Journal of Hospital Infection 86 (2014) 194–200

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Occurrence of fungi in dialysis water and dialysate from eight haemodialysis units in central Italy

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ARTICLE INFO

Article history:
Received 9 July 2013
Accepted 19 November 2013
Available online 8 January 2014

Keywords:
Dialysis solution
Dialysis water
Filamentous fungi
Fungal count
Haemodialysis

SUMMARY

Background: Fungal contamination of dialysis fluids may be a serious problem in therapy, particularly due to the debilitated immune system of haemodialysis patients.
Aim: To investigate the occurrence, distribution, and diversity of fungi in dialysis water and dialysis solution of eight haemodialysis units in a region of central Italy.
Methods: Samples were collected over a one-year period from different points of the haemodialysis circuits in accordance with the guidelines of the Italian Society of Nephrology. Isolation and identification of fungi was performed according to the ISTISAN method Reports (2007/05 and 2008/10).
Findings: Of the 976 samples analysed, 96 grew filamentous fungi, 28 were positive for yeast, and six samples contained both mould and yeast. A wide variety of filamentous fungi (26 genera, of which 15 identified at species level, and 'mycelia sterilia') were recovered, many of which are known as opportunistic pathogens. *Cladosporium* spp. were most frequently found (39%), followed by *Alternaria* spp. and *Tricophyton* spp. Fungal counts in treated water and standard dialysate solution were always below the threshold (<10 cfu/mL), and thus are in agreement with the Italian guidelines for dialysis fluid quality, whereas 10.9% of the samples of ultrapure dialysate solution were contaminated by one or several fungi types, in contravention of the guidelines.
Conclusion: The large variety of opportunistic fungi recovered in the haemodialysis circuits proves the importance of including an analysis of fungi to check the microbial quality of dialysis water and dialysate.

Introduction

During recent years, increasing attention has been paid to fungal infections, especially in hospitals where fungi have emerged as a leading cause of hospital-acquired infections.[1] Several types of fungi are involved in human mycoses; of these, one group is defined as primary pathogens because they can also affect individuals with normal immune systems, whereas another group is known as opportunistic pathogens because they produce illness in debilitated or immune-deficient hosts. Most fungal infections in humans are caused by opportunistic fungi, particularly in the hospital environment due to the presence of a high-risk population such as transplanted patients, the elderly with pre-existing comorbidities, and an increased number of immunocompromised subjects. Patients with chronic renal insufficiency who are undergoing procedures such as haemodialysis or peritoneal dialysis are also at increased risk.[2,3] Dialysis patients often have a compromised immune system and thus are more susceptible to pathogens

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0195-6701/$ – see front matter
http://dx.doi.org/10.1016/j.jhin.2013.11.010

and opportunistic micro-organisms, including fungi. These patients are exposed, during each haemodialysis treatment, to 25 times more water than a healthy subject typically drinks in a day, corresponding to about 400 L each week or to about 20,000 L per year.[4,5] Thus, the microbiological quality of water for dialysis must be assured by treatment processes such as filtration and reverse osmosis. Whereas bacteria such as coagulase-negative staphylococci, *Staphylococcus aureus*, *Pseudomonas* spp. or Enterobacteriaceae and their degradation products including endotoxins are well documented contaminants of haemodialysis circuit water, reports in the literature of the presence of fungi, and filamentous fungi in particular, are scanty .[6,7] However, studies on the occurrence of fungi in haemodialysis units have been conducted in several countries, documenting that various saprophytic fungi species, such as *Candida parapsilosis* and those in the genuses *Trichoderma*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Penicillium*, *Verticillium*, *Chrysosporium*, *Acremonium* and *Phialemonium*, have been isolated from dialysis water and dialysate in different haemodialysis centres.[8–15] Several standards exist for water quality, dialysis fluid quality, and equipment used for the preparation of dialysis solutions. Recently, the International Standards Organization (ISO) has prepared a new document providing guidance for the preparation and quality management of fluids for haemodialysis.[16] In addition to the ISO standards, several groups and organizations have produced standards and guidelines to ensure the quality of dialysis water. Among these are the standards developed by the American Association for the Advancement of Medical Instrumentation, the European Best Practice Guidelines for Haemodialysis, the Directive of the Brazilian Ministry of Health, the Japanese Society for Dialysis Therapy, and the Canadian Standards Association.[17–22] These standards define the maximum permissible levels, although with some variance, of microbiological contaminants permissible in water used for the preparation of dialysis fluid; however, the routine microbiological analysis of dialysis fluids does not include the detection or quantification of fungi. In Italy, the Italian Society of Nephrology (ISN) has developed clinical guidelines that indicate the procedures for the production and monitoring of dialysis fluids aimed to improve dialysis fluid quality.[23] Among the routine of chemical and microbiological checks, the guidelines also include the analysis of moulds and yeasts and indicate that the number of total fungi in treated waters and dialysate should not exceed 10 cfu/mL, whereas this number should be 0 cfu/mL for the ultrapure dialysate. The aims of the present study were to investigate the occurrence, distribution, and diversity of fungi in dialysis water and dialysate solutions in eight haemodialysis centres in a region located in central Italy, following the requirements of established standards of the ISN.[23]

Methods

Sampling

The study was conducted over a period of 14 months, from January 2010 to February 2011, in haemodialysis units of eight hospitals (identified as units A–H), located in the Marche Region, Central Italy. All dialysis unit circuits are equipped with water treatment systems involving hyperchlorination tanks, filtration systems, water softeners, charcoal filters, and reverse osmosis (Figure 1). The circuit units differed in size, in particular the number of the standard monitors ranged from two (unit D) to 17 (unit F) and the ultrapure monitor from four (unit B) to 20 (units A and F). From each dialysis circuit, the monitoring frequency and the collection points were chosen according to the ISN guidelines.[23] Samples were also collected in the junction points between the distribution loop and monitors; the number of these junction points ranged from six (unit B) to 36 (unit A). A total of 976 samples was collected from tap water of the plant inlet (site 1), treated water at the start and end of the distribution loop (sites 2 and 3 respectively), junction points (site 4), and dialysis fluids in the standard (site 5) and ultrapure (site 6) monitors. Briefly, all samples were collected in sterile bottles after 1–5 min of free flow, after disinfection of sample port by 70% ethanol (plastic tubes) or by flaming (metal tubes), and immediately before the disinfection treatment of the distribution loop. For tap water, a solution of 0.1 N of sodium thiosulphate was added to neutralize any residual chlorine. Samples were transported into the laboratory using a portable cooler (4–6°C) and analysed immediately.

Fungal culture and identification

Isolation of fungi was performed according to the ISTISAN method Reports 2007/05, accredited by ACCREDIA.[23] Briefly, 100 mL of each sample of water were filtered with a sterile 0.45 μm membrane, and incubated at 23.5 ± 1.5°C for 7 days on Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (100 mg/L).

Mould colonies grown on SDA were subsequently transferred on to potato dextrose agar for stimulation of conidia production, and examined through macroscopic and microscopic characteristics after incubation at 22–25°C for 3–5 days. The macroscopic observation for each isolate included growth rate, surface appearance and reverse colour. Microscopic observation was performed by slide culture method for a more precise assessment of the conidiogenous process; maintaining a permanent record of the isolate required a cellophane tape mount, placing a drop of lactophenol blue cotton (Lactophenol Blue solution, Fluka, Buchs, Switzerland) on a clean glass slide and examining it under the microscope at different magnifications.[24–26]

Yeasts grown on SDA were directly identified by morphological characteristics of the colonies such as the production of circular, restricted, pasty, or mucoid colonies and microscopic observation of cell size.[27,28]

Results

In all, 976 samples of tap water, treated water for dialysis, and dialysate from the eight haemodialysis centres were collected from January 2010 to February 2011, and investigated for the presence of fungi. Results of analyses are reported in Table I. Fungi were recovered, although to a different extent (from 31.2% to 8.9%), at various collection points of the haemodialysis circuits. Fungi were recovered from 118 samples (12.1%) and their concentrations ranged from 1 to 420 cfu/100 mL. The analysis of the macro- and micromorphology of the colonies indicated that 90 (76.2%) of the 118 positive samples contained one or more genera of filamentous fungi, 22 (18.6%)