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ORIGINAL ARTICLE

MICROBIAL AIR LOAD AT THE TRANSPLANT INTENSIVE CARE UNIT

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Summary

The air does contains microbial agents originally coming from the soil, water, plants or animals, including men. Temperature, light and humidity are the basic factors which has influence of microbial survival and abundance. Different microorganisms travel by aerial transmission and are involved in serious processes causing pneumonia and other diseases.

In our study we decided to investigate microbial load in air at the Transplant Intensive Care Unit of the University Hospital of Hradec Králové, Czech republic for two years period. Air samples were taken from the patient's breathing zone in the single rooms. Air was sampled with Biotest RCS Plus air sampler and material collected on the Total Count strips prepared with Tryptic Soy Agar.

The majority of air samples (54.2%) had microbial air load ≤ 100 CFU.m^{-3}. Very low microbial air concentration from 15 to 30 CFU.m^{-3} was detected in the rooms before admission of new patients. Higher concentration was detected when medical staff was present in the room and investigation or treatment was carried out. The majority of microbial findings in the air were Gram-positive cocci (coagulase-negative *staphylococci*, *Micrococcus spp., Sarcina spp.*). Findings of Gram-negative stems were sporadic (*Pseudomonas aeruginosa*) as well as incidence of microscopic fungi (*Cladosporium spp., Penicillium spp.*).

Key words: transplant intensive care unit; microbial air load; biotest RCS Plus air sampler; immunocompromised patients

INTRODUCTION

The quality of microclimatic conditions in indoor environment belongs to the most important effects on human health and also represents ethical problems in environmental epidemiology (1). Safe exposition limits are exactly determined for a number of components, e.g. aldehydes, ozone, radon, and others. But for the group of other microclimatic factors, such as biological pollutants, the formulation of permissible exposure limits is not easy because of demanding quantitative demonstration of causal connections with health damage. Epidemiological studies of infectious diseases in health service establishments confirmed the importance of quality microclimate for the health of patients and medical staff (2, 3, 4). The character of hospital environment

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is formed under specific conditions which are created in connection with diagnostics, therapy and other activities in patients‘ care. For example, aerosol containing *Legionella spp*. or *Aspergillus spp*. may be produced when patients take a shower (5, 6, 7). For these reasons, regular microbial monitoring of water supply system and surface disinfection in bathrooms are very important in these facilities.

Respecting the above mentioned facts, it is necessary to break the way of transmission of microbial agents and to protect the patients from infectious complications by keeping the anti-epidemic regimes. The possibility how to decrease spreading of microbial components is to establish an air-conditioning device which will fulfill the following tasks: air supply from outdoors and its treatment with regard to the contents of microbial and dust particles, creation of thermal comfort, and creation of optimal air moisture, active air exhaustion from patients` room containing chemical and biological pollutants. But on the other hand, improper procedures during air treatment may become a significant source of new health risk in the environment. The quality of produced air depends on the input air, cleanness of the whole system and functionality of filters. Not only a good air-conditioning system, but also observance of relatively simple sanitary measures including basic requirements for personal hygiene of the patients and staff are important for the quality of indoor air in the hospital. It is necessary to reduce risk of infectious diseases from exogenous sources, especially during treatment of immunocom-promised patients.

The present work was aimed at the monitoring of total microbial air load during a long time period at the Transplant Intensive Care Unit (ICU) of the University Hospital in Hradec Kralove where patients with hematological malignancies are treated. Our experimets were based on using Biotest RCS Air Sampler (Biotest HYCON). That instrument is used for safe and reliable monitoring of ambient air and capable for gentle and effective collection of airborne microorganisms. Monitoring of microbial air load helps to establish hygienic rules at e.g. health units, hospitals, care units.

MATERIALS AND METHODS

The monitoring of microbial air concentration was investigated at the Transplant ICU of the University Hospital in Hradec Kralove where patients are treated in single rooms with toilet and shower. The whole Transplant ICU is linked with central air-conditioning (filtration, heating, cooling, and humidity). Fresh air is sucked from the central chamber built in brick, and the devaluated air is led through an exhaust chamber into the outdoor space. Patients under intensive cytostatic treatment of hemoblastosis, and those after transplantation of peripheral stem blood cells or bone marrow are hospitalized in these units. The facility provides reverse isolation under aseptic regimen.

The monitoring of microbial air load was investigated monthly in six single rooms, and 120 air samples were taken from the patients` breathing zone during two years. The monitoring was carried out all year except July and August. During each monitoring, 200 liters of air were taken from the patients` breathing zone.

Air was sampled using Biotest RCS Air Sampler and collected on Biotest HYCON Agar strips with Tryptic Soy Agar that is a standard growth medium for bacteria. After sampling, agar strips were transported in a thermo box as fast as possible to the laboratory. The numbers of colony forming units (CFU) were counted after 1 and 2 days of incubation at 37 ºC, and then CFU per cubic meter ($CFU.m^{3)}$ were calculated. Further identification of isolated microorganisms was accomplished in the Institute of Clinical Microbiology at the University Hospital in Hradec Kralove using commercial biochemical identification tests (BBL Crystal Identification Systems, Vitek bioMérieux).

The aeroscope Biotest RCS air sampler (Biotest HYCON) relies on the use of the impact principle. Air stream enters the rotor in sucking head where ventilator blades divide the stream in such a way that - due to centrifugal forces - microorganisms are harvested on a foil covered with cultivation medium. When the sampling is over, the foil with cultivation medium is taken out and cultivated in a protective cover (8). Before the air sampling, sterilization of metal parts of the aeroscope was carried out in a hot air sterilizer. Between individual samplings, the aeroscope head was cleaned with napkins dipped in disinfection solution.

Microbial air pollution was evaluated according to the recommendation published in AHEM (Acta Hygienica, Epidemiologica et Microbiologica) No. 1/2002, State Health Institute, Prague (9). The evaluation was carried out in one of the five categories: very low, low, middle, high and very high (Table 1).

Microbial contamination	Bacteria (CFU.m-3)	Fungi (CFU.m-3)
very low	< 50	< 25
low	< 100	< 100
middle	< 500	< 500
high	< 2000	< 2000
very high	> 2000	> 2000

Table 1. Categories of microbial indoor air contamination – a concentration criterion of mixed population of bacteria and fungi (9).

RESULTS

During two years, twenty controls measuring were carried out in six single rooms at the Transplant ICU and 120 air samples were taken. Detailed findings of quantitative microbial air contamination are shown in Table 2. Microbial air contamination had a large range (from 15 to 300 CFU.m^{-3}). The highest concentration of microbial air contamination (300 CFU.m^{-3}) was detected in single room No. 5 when the bed was made. Higher contamination was found also in the rooms where medical staff was present and investigation or treatment was carried out. On the other hand, very low microbial air concentration (15 CFU.m^{-3}) was detected in the single room that had been prepared for admission of a new patient. 54.2 % of all single rooms fulfilled the conditions (≤ 100 CFU.m^{-3}) used as a recommended value during general surgery (10). As to air contamination, the most frequent Gram-positive germs (coagulase-negative *staphylococci*, *Micrococcus spp., Sarcina spp.*,) were diagnosed (Table 3). No significant resistance to antibiotics was found in the recorded germs. Findings of Gram-negative stems were sporadic (*Pseudomonas aeruginosa)* as well as incidence of microscopic fungi. The targeted monitoring of microscopic fungi was carried out during the first year. Detailed results were published earlier (11). The occurrence of microscopic fungi was generally very low. Only *Cladosporium spp., Penicillium spp. and Mucor spp.* were found in low quantities ranging from 2 to 26 CFU.m^{-3}. No isolates of *Aspergillus spp*. were acquired from the single rooms. According to classification presented in AHEM (12), evaluation of the results showed that the microbial air contamination inside single rooms was mostly within the limits of very low and low categories.

DISCUSSION

The care for immunocompromised patients is very demanding and consists of a complex of medical procedures. An integral part of this care is prevention of infection. The submitted study deals with microbial air load in the patients` breathing zone as a source of exogenous infection. The main objective was to test the affectivity of filter-ventilating apparatus on Transplant ICU in a longer time period. For prevention of infection, very important are the aseptic way of treatment and strict observance of all rules in order to prevent transmission of infection on the patient. When we analyzed our results of microbial air contamination at the Transplant ICU, we could admit that the main source of air contamination were all activities in the patient`s room: making the bed, investigation and treatment, presence of medical staff. The patients, medical staff, and seeing of the sick are generally mentioned as the main source of most microorganisms in hospital environment (12). That is why we recommended strict keeping of all antiepidemic precautions; we laid stress on the use of protective clothing of all medical staff and sanitation programmer in the empty room. The majority of microbial air findings were Gram-positive cocci that can be found on the skin of healthy persons. It is known that coagulase-negative staphylococci are opportunistic pathogens which could cause infection in immunocompromised patients. The transmission route for staphylococci is generally by direct contact involving the airborne transportation of microorganisms onto inanimate surfaces (12). Generally we can say that Gram-positive bacteria survive longer in the form of aerosol than Gram-negative bacteria. This is mainly due to the composition of their wall which contains peptidoglycan resistant to desiccation. That is why the question of incidence of Gram-negative bacteria

Sampling		CFU.m^{-3}					
		Room No. 1	Room No. 2	Room No. 3	Room No. 4	Room No. 5	Room No. 6
1st year	January	60	110	■ 280	90	45	100
	February	110	125	50	80	110	65
	March	80	95	■ 220	115	■ 300*	■ 225
	April	130	150	70	105	80	■ 250
	May	130	130	■ 230	35	150	120
	June	25	■ 260	50	20	95	85
	September	80	50	100	70	100	140
	October	■ 225	125	50	25	80	100
	November	85	■ 220	75	110	95	50
	December	130	110	25	85	105	■ 230
1st year	January	55	105	30	90	110	35
	February	140	75	110	225	60	125
	March	115	80	105	45	75	■ 275
	April	125	100	30	85	135	80
	May	70	95	140	120	40	110
	June	110	50	15	125	■ 250	105
	September	75	110	60	135	110	55
	October	120	80	60	45	95	35
	November	30	115	60	110	100	45
	December	80	60	■ 230	155	90	110
Median		**30**					
25th percentile		25					
75th percentile		35					
■ **Median**		**230**					
25th percentile		225					
75th percentile		255					
Median		**100**					
25th percentile		75					
75th percentile		115					

empty room for admission of new patient
■ room with patient and medical staff
room with patient

* making the bed

Table 2. Microbial air load at Transplant ICU in 2004 and 2005.

Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus haemolyticus
Staphylococcus saprophyticus
Staphylococcus capitis
Micrococcus spp.
Pseudomonas aeruginosa
Cladosporium spp.
Penicillium spp

Table 3. Spectrum of microbial findings in the air samples.

as air microflora is sometimes disputed although the sepsis induced by *Acinetobacter spp*. in connection with contaminated air conditioning was described (13). Sometimes *Pseudomonas aeruginosa* can also be part of air aerosol, e.g. in bathrooms. It seems that some microorganisms, especially Gram-negative bacteria, may be non-culturable, but can remain viable in the hospital air. The most feared bacterium that may spread in an aerogenic way and that is risky especially for immunosuprimed patients is *Legionella pneumophilla*. Its catchment in hospital environment is best in the biofilm of water distribution (tap outlets, shower heads etc.) rather than as air contaminant. That is why air contamination monitoring is not aimed at this kind of conditional pathogen (14). Also, we must not forget the occurrence of aerosol during vomiting in diarrheal diseases when not only bacteria, but also viruses may spread into the environment (12).

At the same time we have to mention an important fact: sporadic catchment of some kinds of microscopic fungi, especially *Aspergillus fumigatus*, in the air where patients in deep immunosuppression are treated can be a serious risk factor for the incidence of infectious complication, e.g. invasive aspergillosis (15, 16, 17, 18, 19). For this reason, we focused our investigation on detailed determination of microscopic fungi during one year. No isolates of *Aspergillus fumigatus* were acquired from the single room. Only *Cladosporium spp*. and *Penicillium spp*. were found in low quantities ranging from 2 to 26 $CFU.m^{-3}$ (11).

Aeroscopic investigation of air cleanness suitably completes the results of other controls carried out in hospital hygiene in order to monitor the cleanness and observance of aseptic regimes. Not only proper choice of cultivating media, but also the method and duration of air sampling are important while monitoring the microbial air contamination (20).

Various types of aeroscopes are suitable for detection of air contamination. In our study we used the aeroscope Biotest RCS Plus which acquitted well owing to simple transport and easy manipulation.

CONCLUSION

The range of microbial air load at the Transplant ICU depended on treatment activities in the rooms. No pathogenic germs were found in the rooms during the 2-year monitoring. The strict aseptic regimen during the patient`s hospitalization and the properly working filter ventilation system can represent very efficient preventive measures to reduce the risk of airborne infections.

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