommendations that may decrease the incidence of pneumonia related to use of contaminated humidifiers.

The water of humidifiers is the major environment-associated reservoir for nosocomial pneumonia pathogenes such as *Pseudomonas aeruginosa, Legionella, and Aspergillus.*[1] Inhalation of contaminated aerosols leads to direct inoculation of these pathogenes to the airway,[2] and some free-living amoebae (FLA) serve as natural hosts for legionellae in the environments.[3] At particular risk for acquiring fungal infections are individuals who are immunocompromised.[4] Although many studies have been published on the contamination of humidifier's water with bacteria, few highlighted fungal and amoebal contamination.

MATERIALS AND METHODS

Between April 19 and May 7, 2002, a total of 50 water samples were collected from oxygen humidifier reservoirs of different clinics at the Istanbul University, Istanbul faculty of medicine, Istanbul, Turkey.

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Preparation of media

Amoeba saline (AS) and nonnutrient (NN) agar were prepared as previously mentioned by Isenberg.[5] MWY Legionella media, Tryptic Soy (TS) agar, MacConkey (MC) agar, and Sabouraud Dextrose (SD) agar were prepared as advised by the manufacturer (Oxoid; Basingstoke, Hampshire, England). Gentamicin (40 mg/L; Sigma Inc., St Louis, MO) and chloramphenicol (50 mg/L; Sigma Inc.) were added to SD agar after sterilization.

Sample collections

Approximately 50-mL water samples were collected aseptically from oxygen humidifier reservoirs. Each water sample was divided into 2 equal parts: One part was used for amoebal and another for bacteriologic and mycologic cultures.

Amebal culture. Samples were centrifugated for 10 minutes at 250*g*; supernatant was aspirated, and the sediment was drawn and suspended in about 0.5 mL AS, which inoculated in the center of NN agar plate precoated with *Escherichia coli* ATCC 25922. Plates were examined daily for 10 days. By using bacteriologic loop, thin linear tracks (areas where amoebae have ingested bacteria) were examined for the presence of the amoebae and followed with further identification tests.[5] FLA other than *Acanthamoeba* and *Naegleria fowleri* were reported as an unidentified FLA (UI-FLA).

2. Bacteriologic and mycologic cultures. The method described previously by Ta et al[6] was used. Samples were centrifugated at 1000 rpm/minute for 15 minutes, supernatant was removed, and 0.1 mL of sediment was inoculated on each of the 4 following plates: MWY

Legionella media, SD, TS, and MC agar. All inoculated plates (except SD plates incubated at 28°C) were cultured at 37°C, and incubation times were as follows: 2 days for bacteria, 10 days for amoeba, 14 days for *Legionella* and fungi.

Examination of the cultures

TS and MC plates. TS and MC media were examined within 24 to 48 hours. Gram's stain, biochemical tests, and conventional methods were applied for identification of bacteria. API 32 GN (bioMérieux Inc, France) strips were used for identification of gram-negative bacilli.

MWY plates. MWY Legionella media plates were studied after 48 hours for 14 days. Suspected colonies were subcultured on TS and MWY plates in parallel. Legionella does not grow on TS agar-free cystein but does grow on MWY Legionella media.

SD plates. Fungal cultures were examined after 3 to 14 days, and suspected colonies were stained with lactophenol cotton blue. Conventional and API ID 32 C (bioMérieux, Inc) methods were used for identification of fungi.

RESULTS

A total of 54 different contaminants were isolated from 32 (64%) of 50 oxygen humidifier reservoirs water: 23 (46%) were contaminated with fungi, and 15 (30%) with bacteria. Distribution of these contaminants was as follows:

Pathogenes commonly associated with respiratory tract infections. Ten contaminants were found to contaminate 20% of the humidifiers: 4 *Aspergillus* spp, 2 *Penicillium* spp, 2 *Paeruginosa*, 1 *Flavimonas oryzihabitan*, and 1 *Scedosporium* spp.

Contaminants commonly not associated with respiratory tract infections. A total of 30 organisms was found to contaminate 8 (16%) of the humidifiers' reservoirs: 9 *Bacillus* spp, 6 *Exophiala* spp, 5 *Cladosporium* sp, 4 *Acremonium spp*, 3 gram-negative bacilli (2 *Comamonas testosteroni* and 1 *Pseudomonas mendocina*), 2 *Scopulariopsis* spp, and 1 *Chaetomium* spp.

Fourteen (28%) of the water samples were contaminated with FLA, and 3 of them were identified as *Acanthomoeba* spp; *Legionella* was not detected.

DISCUSSION

The humidifiers reservoirs water were heavily contaminated with fungi (46%) and bacteria (30%). Some of these contaminants such as *Aspergillus* spp, *Scedosporium* spp, *Penicillum* spp, *F oryzihabitans*, and *P aeruginosa* are known as potentially lower respiratory tract pathogenes and life-threatening in immunocompromised hosts and even outbreaks.[1,7,8] Investigating the reasons of water contamination have showed that there were 2 main reasons for contamination of our hospital humidifiers: first, addition of sterile water to the water already in the reservoirs, thus use of the same humidifiers for many weeks without disinfection; second, confusing distilled water with sterile water or use of tap water as an alternative to sterile water. We think that even chlorinated tap/potable water should also not be used in the humidifiers because the water may contain these contaminant organisms in acceptible levels, but the numbers will increase in the reservoirs within a few hours and may be a source of nosocomial infections, especially for patients who have respiratory system disorder or are immunocompromised.

Recommendations have been published from ''The Hospital Infection Control Practices Advisory Committee,'' which provides guidance on prevention of nosocomial pneumonia.[9] The recommendations emphasized the education of health care workers, handwashing, thoroughly cleaning and pasteurizing or use of high-level disinfection for all parts of the oxygen humidifiers, and use of sterile water for rinsing of items after chemical disinfection. Regarding the use of sterile water for refilling of reservoirs, an alternative is to replace the multiple-use humidifier with single-use sterile disposable humidifiers.

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