Short communication

Colonization of a voice prosthesis by Cryptococcus neoformans

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Tracheoesophageal voice prostheses in laryngectomized patients commonly deteriorate due to the presence of yeasts, particularly Candida species. We describe the first case of colonization of such a device by Cryptococcus neoformans in a patient with a history of glottic carcinoma. The isolate showed an identical genomic pattern with C. neoformans from pigeon excreta in the patient’s environment.

Keywords Cryptococcus, voice prosthesis

Introduction

Laryngectomized patients receive a tracheoesophageal voice prosthesis (TVP) for speech rehabilitation. This device consists of a silicone rubber based tube with a one-way low pressure valve and is inserted in a tracheoesophageal fistula. An inner esophageal flange and an outer tracheal one aid in keeping the prosthesis in place (Fig. 1a) [1]. A schematic drawing indicating the position of the voice prosthesis in a patient, and the way the latter handles it, is presented in Figure 1b [2]. The valve permits an airstream into the pharynx where sound is generated by vibration of the pharyngeal walls. During speech, the patient must use a finger to close the stoma. Leakage of fluid and food into the trachea is blocked and thus prevents the development of aspiration pneumonia. Damage to the silicone results in valve failure and leakage and requires the replacement of the TVP. This deterioration is generally associated with biofilm formation and heavy colonization by yeasts, particularly Candida spp. [3–5]. No previous reports exist on the colonization of a TVP by Cryptococcus neoformans, the ubiquitous fungal pathogen causing cryptococcal meningitis/encephalitis. The present paper describes the first case of such colonization and suggests pigeon droppings from the patient’s environment as its source.

Case report

In February 1997, a man was referred to the Department of Head and Neck Surgery for persistent dysphonia. On direct laryngoscopy and biopsy, a glottic carcinoma was diagnosed. The tumour was removed and radiotherapy (66 Gray) was subsequently started. Although the patient tolerated this therapy very well, he developed severe dysphonia in August 1997. A new direct laryngoscopy revealed a recurrent tumour and a total laryngectomy was performed on August 29, 1997. At that moment, a TVP for voice rehabilitation was placed. The prosthesis had subsequently to be removed and changed four times until now.

Mycology

As part of an epidemiological study, mycological examinations were carried out on three of the four removed prostheses, one on June 12, 1998, and the others on February 10, 1999 and April 14, 1999, respectively. The entire prosthesis was placed in 10 ml of peptone, followed by vigorous mixing on a vortex type mixer to release the yeast cells, membrane filtration of the broth and incubation of the membrane filter on Sabouraud glucose agar (SGA; Difco Laboratories, Detroit, MI, USA) supplemented with ticarcillin (4688 μg ml⁻¹) clavulanic acid (312 μg ml⁻¹) (Timentin®; Smithkline Beecham Pharma, Genval, Belgium). Yeasts were detected in an enzymatic two-step method specifically developed for the presumptive differentiation of Candida spp. [6]. Two species were isolated. The
first one was identified as *Candida lusitaniae*. The second isolate, predominantly present, was consecutively sub-cultured on SGA and bird seed agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) for 48 h at 37 °C, the latter medium yielding brown colonies. Microscopic examination of cells with India Ink showed a typical yeast morphology and a clear capsule around the yeast cells, suggesting *C. neoformans*. Further confirmation of this identity was obtained with a yeast identification panel test (API 20C AUX; bioMérieux, Vitek, Hazelwood, MO, USA), a latex-agglutination test (Crypto-LA, Fumouze, France) and incubation in urease broth. All test results were consistent with *C. neoformans*.

Possible sources of *C. neoformans* in the patient’s environment were investigated. As a pigeon fancier, he had a regular exposure to three fancy pigeons. Some old and dehydrated pigeon droppings were collected from the dovecote. They were homogenized and extracted in Sabouraud glucose broth, the extract was filtered and the membrane filter was incubated on bird seed agar for 72 h at 37 °C. Again, brown colonies appeared and microscopic examination with India Ink and confirmation with sugar assimilation and latex-agglutination tests indicated *C. neoformans* var. *neoformans*.

Susceptibility of the isolates to antimycotics was determined by the colorimetric Fungitest (Sanofi Pasteur, Marnes-la-Coquette, France). The isolate was susceptible to fluconazole, amphotericin B and 5-flucytosine at the following minimum inhibitory concentrations (MICs): 8 μg ml⁻¹, 2 μg ml⁻¹ and 2 μg ml⁻¹, respectively.

To determine whether the *C. neoformans* isolates of the patient and the pigeon droppings were clonally related, a polymerase chain reaction (PCR)-based typing technique was used [7–9]. In a previous PCR study on 81 clinical isolates of *C. neoformans* using the simple repetitive sequence (GACA)₄ as a single primer, we discriminated *C. n*. var. *neoformans* serotype A from serotype D [7].

Serotypes A and D encompass seven and three different genotypes, respectively, based on the molecular weights of four major bands. Minor bands allow further subdivision of these main genotypes. In the present study, the same typing technique was used for an epidemiological purpose. PCR was performed in volumes of 50 μl of reaction buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl), containing 25 ng of DNA, 2.5 U of *Taq* DNA polymerase, 160 ng of primer per cycle, 200 μM each of dATP, dCTP, dGTP, dTTP and 2.5 mM of MgCl₂ (GeneAmp PCR Core reagents, Perkin-Elmer Cetus, Norwalk, CN, USA). Samples were overlaid with sterile paraffin oil and amplification was performed in a Thermo Cycler (Perkin-Elmer Cetus) as follows: denaturation at 93 °C (1 min), annealing at 50 °C (1 min), extension at 72 °C (1 min) and final extension at 72 °C for 7 min. A total of 39 cycles was applied. PCR products (20 μl sample⁻¹) were subjected to electrophoresis in 1% agarose gels for 2 h at 5 V cm⁻¹ in 0.5 × TBE buffer (0.045 mM Tris-borate [pH 8.3], 1 mM EDTA), stained with ethidium bromide and visualized by UV transillumination. DNA from two reference strains was included: *C. neoformans* serotype A (A2) and serotype D (D52).
The isolates of the patient and the pigeon droppings exhibited identical banding patterns and, hence were indistinguishable, whereas the epidemiologically unrelated control isolates were clearly different (Fig. 2).

From a clinical standpoint, these findings suggest that the pigeon droppings could have transmitted the yeast to the patient.

**Discussion**

Various studies have demonstrated the rapid colonization of TVPs by yeasts, particularly *Candida* spp., including *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* [1,3–5]. However, to the best of our knowledge, this is the first report of the presence of *C. neoformans* on such a device. Among the 101 patients with a TVP screened over 28 months, only one was found to harbour *C. neoformans*. It is tempting to speculate that the TVP could serve as a reservoir for the organism and induce a life-threatening meningocerehalitis or pneumonia in the patient in connection with future episodes of immunosuppression. To reduce this risk of clinical illness, prophylaxis with fluconazole is being considered. Furthermore, the patient should avoid potential environmental sources of contamination, including his own pigeons and their excreta. Zoonotic transmission of *C. neoformans* to humans is well documented [10–12]. Nosanchuk et al. [10] describe a case in which an immunocompromised patient contracted cryptococcal meningitis from a pet cockatoo. A likely scenario for transmission in the present case is the contamination of the patient’s hands from contact with his pigeons and the subsequent use of an unwashed finger to close the stoma during speech (Fig. 1b). All three TVPs removed over a period of 11 months were found to be colonized by *C. neoformans*. Further follow-up of this patient will reveal if hygienic measures and fluconazole administration will be capable of reversing this colonization.

**References**