

Fungal contamination of ward furnishings and medical equipment used in the treatment and nursing of newborns

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Abstract

Introduction and objective. Newborn babies staying on hospital wards are likely to be colonized by microorganisms, including potentially pathogenic fungi. The aim of the study was to assess the mycological purity of hospital wards and medical equipment utilized in the treatment and nursing of newborns.

Materials and method. The study was conducted in Neonatal High Dependency Units (NHDU) and Neonatal Intensive Care Units (NICU). 539 samples were collected from 24 different sources, 130 from ward furnishings and 289 from medical equipment. The study was carried out following the microbiology research methods for sample collection. Subsequently, the samples (swabs, water from incubators, washings from respirator tubes and nasal cannulas (nCPAP)) were cultivated on Sabouraud agar plates. The stamps were collected with the application of Count-Tact method. The samples were incubated at the temperature of 25+/-2°C and the number of fungi assessed (cfu/cm² of the surface area). The species were identified based on their morphological and biochemical features.

Results. Fungal growth was observed on 60% of samples collected from ward furnishings and 7% of samples collected from medical equipment. The average number of cfu/cm² ranged between 0–8.84 in the case of ward furnishings and between 0–1.22 cfu/cm² in the case of medical equipment. In 180 samples collected from the material which had direct contact with newborns no fungal growth was observed.

Conclusions. The furnishings of the wards on which newborns were treated and nursed were contaminated with fungi to an extent which did not pose a threat to the life and health of the newborns. Medical equipment (respirators, incubators, nCPAP cannulas and masks) which came into direct contact with newborns was free from fungi.

Key words

airborne microorganisms, fungal, medical equipment

INTRODUCTION

The most frequent mould fungi which can be found in the indoor air, including the air of hospital rooms, belong to the *Penicillium* and *Aspergillus* genera. The latter are believed to be the most important etiological factor of hospital infections caused by mould fungi [1]. Reservoirs and sources of fungi in the hospital environment include ventilation systems, plumbing systems, hospital food and also medical equipment and bed linen [2–10]. Medical equipment in hospital rooms might pose a significant microbiological risk. It is particularly true about devices with interior, usually inaccessible, cooling systems (angiograms, portable X-ray machines). Other devices, such as respirators, electronic visual displays or computer equipment cumulate a massive amount of dust which might also contain fungal spores, and consequently, they become a potential source of infections. The most

dangerous parts of medical equipment are those that cannot be disinfected on a daily basis and are exposed to water and higher temperatures. Medical publications show a clear correlation between the presence of airborne fungal spores on medical equipment and in the air of hospital rooms, and the incidence of exogenous infections [1, 4, 9, 11].

Hospital rooms which require the highest standards of microbiological purity include the rooms of the Neonatal Pathology Unit, especially those where premature infants are staying. Therefore, it is essential to ensure that the medical equipment and furnishings of the wards where newborns are staying are not colonized or contaminated by fungi [12].

Generalized fungal infection in very low birth weight newborns (VLBW) is a serious form of hospital infection. According to the latest data connected with the advanced form of fungal sepsis and collected by Neonatal Research Network at the National Institute of Child Health and Human Development, as much as 9% of 1,515 examined newborns with a birth weight below 1,000 g developed a fungal sepsis or fungal cerebrospinal meningitis caused by *Candida albicans* or *Candida parapsilosis*. About 1/3 of these newborns died. Acute invasive fungal infections are connected with impaired

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neurological development and a frequent incidence of the threshold stage of retinopathy of prematurity, compared to a control group of VLBW newborns. Colonization of the digestive system in very low birth weight newborns is frequently preceded by invasive infections, and risk factors responsible for colonization and invasive infection are the same [13].

OBJECTIUE

The study aimed to assess the fungal contamination of hospital ward furnishings and medical equipment utilized in the treatment and nursing of newborns.

MATERIALS AND METHOD

Assessment of the fungal contamination of medical equipment used for newborn's treatment and furnishings of the wards where newborns are staying was conducted in two hospital wards situated in two different hospitals in Krakow: the Neonatal High Dependency Unit (NHDU) and the Neonatal Intensive Care Unit (NICU). 539 samples were collected from 24 different locations in the examined rooms. The material from medical equipment and ward furnishings, as well as from the palms of nurses, was collected following microbiology rules during one week (5 days) twice a day (morning and evening) from each examined site by means of the Count-Tact (Merck) technique. In the case of the samples collected from medical equipment situated in the NICU ward – incubators, respirators, nCPAP cannulas and masks – the material was collected during 7 months. The samples were collected according to the NICU ward rules and epidemiological procedures for medical equipment applied in the treatment of newborns. The examined material in this case was usually disposable and was replaced and reprocessed after examination. The locations from which the samples were collected are listed in Table 1.

The study was carried out in the rooms where newborns were cared for, the cubic area of which was about 15–18 m² and were usually equipped with 3–5 newborn nursing stations. The rooms were air-conditioned following the manufacturers' recommendations and according to the needs connected with the nursing and medical treatment on a particular hospital ward. When the study was conducted, 2–3 people usually stayed in the particular room looking after newborns. When the samples were collected, the doors leading to the rooms were closed.

The period of time from collecting the material until processing the sample for examination did not exceed one hour (especially in the case of water samples, nasal cannulas and masks, parts of breathing tubes collected from respirators and nCPAP devices).

Fungal colonization of nasal cannulas and masks, parts of breathing tubes and water samples collected from incubator humidifiers was assessed quantitatively by growing fungal cultures from the washings obtained from these elements on solid Sabouraud medium (Merck). The same type of medium was used for growing fungal cultures from swabs taken from connectors of incubator humidifiers. The material was collected by means of the Count-Tact (Merck) method following the manufacturer's recommendations.

Microbial colonization of nasal cannulas and masks was conducted by means of a quantitative study. 1 ml of 0.9% NaCl was shaken for a few minutes with a nasal cannula/mask in a sterile container, and subsequently 100 µl (0.1 ml) samples were cultured on agar plates stimulating fungal growth. In the case of breathing tubes, 2 cm pieces were cut off with sterile scissors and placed in a sterile test tube containing 2 ml of 0.9% NaCl. The tube was then rinsed by shaking for a few minutes in a vortex mixer, and 100 µl (0.1 ml) samples were cultured on agar plates. The procedure was similar in the case of water samples from incubator humidifiers.

From the collected capacity of 2 ml, 100 µl samples were cultured on Agar plates which were incubated at the temperature of 27 °C for 14 days, and the growth of microorganisms s monitored periodically. The first reading took place after 72 hours. After 3 days of incubation, the first quantitative and qualitative assessments was conducted. The colonies grown on the plates were counted and their number on one square centimeter was calculated using a formula: $X = a/\Pi r^2$ where a – number of fungal colonies on the sampling plate, r – radius of the plate in centimeters. The findings were expressed in terms of the number of colony forming units on one square centimeter (cfu×cm⁻²). The cultured fungi were identified according to routine mycological diagnostics procedures. The moulds were evaluated macroscopically and microscopically on the basis of the colony appearance and morphological features in the direct preparation stained with lactophenol and methylene blue (Merck). In doubtful cases, slide microcultures were made, and the preparations made were identified. The species were identified by means of identification keys, taking into account their morphology and the way in which their spores were produced [14, 15]. Yeast-like fungi were identified with the application of API 20 C AUX test [14, 15], following the manufacturer's recommendations.

To describe the material collected from the environment, description statistics were determined: minimum, maximum, median, arithmetical mean and standard deviation. χ^2 test was used in statistical analysis and the level of significance was assumed at p<0.05.

RESULTS

Fungi were detected in 167 of 539 (31%) samples: in 145 of 240 stamps collected from ward furnishings (72.5%) and 22 of 299 samples collected from medical equipment (7.4%). The differences in the frequency of fungi isolation were statistically significant (χ^2 test p<2.2E-16) (Fig. 1).

The average number of fungi detected on the furnishings of NHDU ward and on the palms of nurses who worked there ranged from 0–8.84 cfu × cm⁻² of the surface. On the other hand, in 130 materials collected from the furnishings of NICU ward, fungi were detected in the number ranging from 0–1.22 cfu × cm⁻² of the surface. No fungal growth was identified in any sample collected from the equipment such as baby scales on the NICU ward. Moreover, in the samples collected from most locations of both wards, fungal growth was observed only in some part of the samples.

The locations which were the most contaminated with fungal spores and fungal growth were observed in all collected samples included the floor in NHDU and the shelves above the incubator in NICU (median of the number of colonies

Table 1. Sample collection locations and the number of samples collected from the surface and medical equipment of the two examined wards

Lp. Sample collection location	NHDU		NICU		Total
	Surface	Medical equipment	Surface	Medical equipment	
	N	N	N	N	
1 Treatment table/food preparation table	10	-	10	-	20
2 Baby scales	10	-	10	-	20
3 Baby changing mat	10	-	10	-	20
4 Incubator – interior wall	-	10	-	40	50
5 Incubator – exterior wall	-	-	-	20	20
6 Baby bathtub	10	-	-	-	10
7 Washbasin – interior part	10	-	10	-	20
8 Washbasin tap	10	-	-	-	10
9 Wall over washbasin	10	-	20	-	30
10 Wall over incubator/respirator	10	-	10	-	20
11 Floor in the middle of the room	10	-	20	-	30
12 Surface of nurse's palm	20	-	-	-	20
13 Respirator screen	-	-	-	10	10
14 Door handles	-	-	10	-	10
15 Shelf over incubator	-	-	10	-	10
16 Surface of nurses' desk	-	-	10	-	10
17 Surface of computer terminal	-	-	10	-	10
18 Incubator mattress	-	-	-	34	34
19 Water from incubator humidifier	-	-	-	35	35
20 Swabs from tubes supplying water to incubator humidifier	-	-	-	5	5
21 CPAP breathing tubes	-	-	-	13	13
22 Mechanical respirator breathing tubes	-	-	-	19	19
23 NCPAP nasal cannulas	-	-	-	108	108
24 NCPAP masks	-	-	-	5	5
Total	110	10	130	289	539

N – number of collected samples; NHDU – Neonatal High Dependency Unit; NICU Neonatal Intensive Care Unit

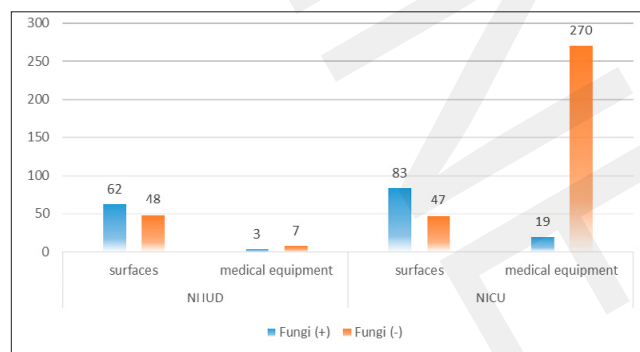


Figure 1. The number of materials (surfaces, medical equipment) in which fungi were detected.

NHDU – Neonatal High Dependency Unit; NICU – Neonatal Intensive Care Unit

per cm² of the surface were 0.33 cfu/cm² vs 0.34 cfu/cm², respectively. The cleanest surfaces (where fungi were not identified at all) were: on the NHDU ward the wall over the incubator, the wall over the washbasin and the surface of the palms of nurses who were working there, whereas on the NICU ward the cleanest areas were baby scales and the wall over the washbasin (medians of the number of colonies per cm² of the surface were 0). Further detailed data shown in Table 2.

Figure 2 shows the medians of the numbers of colony-forming units (cfu) detected on the furnishings of the NHDU

and NICU wards. The values were ranked from the lowest to the highest medians, calculated for the fungal colony-forming units. The highest growth of fungi was observed on the washbasin and the floor of the NHDU ward and the mattress, wall and shelf over the incubator on the NICU ward.

A total of 299 samples was collected from medical equipment on the NHDU and NICU wards (Tab. 3). It is particularly interesting that no fungal growth was found in the materials collected from CPAP breathing tubes, mechanical respirator breathing tubes, nCPAP nasal cannulas, nCPAP masks, and the samples of water from respirator humidifiers – a total of 180 samples.

The highest number of fungi by far was detected on the stamps collected from the respirator screen (max = 1.22 cfu × cm²) and the swabs from tubes supplying water to the respirator humidifier (max = 1.0 cfu × cm²).

In the study, as many as 281 different fungi were isolated and classified as 17 genera: 3 yeast-like fungi and 15 mould fungi. Figure 3 shows the percentage distribution of genera and/or species of fungi isolated from all examined locations.

In the examined material, the dominant fungi belonged to the following genera: *Penicillium* (23.5%), *Cladosporium* (19.2%) and *Aspergillus* (14.6%). Also, yeast-like fungi belonging to *Candida* sp. genera made up a significant percentage – 10.3% (Fig. 3). Some fungi were isolated only from single samples collected from a particular site or item of

Table 2. Numbers of colony-forming units (CFU) detected on the examined surfaces on NHUD and NICU wards including the number of samples, average values and measures of statistical dispersion

Lp.	Location of stamp collection	Numbers of fungal colony-forming units (CFU)* obtained from stamps collected from examined surfaces											
		NHUD*					NICU						
		N	Min	Max	Average	Median	SD	N	Min	Max	Average	Median	SD
1	Treatment table /food preparation table	10	0	0.63	0.138	0.04	0.216	10	0	0.37	0.082	0.04	0.121
2	Baby scales	10	0	0.46	0.112	0.04	0.141	10	0	0	0	0	0
3	Baby changing mat	10	0	3.7	0.407	0.04	1.158	10	0	0.84	0.288	0.255	0.251
4	Baby bathtub	10	0	3.53	0.895	0.085	1.385	-	-	-	-	-	-
5	Washbasin – interior part	10	0	1.68	0.409	0.275	0.496	10	0	0.25	0.083	0.08	0.074
6	Washbasin tap	10	0	8.84	0.985	0.105	2.762	-	-	-	-	-	-
7	Wall over washbasin	10	0	0.21	0.021	0	0.066	20	0	1.22	0.079	0	0.270
8	Wall over incubator/respirator	10	0	0.5	0.066	0	0.155	10	0	0.55	0.308	0.32	0.152
9	Floor in the middle of the room	10	0.08	1.68	0.517	0.335	0.488	20	0	0.37	0.108	0.06	0.1234
10	Surface of nurse's palm	20	0	0.13	0.021	0	0.043	-	-	-	-	-	-
11	Door handles	-	-	-	-	-	-	10	0	0.13	0.017	0	0.042
12	Shelf over incubator	-	-	-	-	-	-	10	0.08	0.5	0.303	0.34	0.137
13	Surface of nurses' desk	-	-	-	-	-	-	10	0	1.13	0.15	0.04	0.346
14	Surface of computer terminal	-	-	-	-	-	-	10	0	0.21	0.08	0.06	0.076
	Min	10	0	0.13	0.021	0	0.043	10	0	0	0	0	0
	Max	20	0.08	8.84	0.985	0.335	2.762	20	0.08	1.22	0.308	0.34	0.346
	Total	110						130					

* Values presented in cfu per cm² of the surface

N – Number of collected samples; SD – standard deviation.

Table 3. Numbers of fungal colony-forming units (CFU) identified on medical equipment of NHUD and NICU wards including number of samples, average values and measures of statistical dispersion.

Ward	Sample collection location	Numbers of fungal colony-forming units (CFU)* obtained from materials collected from medical equipment					
		N	Min	Max	average	Median	SD
NHUD	Incubator – interior wall	10	0	0.08	0.016	0	0.028
	Incubator – interior wall	40	0	1.0	0.025	0	0.160
	Incubator – exterior wall	20	0	0.17	0.037	0	0.058
	Respirator screen	10	0	1.22	0.181	0.06	0.372
	Incubator mattress	34	0	1.0	0.029	0	0.174
	Water from incubator humidifier	35	0	0	0	0	0
NICU	Swabs from tubes supplying water to incubator humidifier	5	0	1.0	0.6	1.0	0.547
	nCPAP breathing tubes	13	0	0	0	0	0
	Mechanical respirator breathing tubes	19	0	0	0	0	0
	nCPAP nasal cannulas	108	0	0	0	0	0
	nCPAP breathing masks	5	0	0	0	0	0
	Min.	5	0	0	0	0	0
	Max.	108	0	1.22	0.6	1.0	0.547
	Total	299					

medical equipment. No fungi were isolated from 35 samples of water collected from incubator humidifiers, whereas from 5 swabs collected from the tubes supplying water to the respirator humidifiers, mould fungi belonging to *Penicillium* sp. and *Paecilomyces* sp. genera were grown in 3 cases. The

least varied mycobiota was found on the materials collected from door handles and mattresses inside the incubators, of which the most varied was observed on the materials collected from the floor, washbasins and baby changing mats. Unfortunately, the fungi cultures grown from the materials

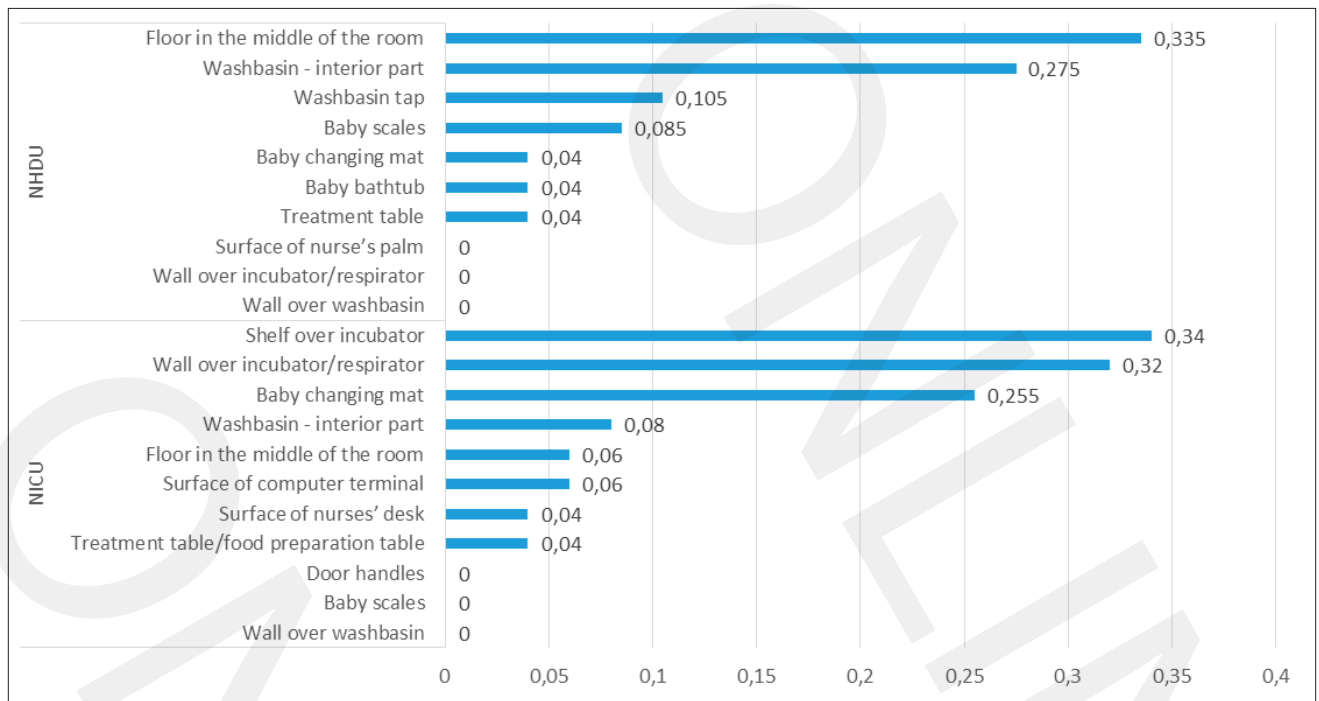


Figure 2. Medians of numbers of colony-forming units (cfu) obtained from the stamps collected from the examined surfaces of NHDU and NICU. NHDU – Neonatal High Dependency Unit; NICU – Neonatal Intensive Care Unit

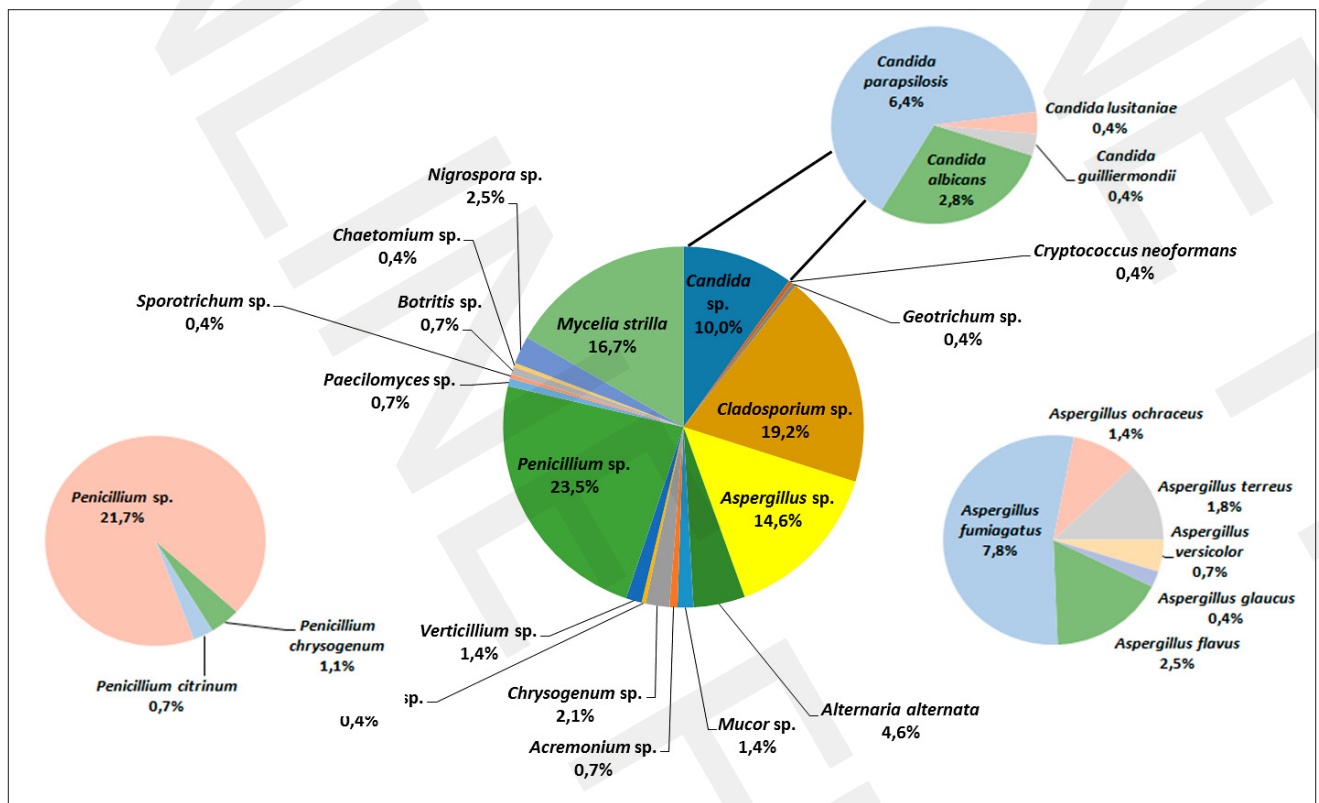


Figure 3. Percentage distribution of fungi grown from examined surfaces and medical equipment

also include those which may pose a significant threat to the health of newborns, for example, fungi belonging to *Stachybotrys*, *Botrytis* genera or *Aspergillus fumigatus* species.

DISCUSSION

Inspecting the environment of hospital rooms is one of the ways of monitoring the hygiene of this environment, which is closely connected with the control over hospital infections [16]. According to Gangneux, an optimal control over the cleanness of the rooms requires the application of efficient

devices for collecting not only air samples, but also materials from other surfaces. Gangneux does not recommend the swab method for assessing mycological contamination of the surface, except for inaccessible places, for which the stamp method is insufficient [17]. In the presented study, in order to assess the cleanliness of the hospital environment, the authors applied both the recommended Count-Tact stamp method and swab method, which were used for collecting material from inaccessible and damp surfaces, such as incubator humidifiers. Thanks to these methods, the results obtained can be considered satisfactory because fungi were detected only in 60% of the stamps collected from the furnishings, and 7% of samples collected from medical equipment. The highest observed number of fungal colony-forming units per 1 cm^{-2} of the surface was $8.84\text{ cfu}\times\text{cm}^{-2}$ in the case of furnishings (washbasin tap on the NHDU ward) and $1.22\text{ cfu}\times\text{cm}^{-2}$ in the case of medical equipment (respirator screen on the NICU ward). The highest median values for the average numbers of fungi grown on all the materials collected from ward furnishings and medical equipment reached $0.34\text{ cfu}\times\text{cm}^{-2}$ vs $1.0\text{ cfu}\times\text{cm}^{-2}$, respectively, whereas the maximum arithmetical mean for the number of fungi isolated from the samples collected from ward furnishings was $0.985\text{ cfu}\times\text{cm}^{-2}$ (washbasin tap on the NHDU ward), and in the case of medical equipment – $0.6\text{ cfu}\times\text{cm}^{-2}$ (swab from the incubator humidifier on the NICU ward). These values were usually less than $1\text{ cfu}\times\text{cm}^{-2}$, which is the value recommended by the French ASPEC Association (*Association pour la protection et l'étude de la contamination*) [18, 19]. Therefore, it can be assumed that, as far as epidemiology is concerned, the cleanliness of the environment was satisfactory, especially because no fungi at all were grown on some parts of the examined material (180 samples) collected from medical equipment. The equipment which came into direct contact with the patient (breathing tubes from respirators and nCPAP devices, nasal cannulas and masks applied in nCPAP therapy and water samples from incubator humidifiers) was also not contaminated with fungi. Such a condition proves that the procedures for treating patients with stimulated breathing are scrupulously observed. Nevertheless, further research into the contamination of medical equipment needs to be carried out in order to control contamination of incubator humidifiers. Mould fungi were observed in 3 of 5 samples collected from these devices. It should be pointed out that incubator humidifiers, depending on their type and manufacturer, can be either replaceable (they can be sterilized) or permanently fitted.

Microbial contamination of the hospital environment depends on numerous factors. No matter whether the patients staying in the hospital environment are immunocompromised or do not belong to any risk groups, hospital staff must follow strict procedures, especially those connected with hand hygiene. However, it is impossible to eliminate this way of spreading hospital infections, and the hands of the staff are still considered to be a significant risk factor [20, 21, 22]. Also, medical equipment and ward furnishings which surround every patient, including newborns, are not sterile and may be contaminated with microorganisms despite proper medical procedures and cleaning the rooms.

The study showed that lower numbers of fungi were identified on the equipment and furnishings from the NICU ward than from the NHDU ward. Obviously, these findings can be seen as satisfying, taking into consideration

the necessary procedures which must be performed on the ward where seriously immunocompromised newborns are staying, including pre-term newborns with a birth weight below 1,000g. On the other hand, the newborns staying on the NHDU ward were usually term newborns who did not require complicated medical procedures, for example, mechanical ventilation, as much as the newborns from the NICU ward.

The study should be complemented with evaluation of the ways of spreading microorganisms and evaluation of their reservoirs, indicating the sources of pathogens in particular rooms. However, it should be pointed out that moulds are spread mostly by the air, and thus the quality of the air in hospital wards has a big impact on their occurrence also on the equipment and furnishings located in those rooms [23, 24].

In this study, mould cultures were grown from the samples collected from incubator humidifiers which could not be replaced. Although these fungi belong to the low pathogenicity genera *Penicillium* sp. and *Paecilomyces* sp., they have been known to cause infections also in children [25, 26]. It is essential that all elements which might constitute a potential source and reservoir of newborns' infections should be decontaminated in order to limit colonization of these places by microorganisms. There are two goals of the microbiological control of patient's inanimate environment, one of which is monitoring hygienic standards and following epidemiological procedures, and the other is detecting specific hospital pathogens which might be a source of infections in places which might be their reservoirs [12]. Therefore, microbial contamination is a signal to take proper preventive measures against potential infection, as well as to continue research in this area. This becomes even more important as it has been confirmed that microorganisms are transmitted from one piece of equipment to another one on hands of medical staff, which may pose a risk to the health of hospitalized patients.

The numbers of fungi grown from the samples collected from furnishings of the wards on which newborns were staying resemble those obtained in research conducted by Caggiano et al. [27]. Their study involves a 3-year observation of the incidence of fungi in the operating theatre environment. The study showed that fungal growth was observed in only 29.1% of materials collected from various surfaces by means of the stamp method. The average value was $0.35\text{ cfu}\times\text{cm}^{-2}$. It was also confirmed that the dominating species in the examined samples was *Aspergillus fumigatus*. Also in the authors' own study, moulds belonging to *Aspergillus* genus made up a significant percentage of the identified fungi, including *A. fumigatus*, which was observed in over a half of the isolates of this type. The fungi belonging to *Aspergillus* genus might present a significant threat to patients' health, especially those with impaired immunity, and particularly newborns [28, 29, 30]. They may also pose a threat to the staff working in these rooms because they are included in the group of 2 biological factors which may have negative effects on people exposed to them for prolonged periods [31].

Fungal infections are more likely to affect low birth weight newborns. Children suffering from fungal infections were hospitalized longer than those suffering from bacterial infections [32, 33]. According to Groll et al., any culture positive for moulds obtained from a neonate should be considered seriously, and prompt empirical treatment should

be instituted until infection can be reliably excluded [34]. This is particularly important because 12.3% of the cases of primary aspergillosis are diagnosed in newborns [35]. British researchers confirmed, thanks to the typing based on a sequence of microsatellite regions of *A. fumigatus* isolates obtained from newborns diagnosed with cutaneous aspergillosis and environmental isolates obtained from water containers in incubators, that the examined water containers were a source of fungal infections for newborns [36]. Microsatellite typing confirmed a genotypical connection between *A. fumigatus* isolates found in newborns and the isolates grown from the samples collected from incubator humidifiers, which means that incubator humidifiers used on NICU wards may have been a source of fungal infections.

Study limitations. The authors are aware of the limitations of the presented study as far as the applied laboratory methods are concerned. No evaluation of the toxicity of metabolites produced by moulds was conducted, which could have raised the value of the study for medical practice. Another essential drawback is the limited number of samples collected for examination, especially in the group of samples collected from incubator humidifiers and/or swabs from the tubes conveying water to incubator humidifiers. This situation was caused by the different access the researchers had to different incubators in which the newborns were staying. Different construction of incubators affected access to them and the possibility of sample collection (water from incubators or swabs from the tubes). On the other hand, an advantage of this study might be the fact that it was conducted in 2 different hospital wards, using the same procedures regarding the cleaning and the frequency with which medical equipment used for newborns' treatment was exchanged.

Continuation of this study seems to be justified and should take into consideration the ways in which microorganisms are spread, including mainly following the rules of hand hygiene during medical procedures in which there is a risk of contaminating medical equipment that subsequently comes into contact with patients' skin or mucosa.

This kind of study should be published not only in the journals dealing with the clinical aspects of nursing children, but also in journals connected with health sciences and environmental engineering which deal with the presence of microorganisms in the interior air.

CONCLUSIONS

1. The furnishings of the wards on which newborns are treated and nursed were contaminated with fungi to an extent which did not pose a mycological threat to the life and health of newborns. Medical equipment (respirators, incubators, nCPAP cannulas and masks) which comes into direct contact with newborns was free from fungi.
2. Maintaining proper microbiological purity standards of hospital environment helps to significantly decrease the risk of the incidence of hospital infections.

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