

Original papers

Mycobiota of the air in hospital rooms and the fungal colonisation of tracheostomy tubes used by patients diagnosed with larynx cancer – preliminary research

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ABSTRACT. A tracheotomy tube, as well as the stoma through which it is inserted into the patient's throat, may represent a potential risk of fungal infections for patients suffering from larynx cancer. The study was aimed at evaluating the influence of the hospital room environment on the fungal colonisation of tracheotomy tubes in the case of patients diagnosed with larynx cancer and operated on in the Laryngology ward. The mycological research was carried out in the rooms of the Laryngology ward, from which 105 air samples were collected. Twenty-two Portex and metal tracheostomy tubes collected from 13 patients diagnosed with larynx cancer. Fungi were cultured on 15 tracheostomy tubes: moulds were isolated from 3 of these tubes, and fungi belonging to the genus *Candida* from the remaining 12. The simultaneous occurrence of the same moulds in the air and on the tracheotomy tubes was observed only in one case (*Aspergillus flavus*). In conclusion, the same moulds observed in the air can sometimes also be found on the tracheotomy tubes used by patients diagnosed with larynx cancer. Yeast-like fungi are isolated from tracheotomy tubes much more frequently than moulds, and this requires further mycological research.

Key words: mould fungi, tracheotomy tubes, larynx cancer

Introduction

More and more opportunistic infections caused by moulds have been observed in recent years. The dominant type of fungus responsible for exogenous infections is genus *Aspergillus.* Yet, the epidemiology of mould infections has undergone continuous change and new infections caused by moulds belonging to the genera *Rhizopus* or *Mucor* (order Mucorales) have appeared [1,2]. The respiratory system and openings in the skin resulting from surgery, for example tracheotomy, might be potential routes of entry for moulds to cause infection. Infections of the respiratory system result from inhalation of airborne fungal spores, and aspergillosis or mucormycosis are often preceded by fungal spores colonizing the nasopharyngeal cavity. Patients who are diagnosed with cancer, especially when the disease spreads in larynx and pharynx, are much more susceptible to fungal infections [3]. The risk of fungal infections increases when the patient must have a tracheotomy tube inserted, as the area of potential infection spreads onto the tracheotomy tube and the flange (neck plate) into which it is fitted. The problem of tracheotomy tubes being colonised by moulds and the influence of the environment on such colonisation have not been reported so far. Some studies imply that a biofilm made up of yeast-like fungi and Gram-negative bacteria might appear on laryngotracheal stents or other airway prosthesis. However, no results concerning moulds can be found.

The aim of the present study was to evaluate the influence of hospital room mycobiota on the fungal colonisation of tracheotomy tubes fitted to patients diagnosed with larynx cancer and operated on in the Laryngology ward.

Materials and Methods

The study was carried out in the Department and Clinic of Otolaryngology, Jagiellonian University Medical College in Cracow between November 2012 and March 2013. Permission for the study was given by the Committee of Bioethics of the Jagiellonian University (KBET/236/B/2012). The research involved an evaluation of the fungal contamination of the air in three hospital rooms (patients' room, corridor and toilet) and examination of tracheotomy tubes collected from 13 patients diagnosed with larynx cancer who were staying in these rooms.

The patient diagnosed with larynx cancer was interviewed on the day preceding the tracheotomy operation and air samples from the room where the patient was staying, the corridor and the toilet were collected by means of a MAS 100 air monitoring system (Merck). On the first day following the surgery, when the Portex tracheotomy tube was replaced with a metal one, the whole tube was collected under sterile conditions and transported to a laboratory within an hour. On the same day, air samples were collected from 3 rooms on the ward. The third air sampling took place on the Laryngology ward when the tracheotomy tube was exchanged for the second time; this was on average 15 days after the replacement of the first tube – between 9 and 22 days. On the same day, the metal tracheotomy tube was collected and transported to the laboratory for mycological examination, using the same procedure for the Portex tube.

In total, 22 tracheotomy tubes were collected: 13 Portex and 9 metal tubes. Although both Portex and metal tracheotomy tubes were collected from 9 patients, only Portex tubes were examined from the remaining 4 patients due to technical problems.

A total number of 105 Petri plates with Sabouraud (bioMérieux) medium were used to culture air samples during the research cycle. They were incubated at a temperature of 27°C for three days. The fungal colonies which grew on the plates were counted, and their number was presented as CFUs (colony forming units) calculated per m^3 of air tested in each separate sample.

The identification of fungal colonies was carried out following the routine rules of mycological diagnostics. As far as the Portex tracheotomy tubes were concerned, a one-centimetre-long part was cut from the trachea end of the tube. Next, the material was placed in 5 ml of physiological saline and shaken for about 1.5 min. After that, a 10 µl drop of the liquid was placed on SGA (Sabouraud) medium. In addition, the tube part was also placed in liquid Sabouraud medium and incubated at a temperature of 27°C for up to 2 weeks.

The procedure for metal tubes consisted in taking a swab from the inner part of the tube and placing it in SGA medium. The tubes were also delicately shaken in liquid Sabouraud medium. The material which was obtained this way was incubated under the same conditions as for the Portex tubes. Isolated yeast-like fungi were first identified on the basis of their ability to form chlamydospores in rice extract agar.

Results

The total of 22 tracheotomy tubes were collected from 13 patients diagnosed with larynx cancer, and the incubation of the material from 15 of these tubes resulted in fungal growth. Yeast-like fungi belonging to genus *Candida* were isolated from 12 tubes: *Candida albicans* sp. from 6 tubes and other *Candida* species from the other 6. In the material from the remaining 3 tubes, all of which were Portex tubes, *Aspergillus fumigatus* and *Aspergillus flavus* species of moulds were found.

Although the Portex tubes were used only for one day and the metal ones for 15 days, on average, the frequency with which fungi were isolated was similar for both types of tubes (Chi^2 test p=0.861): fungi were isolated from 9 out of 13 Portex tubes and 7 out of 9 metal tubes. From the tubes collected from two patients (patients 3 and 12) no fungi were isolated at all. There was only one example of identical fungi belonging to *C. albicans* being isolated from two tubes collected from the same patient. A detailed description of the types of fungi isolated from particular tracheotomy tubes is presented in Table 1.

Airborne fungi were detected in 96 (out of 105) samples, in which the average number of colony forming units ranged between 5 and 235 CFUs in m^3 of the air depending on the room. The lowest number of fungal CFUs was found in the samples collected in the patients' rooms, where the median

Table 1. Occurrence of fungi in the air and on tracheotomy tubes, and their systematic classification depending on particular measurements and the number of patients

N	Air			Tracheostomy tubes	
	Survey	CFU	Systematic classification of fungi	Survey	Systematic classification of fungi
1	I	160	*Rhizopus* sp., *Rhodotorula* sp., other moulds	I	*Aspergillus fumigatus*
	II	30	*Rhizopus* sp., *Aspergillus niger*, other moulds		
	III	92	*Rhizopus* sp., *Rhodotorula* sp., other moulds	II	*Candida albicans*
2	I	20	*Aspergillus fumigatus*, other moulds	I	no fungal growth
	II	27	*Aspergillus ochraceus*, *Aspergillus ustus*, *Aspergillus fumigatus*, other moulds		
	III	34	*Aspergillus ochraceus*, *Aspergillus fumigatus*, *Candida* sp., other moulds	II	*Candida* non-*albicans*
3	I	57	*Aspergillus fumigatus*, *Aspergillus flavus*, other moulds	I	no fungal growth
	II	22	*Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Rhodotorula* sp., other moulds		
	III	32	*Aspergillus clavatus*, *Aspergillus ochraceus*, *Candida* sp., other moulds	II	no fungal growth
4	I	52	*Rhizopus* sp., *Aspergillus fumigatus*, other moulds	I	*Candida albicans*
	II	14	*Aspergillus niger*, *Candida* sp., other moulds		
5	I	20	*Aspergillus fumigatus*, *Aspergillus ochraceus*, *Candida* sp., other moulds	I	*Candida* non-*albicans*
	II	19	*Candida* sp., other moulds		
6	I	30	*Aspergillus terreus, Rhizopus* sp., *Candida* sp., other moulds	I	*Aspergillus fumigatus*
	II	12	*Aspergillus nidulans*, *Candida* sp., other moulds		
	III	23	*Aspergillus ochraceus*, *Aspergillus fumigatus*, other moulds	II	*Candida* non-*albicans*
7	I	45	other moulds	I	*Candida albicans*
	II	12	*Aspergillus terreus*, *Aspergillus niger*, other moulds		
8	I	15	*Aspergillus niger*, *Aspergillus flavus*, *Candida* sp., other moulds	I	*Candida* non-*albicans*
	II	19	*Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus terreus*, *Rhodotorula* sp., *Candida* sp., other moulds		
	III	12	*Aspergillus niger*, *Aspergillus terreus*, *Rhodotorula* sp., *Candida* sp.	II	*Candida albicans*
9	I	27	*Aspergillus ochraceus*, other moulds	I	no fungal growth
	II	25	*Candida* sp., *Rhodotorula* sp., other moulds		
	III	10	other moulds	II	*Candida* non-*albicans*
10	I	33	*Candida* sp., other moulds	I	*Aspergillus flavus*
	II	38	*Aspergillus flavus*, *Aspergillus ochraceus*, *Rhodotorula* sp., other moulds		
	III	28	*Aspergillus flavus*, *Candida* sp., other moulds	II	*Candida* non-*albicans*
11	I	103	*Aspergillus ochraceus*, *Aspergillus fumigatus*, *Rhizopus* sp., other moulds	I	*Candida albicans*
	II	20	*Aspergillus fumigatus*, other moulds		
	III	10	other moulds	II	*Candida albicans*
12	I	37	*Aspergillus versicolor*, other moulds	I	no fungal growth
	II	7	other moulds		
	III	20	*Aspergillus niger*, *Aspergillus candidus*, *Aspergillus fumigatus*, other moulds	II	no fungal growth
13	I	52	*Aspergillus fumigatus*, *Mucor* sp., other moulds	I	no fungal growth
	II	5	*Aspergillus niger*, other moulds		

Explanations: N – consecutive patient numbers; **CFU** – average number of fungi calculated on the basis of 3 measurements (patients' room, corridor, toilet); **other moulds** – moulds other than *Aspergillus*, *Rhizopus*, *Mucor* detected in air samples; **I, II, III** – consecutive measurements

amounted to 15 CFUs (between 5 and 160 CFUs in m^3), while the highest number was found in the samples collected from the corridor, with a median of 30 CFUs (between 5 and 125 CFUs in m^3). The average number of fungi, calculated on the basis of 3 measurements carried out on the same day in various rooms, ranged between 5 and 160 CFUs per m^3, giving a median value of 25 CFUs per m^3 of air. *Penicillium* sp. and *Aspergillus* sp. were the most frequently detected genera in the air of the hospital rooms, however, fungi belonging to *Rhizopus* sp. were isolated from six air samples, and a *Mucor* fungus was found in one case. Only one simultaneous occurrence of the same mould (*Aspergillus flavus*) was observed in both the air sample and the material from the tracheotomy tubes (patient 10) (Table 1).

Discussion

The moulds which are the most frequently isolated in the indoor hospital air belong to the genera *Penicillium* and *Aspergillus*, the latter of which is said to have the greatest clinical significance. The source of this kind of infection might be another patient suffering from a fungal disease but usually the hospital reservoirs are at fault [6]. In the present study, only in one case was the same type of fungi isolated from both the Portex tracheotomy tube and the air of the ward in which the patient was staying. Although it is difficult to conclude that the occurrence of genus *Aspergillus* in the hospital environment or its colonisation of tracheotomy tubes might be a decisive risk factor as far as mould infections are concerned, such an option cannot be eliminated.

Although very little support for these results, if any, can be found in the literature, some cases have been reported. An immunosuppressive patient suffering from pneumonia, who was originally diagnosed with larynx cancer, developed zygomycosis caused by the genus *Mucor* [7]. In another patient originally diagnosed with prostate gland cancer, Laryngopharyngeal zygomycosis was caused by fungi belonging to the species *Rhizopus rhizopodiformis* [8]. The problem is a significant one, as a significant number of patients who undergo laryngectomy for larynx cancer must, subsequently, undergo chemotherapy and radiotherapy [9].

These preliminary results imply that research should be continued, firstly, in order to verify the simultaneous co-occurrence of fungi, in both the air of the hospital rooms and on tracheotomy tubes, and secondly, to determine whether these moulds, detected in the air and on the tubes, have the same genotype and are cytotoxic in laboratory conditions. It should be noted that the type of fungus which was often isolated from the tracheotomy tubes in the present study was identified as a yeast-like fungus belonging to the genus *Candida* (detected in 12 out of 22 cases). These fungi might be a significant risk factor of fungal infections for patients diagnosed with larynx cancer, especially for those who need further immunosuppressive treatment. It needs to be emphasised, however, that the main source of infections caused by yeast-like fungi is not the air of the hospital rooms in which the patients are staying, but the resident flora of the oral cavity and respiratory tract of the patient. In some cases, medical staff or contaminated personal belongings might also be to blame.

The number of fungal CFUs estimated for the air of examined hospital rooms was very small: its median was only 25 CFUs per m^3 of air. The calculations were performed for average values of 3 examinations carried out in various rooms on the same day for all 13 patients separately. Such a result was similar to the median calculated for the number of fungi detected in the air of hospital wards in which oncological patients were staying [10].

Conclusions

The same types of moulds can sporadically be observed both in hospital air and on the tracheotomy tubes used by the patients diagnosed with larynx cancer. Yeast-like fungi are much more frequently isolated from tracheotomy tubes than moulds, and this should be the area of interest for further mycological research.

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