

Original article

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The additional reservoir of hospital environment microorganisms at healthcare facilities

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Abstract

Introduction. Most of healthcare-associated infections (HAIs) develop through colonization of patients' loci with hospital-associated pathogenic strains.

The aim of the research is to study an additional reservoir of microorganisms at healthcare facilities.

Materials and methods. A total of 28 samples of hospital environment dust were examined by using scanning electron microscopy and dynamic light scattering, energy dispersive X-ray spectrometry and high-temperature catalytic oxidation techniques; to study microbial diversity, 97 samples were examined by using a polymerase chain reaction and a VITEK[®]2 Compact microbial identification system. Biological films ($n = 29$) were detected with a catalase indicator; the total number of microorganisms in one cubic meter of air was estimated.

Results. The dust from ventilation grilles was contaminated in 71.13% of cases; antibiotic-resistant bacterial strains were detected in 69.44% of cases; biofilm-forming bacteria were present in 48% of cases. The biodiversity is represented by 21 genera of microorganisms persisting for 6 months. All the samples contained a nanosized fraction. The organic compound is represented by carbon (16.26–50.69%), nitrogen (1.59–25.03%), hydrogen (2.03–6.67%), sulfur (0.15–2.38%), and oxygen (20.02–37.50%). The total microbial counts before and after the doors and windows were opened - 276 and 462 CFU/m³ respectively ($p = 0.046$). The mineral component contained sodium (0.07–1.86%), magnesium (0.11–1.40%), aluminum (0.36–1.78%), silica (0.21–4.64%), phosphorus (0.04–0.81%), chlorine (0.05–2.83%), potassium (0.04–0.85%), calcium (0.19–7.49%), and iron (0.08–1.61%).

Discussion. A wide range of microorganisms, the presence of an organic substrate and trace elements suggest that HAI-causing pathogens can persist, accumulate, and return to the hospital environment.

Conclusion. At healthcare facilities, exhaust ventilation and air-duct grilles can serve as an additional reservoir of microorganisms causing HAIs and can contribute significantly to persistence of a HAI epidemic process.

Keywords: *reservoir, microorganisms, dust particles, healthcare-associated infections*

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Дополнительный резервуар госпитальных микроорганизмов в медицинских организациях

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Введение. Подавляющее большинство инфекций, связанных с оказанием медицинской помощи (ИСМП), развиваются в результате колонизации локусов пациентов госпитальными штаммами возбудителей.

Цель исследования — изучение дополнительного резервуара микроорганизмов в медицинских организациях.

Материалы и методы. Исследовано 28 образцов больничной пыли методами сканирующей электронной микроскопии и динамического рассеяния света, энергодисперсионной рентгеновской спектроскопии и высокотемпературного каталитического окисления, 97 проб — полимеразной цепной реакцией и на бактериологическом анализаторе «VITEK®2 Compact» с целью исследования микробного разнообразия. Проведены индикация биологических плёнок ($n = 29$) с помощью каталазного индикатора, определение общего количества микроорганизмов в 1 м³ воздуха.

Результаты. Пыль вентиляционных решеток контаминирована в 71,13% случаев, доля резистентных к антибиотикам штаммов бактерий — в 69,44%, бактерий, образующих биопленки, — в 48%. Биоразнообразие представлено 21 родом микроорганизмов, сохранившимся в течение 6 мес. Во всех образцах присутствовала наноразмерная фракция. Органический субстрат представлен углеродом (16,26–50,69%), азотом (1,59–25,03%), водородом (2,03–6,67%), серой (0,15–2,38%) и кислородом (20,02–37,50%). Общее микробное число до и после открывания дверей и окон — 276 и 462 КОЕ/м³ соответственно ($p = 0,046$). Минеральный компонент содержал натрий (0,07–1,86%), магний (0,11–1,40%), алюминий (0,36–1,78%), кремний (0,21–4,64%), фосфор (0,04–0,81%), хлор (0,05–2,83%), калий (0,04–0,85%), кальций (0,19–7,49%), железо (0,08–1,61%).

Обсуждение. Широкий спектр микроорганизмов, наличие органического субстрата и микроэлементов свидетельствуют о возможности сохранения, накопления и возврата в больничную среду возбудителей ИСМП.

Заключение. На решетках вытяжных вентиляционных систем и прилежащих частей воздуховодов в условиях медицинских организаций может формироваться дополнительный резервуар микроорганизмов — возбудителей ИСМП, играющий значимую роль в поддержании эпидемического процесса ИСМП.

Ключевые слова: резервуар, микроорганизмы, пылевые частицы, инфекции, связанные с оказанием медицинской помощи.

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The intensive development of medical technologies is inextricably entwined with the importance of efficient control measures for preventing healthcare-associated infections (HAIs). In a present-day facility,

most (60%) of HAIs develop through colonization of patients' and healthcare workers' loci with hospital-associated pathogen strains (clones) [1]. The body of patients is the main reservoir of HAI-causing pathogens, providing selection and accumulation of epidemic vari-

ants of pathogens. In the meantime, 30% of all HAIs are caused by sapronotic pathogens with their reservoir supported by a hospital environment [2]. Such microorganisms (MOs) are characterized by high tolerance to unfavorable factors; they multiply and accumulate in water, solutions, and moist environments [3, 4]; they can persist on equipment [5] and even on dry surfaces for a long time [6]. The role of airborne dust transmission in the spread of multi-resistant MOs has been poorly studied and is controversial [7].

The aim of the work is to study an additional reservoir of MOs at healthcare facilities.

Materials and methods

The study was performed at various healthcare facilities in the Kemerovo Region: in surgery, resuscitation, pediatric care, and infectious diseases departments. Dust was collected in operating, treatment, dressing, examination rooms, and sterilization centers; intensive care units, wards of purulent, general and pediatric surgery departments, oncology, chemotherapy, pulmonary departments, divisions of pediatric infectious diseases, and the COVID-19 specialized hospital.

Dust samples ($n = 97$) were collected with sterile gloves in sterile containers from the inner side of vent grilles and adjacent air ducts of exhaust ventilation systems at different healthcare facilities.

The shape, size, and elemental composition of dust particles ($n = 28$) were examined with a Jeol JSM-6390 LA microscope (Jeol) by using scanning electron microscopy. The high-temperature catalytic oxidation technique (a CHNSO analysis) performed with a Flash 2000 elemental analyzer (ThermoScientific) was used for estimating the content of elements (C, H, N, S) in the organic matter. The average size and size distribution of dust particles in the solution ($n = 28$) were measured with a Zetasizer Nano ZS laser analyzer (Malvern Instruments) by using a dynamic light scattering technique. Prior to the photography, the examined particles were resuspended in filtered (220 nm) sterile bi-distilled water and were exposed to ultrasound for 20 min until stable disperse systems were obtained. Then, large particles were removed by filtration through a paper filter and filtering cartridges with 450 and 220 nm pore diameter. 10–50 measurements were made for each sample until at least 5 convergent results were obtained. During the measurement process, the temperature was set at 25°C (with preliminary 20-minute thermostating).

For detection of the RNA of rotaviruses group A, astroviruses, and noroviruses genotype II in all samples ($n = 97$), we used a polymerase chain reaction (PCR) with hybridization-fluorescence detection with an AmpliSensRotavirus/Norovirus/Astrovirus-FL kit (Central Research Institute of Epidemiology, Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing). The similar technique was used for identification of RNA of enteroviruses ($n = 97$,

the AmpliSens Enterovirus-FL kit), RNA of hepatitis A virus ($n = 97$, the AmpliSens HAV-FL kit), RNA of SARS-CoV-2 ($n = 22$, the RealBest RNA SARS-CoV-2, Vector-Best), and for identification of DNA of *Shigella spp.*, enteroinvasive *E. coli*, *Salmonella spp.* and thermophilic *Campylobacter spp.* ($n = 97$, AmpliSens *Shigella spp.* and EIEC/*Salmonella spp./Campylobacter spp.*-FL kits).

The authors' unique method was used to examine the bacterial composition: Dust samples ($n = 97$) were seeded in 1% sugar broth and incubated for 24 hours at 37°C. Then, the cultures were reseeded on blood agar, Candida agar, Orientation agar, and further incubated at the same temperature during the same time. For differential diagnosis of gram-negative MOs (enterobacteria and non-fermenting bacteria), the cultures were reseeded on the Kligler medium to be further grown in an incubator following the protocol. The species identification was performed with VITEK®2 Compact, a bacteriological automated analyzer (BioMerieux), and VITEK®2GN cards designed for identification of clinically significant fermenting and non-fermenting gram-negative rods and including 47 individual bacteriological tests as well as VITEK®2GP cards designed for identification of 120 gram-positive MOs. To fill out the respective instrument cards, the obtained cultures were used to prepare a suspension with an optical density of 0.50–0.63 McFarland standard in accordance with the manufacturer's instructions (BioMerieux). The antimicrobial susceptibility was tested with a VITEK®2 Compact analyzer.

The indication of biological films on surfaces of 29 grilles of exhaust ventilation systems was performed with a BFR peroxyfilm catalase indicator (BFR laboratories) in accordance with MR 4.2.0161-19, Indication Techniques for biological films of MOS on abiotic items.

The total number of MOs in one cubic meter of air (CFU/m³) was measured in different functional divisions of a healthcare facility, within 1 m from the ventilation grilles, before and after the windows and doors were opened; the measurements were made by using the aspiration technique, including the Flora-100 microbial air sampler ($n = 18$). The specified air volume was 250 dm³.

The results were statistically processed with the GraphPad Prism7 program (GraphPad Software). Two independent groups were compared by using the Mann-Whitney U test. The differences between the variables were assessed by using the Wilcoxon test, χ^2 at the confidence parameter $p < 0.05$.

Results

It was found that 69 dust samples contained MOs (71.13%). Bacteria (68.04%) were identified in 66 samples out of 97 samples; viruses (13.4%) were identified in 13 samples. 11 samples (the sixth of all

MO-containing samples) had both viruses and bacteria (11.3%). Only 5 samples had mold fungi (5.15%). The bacterial diversity is represented by 21 genera and significant prevalence of gram-negative bacteria (76.74%) over gram-positive (23.26%) bacteria; the examination detected 69.44% of antibiotic-resistant strains by the bacterial composition; development of biofilms was detected in 48% of cases. Sporous types of bacteria accounted for 41.1%. The examination identified *Staphylococcus pseudintermedius*, *Staphylococcus hominis ssp. hominis*, *Micrococcus spp.*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus durans*, *Moraxella lacunata*, *Raoultella ornithinolytica*, *Rhizobium radiobacter*, *Klebsiella pneumoniae*, *Kluyvera intermedia*, *Pantoea*, *Pasteurella canis*, *Pasteurella testudinis*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, *Aeromonas sobria*, *Sphingomonas paucimobilis*, *Brevundimonas diminuta*, *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Shewanella putrefaciens*, *Serratia plymuthica*, *Bordetella bronchiseptica*, *Salmonella spp.*, *Campylobacter spp.*, *Chromobacterium violaceum*, *Cronobacter dublinensis*. The most frequent dust contaminants were *Enterococcus spp.* (16.28%). Bacteria of the sapronoses group accounted for 51.16%; *Sphingomonas paucimobilis* (13.96%) and *Acinetobacter spp.* (6.98%) demonstrated the highest occurrence.

The RNA of rotaviruses A group was detected in 13 (13.40%) dust samples out of 97 samples; the RNA of noroviruses genotype 2 was found in 3 (3.09%) samples. Viruses were identified at pediatric in-patient facilities ($p = 0.0004$). SARS-CoV-2 RNAs were not detected in any of the examined samples ($n = 22$) collected at the COVID-19 hospital and at non-infectious healthcare facilities having the imported cases of COVID-19.

The re-examination (in 6 months) of the microflora of the inner surface of identical ventilation grilles and adjacent air ducts detected bacteria in all cases, though no presence of viral nucleic acids was detected.

It was found that the total microbial count during the window and door opening was 20-736 CFU/m³; the mean values before and after windows and doors were opened amounted to 276 and 462, respectively. The total microbial count during the window and door opening changed from a few numbers to 10 times, $p = 0.046$ (Fig. 1).

In surgery departments, the occurrence of bacteria in dust was 4 times as high as in non-surgery departments ($p = 0.0001$), while no significant differences in the occurrence of duct contaminated with viruses were found ($p = 0.361$).

The dust in medical technology areas was contaminated with MOs 2.45 times more rarely than the dust in patients' area (wards), $p = 0.0001$.

The examined samples had two morphological types of dust (Fig. 2): Those with globular particles

(60.71%) and micro-sized fibers (39.29%) regardless of the specialization of departments and the location of healthcare facilities. Although the occurrence rate of MO contamination of dust with micro-sized fibers is higher (OR = 1.78 [0.23–15.06]; $\chi^2 = 0.431$; $p = 0.512$), its statistical significance was not confirmed.

All the samples had a nanosized fraction of dust. The dependence between sizes of dust particles and department specialization has not been found.

The examination confirmed the presence of an organic substrate in the samples; the proportions of elements were as follows: carbon (16.26–50.69%), nitrogen (1.59–25.03%), hydrogen (2.03–6.67%), sulfur (0.15–2.38%) and oxygen (20.02–37.50%); the dust collected in resuscitation and pediatric care departments contained a higher weight percentage of nitrogen than the dust collected in surgery departments ($p = 0.003$). The dust collected in wards contained a higher weight percentage of nitrogen than the dust collected in operating rooms ($p = 0.05$; Fig. 3).

The examination of the mineral constituent of dust samples revealed the presence of sodium (0.07–1.86%), magnesium (0.11–1.40%), aluminum (0.36–1.78%), silica (0.21–4.64%), phosphorus (0.04–0.81%), sulfur (0.19–2.58%), chlorine (0.05–2.83%), potassium (0.04–0.85%), calcium (0.19–7.49%), and iron (0.08–1.61%). A high weight percentage of potassium ($p = 0.05$) and carbon ($p = 0.02$) was found in resuscitation and pediatric care departments (Fig. 4). The dust containing adsorbed viruses had a higher weight percentage of oxygen ($p = 0.003$) in the inorganic portion and a higher weight percentage of nitrogen ($p = 0.04$) in the organic portion than virus-free dust (Fig. 5).

The summarized characteristics of the correlation relationships are shown in the heat map (Fig. 6).

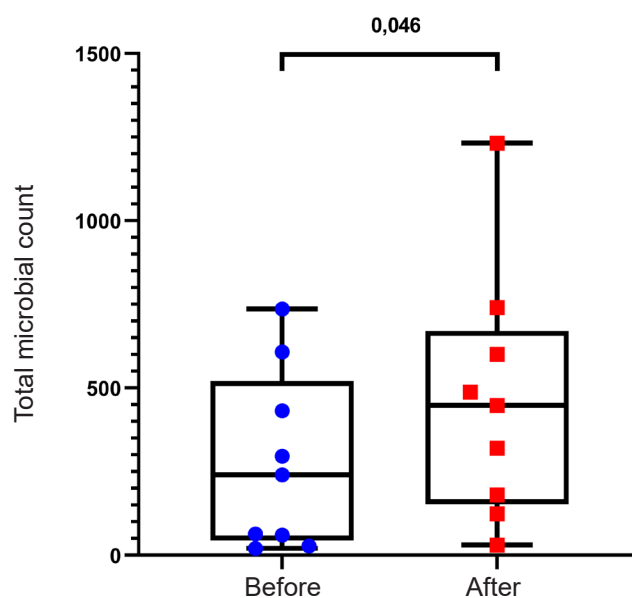


Fig. 1. The total microbial count in the wards of healthcare settings before and after opening doors and windows.

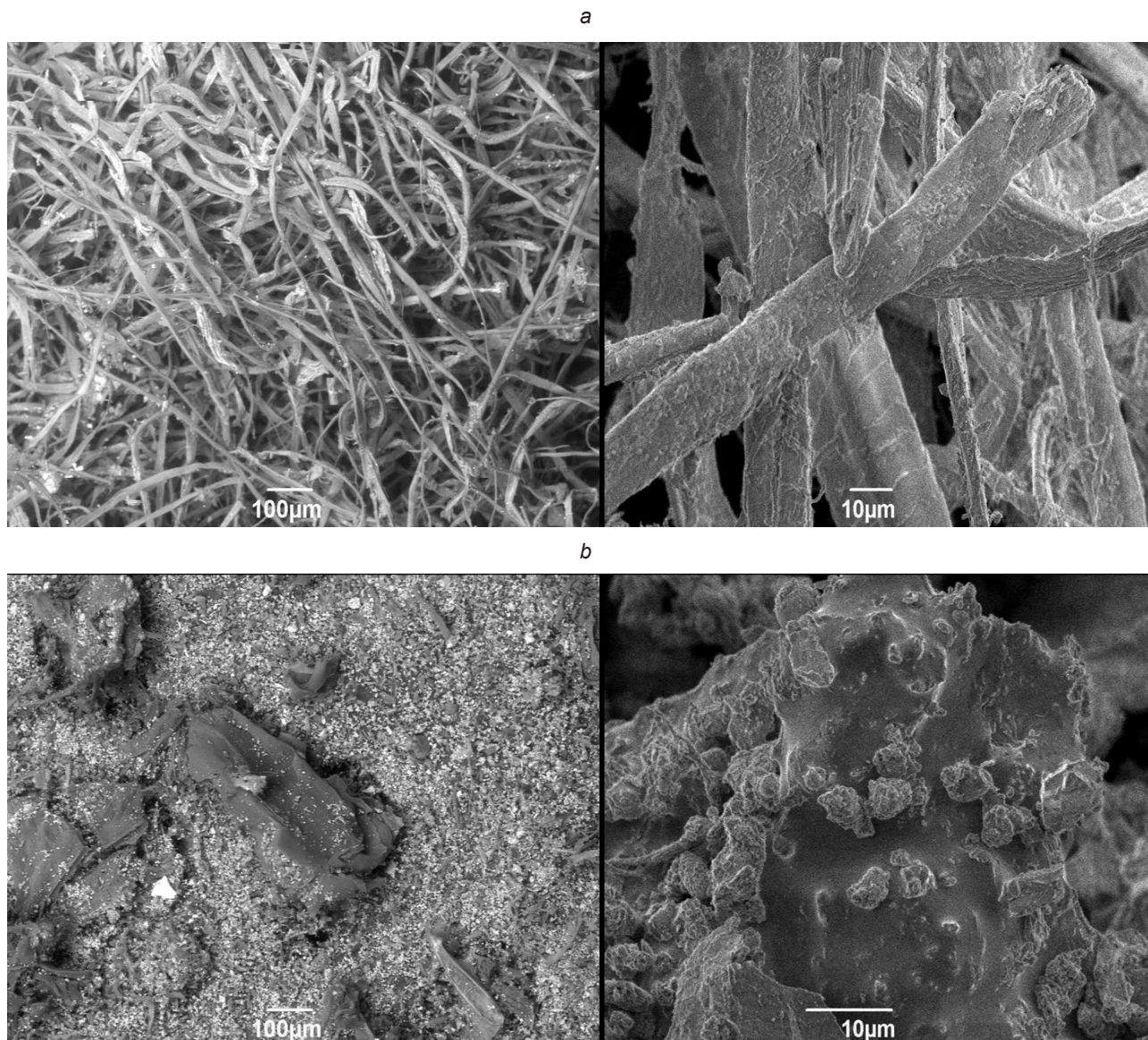


Fig. 2. Morphological characteristics of dust samples with micro-sized fibers (a) and globular particles (b).

Discussion

The hospital facility is a specific environmental system characterized by continuous selection of strains, which are able to compete efficiently with other MOs and to form hospital clones possessing a high epidemic potential as well as the ability to cause severe forms of infections and persist in a hospital environment for a long time [1]. At present, there have been a lot of studies addressing the risk, conditions of retention, and accumulation of HAI pathogens on different items in a hospital environment, equipment, drug, and disinfectant solutions. It has been found that the retention of viable MOs depends on the environmental temperature, humidity [8], and pH as well as on the presence of other MOs, chemical substances, and surface materials (metal, plastic, rubber, etc.) [9]. Detailed studies have been performed regarding different transmission factors such

as water, hands, tools [10], solutions, and structural parts of devices and instruments [11, 12].

It is assumed a priori that the presence of dust at healthcare facilities is very low and, subsequently, its role in transmission of HAI pathogens is insignificant. However, in recent years, the aerosol transmission of HAI pathogens has attracted increasing attention. In their studies, Bourouiba et al. convincingly demonstrated the turbulent nature of the aerosol flow propagation resulting from physiological and pathological respiratory acts, and its directed movement not only in sagittal plane, but also into upper layers of the indoor air, including air ducts, as well as indefinitely long circulation of nanosized particles and the ability of the aerosol cloud fraction to move over large distances [13]. Air ducts are subject to regular disinfection. However, inner surfaces of ventilation grilles and adjacent air-duct

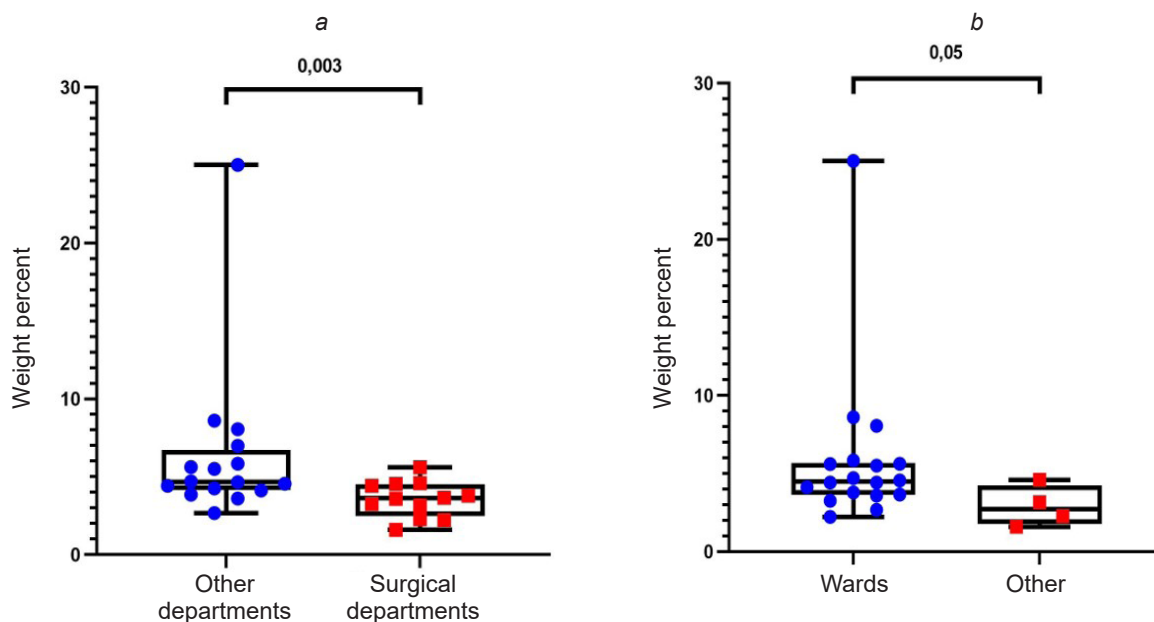


Fig. 3. The content of nitrogen (weight percent) in the dust of various types of healthcare settings (a) and functional units (b) in healthcare settings.

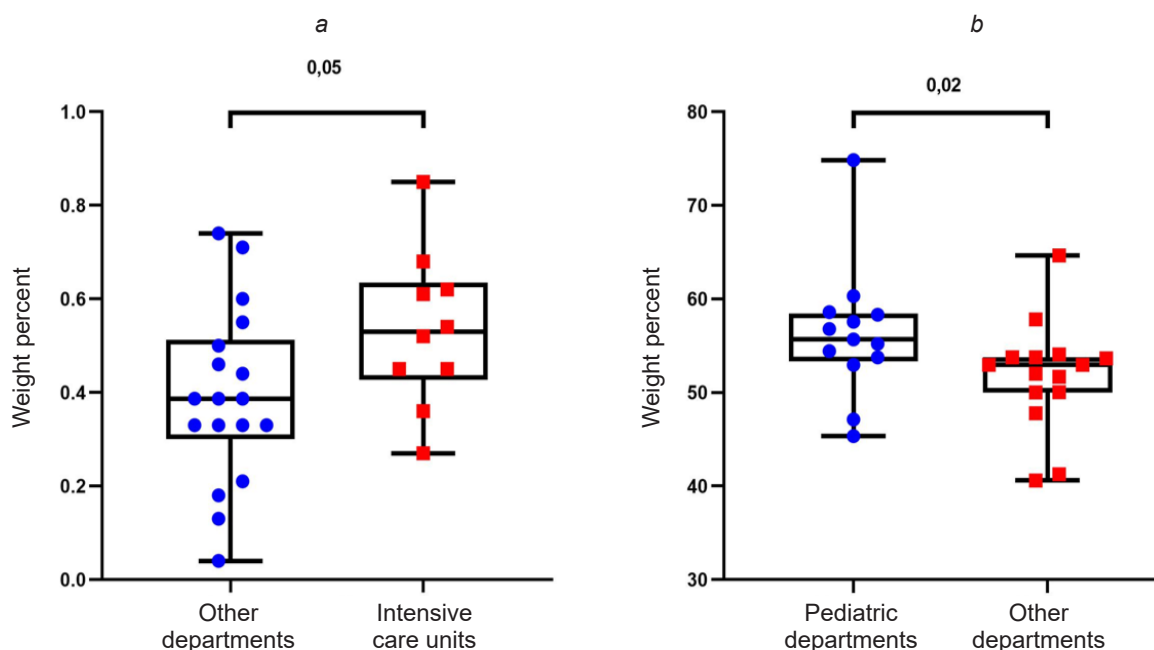


Fig. 4. The content of potassium (a) and carbon (b) in the dust of various types of compartments in healthcare settings (weight percent).

sectors are frequently contaminated with dust, as they are inaccessible for regular cleaning, dust removal and disinfection. As a result, a reservoir for MOs is created, and these MOs forming the dust particle-MO complex can be redelivered along with air flows into patients' breathing zone, entering the patients' nasal pharynx and respiratory tract. The discovered wide range of MOs, including multi-resistant strains of bacteria, RNAs of rota- and noroviruses, and high occurrence of dust contamination on ventilation grilles, suggests the pos-

sibility of not only retention, but also accumulation and redelivery of actual HAI pathogens to the hospital environment. The experiment involving opening of windows and doors confirmed a significant increase in the microbial content of the ward air within 1 m under the ventilation grille, thus proving the possibility of contamination both of a patient's breathing zone and surfaces in the hospital setting as well as the risk of colonization of a patient's loci. Such redelivery of rotaviruses into the hospital environment can lead to

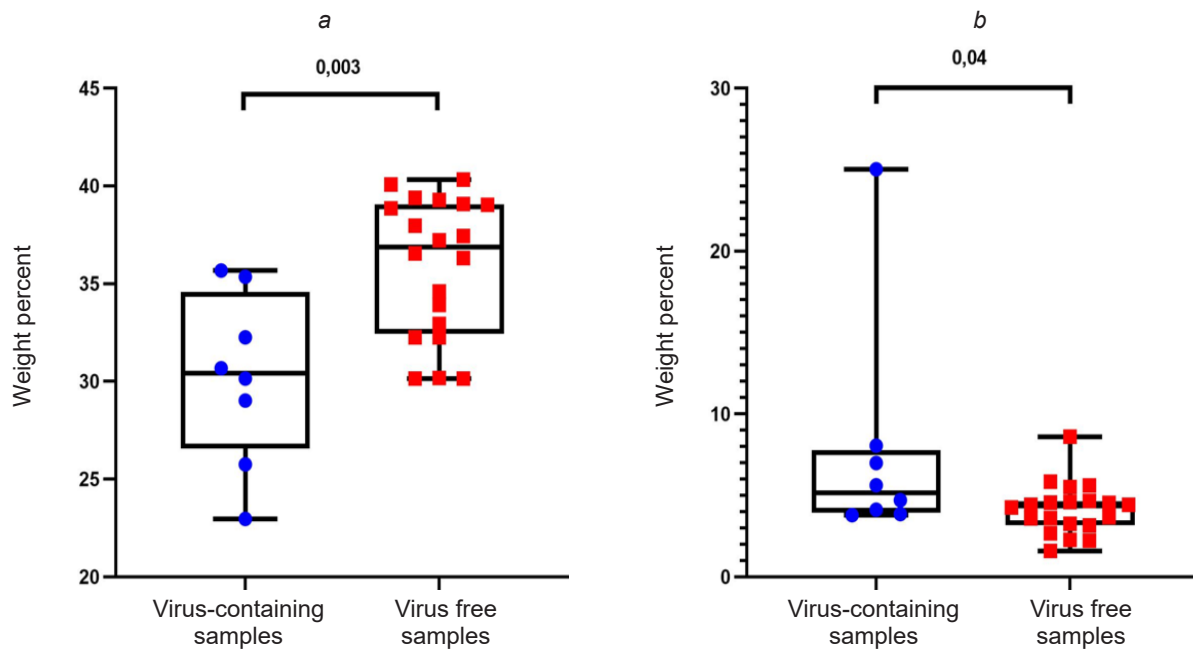


Fig. 5. The content of oxygen (a) and nitrogen (b) in the dust with and without adsorbed viruses (weight percent).

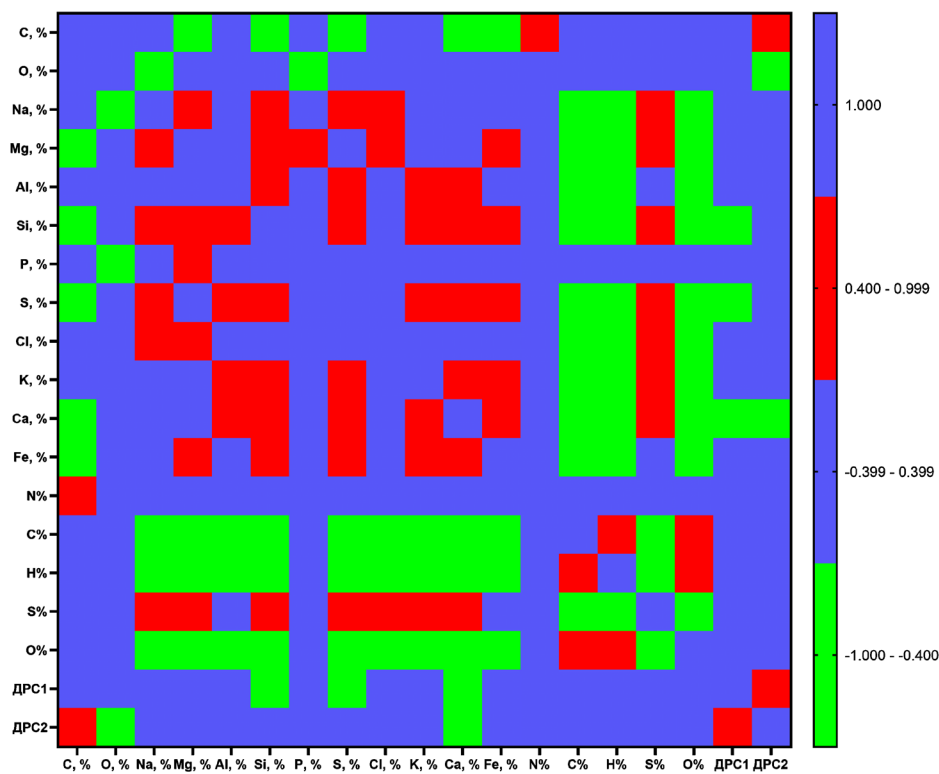


Fig. 6. Heatmap. The color indicates the correlation coefficients.

group incidence of nosocomial rotavirus infection, as the infecting dose for disease development can be very small. The nanosized fraction ($< 5 \mu\text{m}$) found in all the examined dust samples confirmed the risk of longtime circulation of the particle-MO complex in the air environment and its penetration to the lower respiratory

tract, which, at minimum, opens the way to colonization of this locus. The obtained data correlated with the findings of other researchers [14].

Apparently, unfavorable conditions typical of dry dust shaped the selectivity of MO retention: A significant proportion of the bacteria isolated from dust were

gram-negative (76.74%) and demonstrated antibiotic resistance (69.44%), nearly half of them belonged to the sapronoses group, were of sporous type, and formed biofilms, thus contributing to the role of dust in retention of MO strains characterized by widespread occurrence. However, bacterial survival on items of a hospital environment cannot be seen as a sufficient criterion of existence of an additional reservoir in hospital-associated dust. In the meantime, the lifetime of HAI pathogens in dust (6 months), the confirmed presence of an organic substrate (carbon, hydrogen, nitrogen, sulfur, and oxygen), and the observed diversity of trace elements (sodium, magnesium, aluminum, silica, phosphorus, potassium, calcium, and iron) imply that the dust accumulated on inner surfaces of ventilation grilles and adjacent air-duct sections of exhaust systems can be seen as an additional reservoir of resistant hospital-associated MO strains. Previously, we demonstrated a trigger role of carbon microparticles present in dust in retention of HAI pathogens [15]. As for the relationship between the morphology of dust particles and the frequency/type of MO contamination, it needs further exploration. At the moment, we can assert that the significance of this additional reservoir is different for different structural and functional divisions of an in-patient facility as well as for MO types.

Conclusion

At healthcare facilities, exhaust ventilation and air-duct grilles can serve as an additional reservoir of microorganisms causing HAIs and contribute significantly to persistence of a HAI epidemic process.

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