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MICROBIOLOGICAL AIR CONTAMINATION IN PREMISES OF THE PRIMARY HEALTH-CARE

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Abstract: The aim of this research was to evaluate the microbiological indoor air contamination level in chosen facilities of the primary health-care for adults and children. The total numbers of mesophilic bacteria, staphylococci, coli-group bacteria and moulds in both surgery rooms and patients' waiting rooms were determined. Air samples were collected with a MAS 100 impactor and the concentration of microorganisms was estimated by a culture method. The microbiological air contamination level was diverse: the number of mesophilic bacteria ranged from 320 to 560 CFU/m^3, number of staphylococci – 10–305 CFU/m^3, coli group bacteria – 0–15 CFU/m^3 and moulds – 15–35 CFU/m^3. The bacteriological contamination level of the air in examined community health centers was higher than described in the literature for hospitals and exceeded the acceptable values proposed for the surgery objects.

INTRODUCTION

The indoor air quality monitoring is an important factor [6], especially in the case of health-care premises. Patients are one of the sources of microbial contamination in health-care premises, and they may increase the threat of air pollution with potentially pathogenic bacteria and fungi. The additional problem is the appearance of microorganisms of modified properties, mainly antibiotic resistant bacteria.

It was revealed that the nasal mucus or saliva may contain up to 10^7 of microorganisms in 1 ml, which are spread in the air by sneezing, coughing and talking [15]. Coughing causes the appearance of 3,000 droplets and sneezing – about 40,000 droplets, in the range of 0.5–5 μm [3]. During the normal breathing, these droplets are spread in the indoor air within a distance of 1 m [25].

The infection dose of some microorganisms is extremely low, for example, only a few cells of bacteria *Francisella tularensis,* or *Mycobacterium tuberculosis,* transferred by the air, may cause an infection [3]. The important factor is the lowered patients' resistance. Such people are not only more susceptible to potential infections but also may serve as an additional source of microbial emission, due to decreased control of immunological system

of the organism [25]. A problem related to the health conditions of the medical staff is also observed. According to Cole and Cook [3], 31% of intensive healthcare workers were infected after 5-day contact with non-diagnosed tuberculosis patient.

The additional sources of the secondary potentially pathogenic microbial air contamination are surfaces, clothes and the equipment in the health-care premises [11].

The spreading of infections through the air is influenced by their origin, aerodynamic properties, ability to survive and virulence [3]. Wan *et al*. [26] and Dascalaki *et al*. [4] pointed out the role of the ventilating system and medical procedures in the microbial transfer in an hospital environment.

Microorganisms present in hospital facilities are very diverse. The most frequently isolated bacteria of surgery rooms belonged to genera: *Micrococcus, Sarcina, Staphylococcus, Enterococcus, Bacillus, Corynebacterium, Brevibacterium, Legionella, Alcaligenes, Achromobacter, Flavobacterium, Pseudomonas, Serratia, Klebsiella, Escherichia*; moulds were represented by genera *Aspergillus, Penicillium, Fusarium, Cladosporium, Alternaria, Stachybotrys, Rhizopus, Mucor* and yeasts – *Candida, Rhodotorula* and *Torulopsis* [15]. Bacteria from genera *Staphylococcus, Micrococcus* and *Bacillus* were also observed by Shintani *et al*. [21] and Sudharsanam *et al*. [24] who examined the air samples taken in hospitals in India and found bacteria of genera: *Staphylococcus, Micrococcus, Klebsiella, Pseudomonas* and moulds: *Aspergillus niger* and *A. flavus*. Łebkowska [14] cites the results of the research of Holzheimer *et al.*, revealing that the most frequent reason of hospital infections are bacteria *Escherichia coli, Enterococcus faecalis*, coagulase-negative staphylococci, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp* and *Proteus spp.*

The most frequently published data of the microbiological air quality in health-care objects regard the research carried out in hospitals, mainly in operating rooms and various types of rooms classified as "clean", [1], [2], [4], [9], [10], [11], [15], [16], [24]. There is lack of precise information concerning the microbiological air contamination level in the premises of the basic, primary health-care such as community health centers, while the potential impact on the human health in the case of such objects is comparable.

The aim of this research work was the evaluation of the microbiological contamination level of the indoor air in chosen premises of the primary health-care for adults and children: patients' waiting rooms and surgeries.

SAMPLING AND ISOLATION PROCEDURE

The research was carried out in autumn (November), in selected community health centers in Warsaw. Two waiting rooms as well as two surgeries were examined. The rooms were equipped with the gravity ventilation system. In waiting rooms samples were taken while about 10 patients were present. The surgeries were examined after the end of work. Parameters such as relative humidity and temperature were determined with portable thermometer-hygrometer LB-705 (LAB-EL, Poland).

Air samples (100 dm^3 in volume) were taken using MAS 100 sampler (Merck), a 400-hole single stage impactor, corresponding to the 5^{th} stage of the Andersen air sampler, which guarantees that all particles ≥ 1 μm were collected. After sampling Petri dishes were transported to the laboratory at 4°C and incubated. Microbiological culture media and culture conditions were applied as presented in Table 1.

The number of colonies were counted after incubation. The number of microorganisms obtained were converted by the "positive hole correction" method, according to the conversion table attached to the impactor.

Table 1. Culture conditions for microorganisms

Microorganisms	Culture medium	Incubation conditions	
		Temperature (°C)	Time (h)
Total number of mesophilic bacteria	Nutrient agar (Merck)	37	48
Number of mannitol-positive staphylococci	Chapman agar (BTL)	37	48
Number of coli group bacteria	Endo agar (Merck)	37	48
Number of moulds	Rose Bengal Chloramphenicol (RBC) Agar (Merck)	26	7 days

Air sampling was repeated twice at each place. The average number of bacteria and fungi was calculated as colony-forming units in 1 m^3 of the air (CFU/m^3).

Based on morphological criteria and microscopic analysis, the preliminary identification of moulds was accomplished [7], [20], [23].

RESULTS

During the sampling the temperature of the air was 20 ± 2°C and the relative air humidity was 48–50% . Such conditions are considered as facilitating the growth of bacteria and fungi [19].

The numbers of particular groups of microorganisms are presented in Figs 1–4. Error bars show standard deviation.

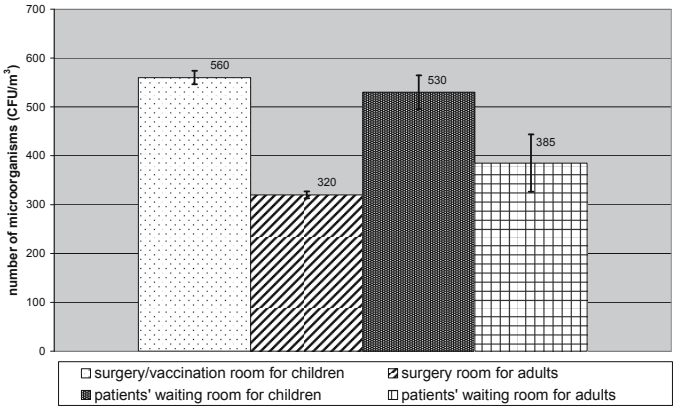

Fig. 1. Number of mesophilic bacteria in health care facilities

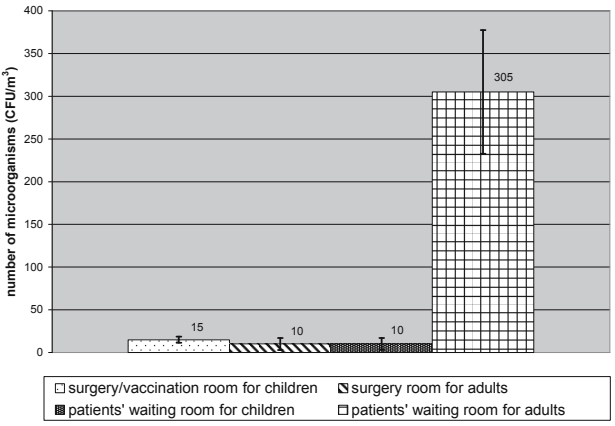

Fig. 2. Number of staphylococci in health care facilities

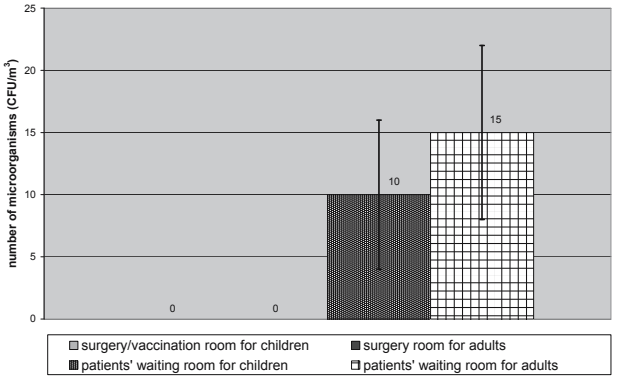

Fig. 3. Number of coli group bacteria in health care facilities

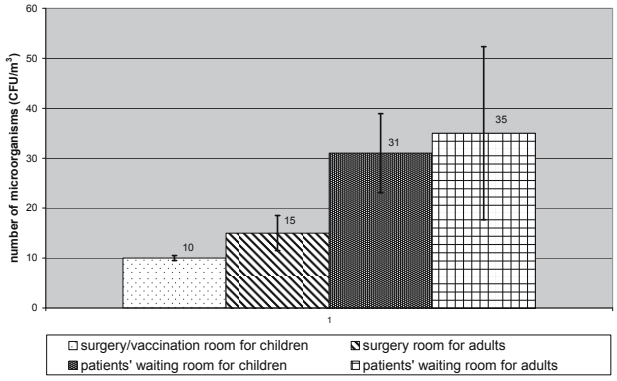

Fig. 4. Number of moulds in heath care facilities

The total number of bacteria growing at 37°C (mesophilic bacteria) ranged from 320 CFU/m^3 in adults' surgery to do 560 CFU/m^3 in children's surgery/vaccination room. The observed microbiological contamination level of health-care premises for children was significantly higher than in rooms for adult patients.

The highest number of staphylococci (305 CFU/m^3) was detected in adults' waiting room. In the case of other sampling places the concentration of these microorganisms did not exceed 15 CFU/m^3. Several coli group bacteria were detected in air samples taken in patients' waiting rooms, but not in surgeries.

The number of moulds in the air did not exceed 35 CFU/m^3, the highest value was observed in adult patients' waiting room.

The preliminary microscopic analysis revealed the predomination of moulds belonging to genera: *Aspergillus, Penicillium* and *Cladosporium* in the air of tested rooms.

DISCUSSION AND CONCLUSIONS

The literature data prove that the air of health-care facilities may be heavily contaminated with microorganisms. Augustowska and Dutkiewicz [1] stated that average monthly concentration of bacteria in hospital rooms was about 257.1–436.3 CFU/m^3, and in the case of fungi 9.9–96 CFU/m^3. Gram-positive cocci were the most commonly detected bacteria (about 46% of the total number of microorganisms). The concentration of microorganisms in pediatric hospital rooms according to Li and Hou [12] did not exceed 160 CFU/m^3 for bacteria and 260 CFU/m^3 for fungi. In most cases the observed number of bacteria was in the range of 35–55 CFU/m^3 and of fungi – 13–53 CFU/m^3. The research of Klánová and Hollerová [10] in pediatric hospital revealed the total number of bacteria in infectious patients' rooms about 40–55 CFU/m^3, and in non-infectious patients' rooms – 65–100 CFU/m^3, while the number of staphylococci was 5–55 CFU/m^3 and 35–70 CFU/m^3, respectively.

The results obtained in this research work, in premises of the primary health-care are similar to the literature data regarding moulds, but the number of bacteria was comparatively higher. It should be stressed that in the study the air contamination with mesophilic bacteria, informing about the presence of potentially pathogenic strains, was determined. The concentration of mannitol-positive staphylococci in one of the waiting rooms was several times higher comparing with the results obtained by Klánová and Hollerová [10].

Among fungi isolated from the air samples of tested rooms moulds belonging to genera *Aspergillus, Penicillium* and *Cladosporium* predominated, what is similar to the literature data. According to Augustowska and Dutkiewicz [1] the mould *Aspergillus fumigatus* was the predominating fungal strain in hospitals. The research of Li and Hou [12] revealed that in the case of pediatric health-care rooms, the most frequently occurring moulds were those belonging to genus *Penicillium*. Perdelli *et al*. [16] stated that *Aspergillus fumigatus* and *Aspergillus flavus* were the most common reason of the aspergillosis disease in hospitals. Pathogenicity of moulds is a consequence of synthesis of mycotoxins – secondary metabolites of low molecular weight (below 1000 daltons). Mycotoxins are usually present in spores, sometimes in hyphae or phialides. They cause irritation of eye, nose and mouth mucous membranes, acute or chronic damages of respiratory system (bronchitis, allergic alveolitis, lung mycotoxicosis) or cancerogenic

effects. Certain genera produce 3–8 different mycotoxins. *Cladosporium* genus fungi are mostly regarded as phylloplane organisms but certain species produce toxic substances – harmful to birds, horses and, occasionally, to humans [13]. Moreover, it was proved that the dose of mycotoxin required to cause health effect is usually one order of magnitude lower when taken by the airways than by digestive system [17]. It is suggested that about 30% of air quality-related problems are caused by moulds [6]. Realizing the threat WHO has concluded that the indoor air cannot contain any fungi that produce toxins and proposed 150 cfu/m^3 as a limit value if different mould species were identified [8]. Taking above into account, not only quantitative but also qualitative analysis of moulds in indoor air should be carried out.

The studies revealed the differences in microbiological air contamination level, which were proved by statistical analysis of research results (Table 2).

Table 2. Statistical analysis of data concerning the number of chosen groups of microorganisms isolated from the air of the examined health-care facilities

Microorganisms	Average number CFU/m^3	Standard deviation	Variation coefficient (%)
Mesophilic bacteria	$(4.5\pm0.7)\times10^2$	1.2×10^2	25.0
Staphylococci	$(8.5\pm8.7)\times10^1$	1.5×10^2	172.0
Moulds	$(2.1\pm0.6)\times10^1$	1.1×10^1	52.2

In Poland there are no regulations concerning the acceptable level of microorganisms concentration in indoor air. However, according to the instructions for hospital projects [19], three classes of hospital rooms, depending on the microbiological air quality, were distinguished:

I class – highest aseptic level – up to 70 bacterial cells/m^3

II class – low bacterial concentration level – up to 300 bacterial cells/m^3

III class – normal bacterial concentration level – up to 700 bacterial cells/m^3.

Górny [8] describes some proposals of microbiological air quality standards: Topley's, with the highest acceptable concentration of microorganisms in surgeries including both bacteria and fungi of 700 CFU/m^3 and Rabino's, which defines the air quality as "good" if the CFU number does not exceed 125/m^3. According to American Conference of Governmental Industrial Hygienists the concentration of microorganisms in rooms of advanced cleanliness may be stated as low if it is < 100 CFU/m^3 [8]. Italian Health Institute classifies clean rooms into three classes with maximum value of 500 CFU/m^3 in C class, 200 CFU/m^3 in B class and 10 CFU/m^3 in A class room [8].

The primary health-care facilities examined in this research work belong to the III class of hospital projects classification [19] but the microbiological air contamination was too high comparing with the acceptable values of other cited standards. Although, we should be aware of the fact that the values were stated for the typical hospital/clean rooms. The Topley's proposal for the surgeries was not exceeded [8]. However, because of the fact that two of rooms were located in a children health-care centre, the air quality should fit the higher quality standards. Moreover, the level of bacteriological air contamination

in the examined health centers was higher than described in the literature concerning the hospitals.

It should be stressed that most of airborne bacteria are difficult to detect using culture methods. Therefore, a real danger connected with the microbiological air contamination is probably significantly higher [2]. According to this, it is suggested that some estimation methods based on molecular biology would be useful for the determination of indoor air quality. Aerosol and bioaerosol measurements in determination of acceptability of indoor air quality may be also accompanied by other methods like measurements of CO_2 concentration [18], volatile organic compounds [5] or dust particles [27]. Some mathematical models are also useful to predict a propagation of air contaminants [22].

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OCENA POZIOMU ZANIECZYSZCZENIA MIKROBIOLOGICZNEGOPOWIETRZA W WYBRANYCH POMIESZCZENIACH PLACÓWKI PODSTAWOWEJ OPIEKI ZDROWOTNEJ

Przedmiotem pracy była analiza liczebności mikroorganizmów w wytypowanych pomieszczeniach służby zdrowia w placówce podstawowej opieki zdrowotnej. Zakres badań obejmował określenie stężenia bakterii mezofilnych, gronkowców, bakterii grupy coli oraz grzybów pleśniowych w powietrzu poczekalni dla chorych, gabinetu lekarskiego oraz punktu szczepień. Próbki pobierano za pomocą aparatu MAS 100 firmy Merck a liczebność mikroorganizmów szacowano metodą hodowlaną. Stwierdzono, iż poziom zanieczyszczenia mikrobiologicznego powietrza badanych pomieszczeń był zróżnicowany. Liczebność bakterii mezofilnych kształtowała się na poziomie 320–560 JTK/m^3, gronkowców – 10–305 JTK/m^3, bakterii grupy coli – 0–15 JTK/m^3 a grzybów pleśniowych – 15–35 JTK/m^3. Poziom zanieczyszczenia bakteriologicznego w badanej placówce opieki zdrowotnej był wyższy niż ten dopuszczalny dla pomieszczeń szpitalnych w tym sal operacyjnych.