

# IDENTIFICATION AND CHARACTERISTICS OF BIOLOGICAL AGENTS IN WORK ENVIRONMENT OF MEDICAL EMERGENCY SERVICES IN SELECTED AMBULANCES

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## Abstract

**Objectives:** Assessment of microbial air quality and surface contamination in ambulances and administration offices as a control place without occupational exposure to biological agents; based on quantitative and qualitative analysis of bacteria, yeasts and filamentous fungi found in collected samples. **Material and Methods:** The sampling was done by wet cyclone technology using the Coriolis recon apparatus, imprint and swab methods, respectively. In total, 280 samples from 28 ambulances and 10 offices in Warszawa were tested. Data was analyzed using Shapiro-Wilk normality test, Kruskal-Wallis test with  $\alpha = 0.05$ . P value  $\leq 0.05$  was considered as significant. **Results:** The levels of air contamination were from 0 to  $2.3 \times 10^1$  colony-forming unit (CFU)/m<sup>3</sup> for bacteria and for yeast and filamentous fungi were from 0 to  $1.8 \times 10^1$  CFU/m<sup>3</sup>. The assessment of office space air samples has shown the following numbers of microorganisms: bacteria from  $3.0 \times 10^1$  to  $4.2 \times 10^1$  CFU/m<sup>3</sup> and yeast and filamentous fungi from 0 to  $1.9 \times 10^1$  CFU/m<sup>3</sup>. For surface contamination the mean bacterial count in ambulances has been between  $1.0 \times 10^1$  and  $1.3 \times 10^2$  CFU/25 cm<sup>2</sup> and in offices – between  $1.1 \times 10^1$  and  $8.5 \times 10^1$  CFU/25 cm<sup>2</sup>. Mean fungal count has reached the level from  $2.8 \times 10^0$  to  $4.2 \times 10^1$  CFU/25 cm<sup>2</sup> in ambulances and  $1.3 \times 10^1$  to  $5.8 \times 10^1$  CFU/25 cm<sup>2</sup> in offices. The qualitative analysis has revealed the presence of *Acinetobacter* spp. (surfaces), coagulase – negative *Staphylococci* (air and surfaces), *Aspergillus* and *Penicillium* genera (air and surfaces). **Conclusions:** The study has revealed a satisfactory microbiological quantity of analyzed air and surface samples in both study and control environments. However, the presence of potentially pathogenic microorganisms in the air and on surfaces in ambulances may endanger the medical emergency staff and patients with infection. Disinfection and cleaning techniques therefore should be constantly developed and implemented. Int J Occup Med Environ Health 2017;30(4):617–627

## Key words:

Bacteria, Fungi, Work environment, Harmful biological agents, Antimicrobial resistance, Ambulances

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## INTRODUCTION

Risk assessment of the workplace microbiological contamination informs employers and workers about existing biohazards, allows determination of health safety and workplace hygiene of paramedics, and thus implies the necessity of protection against harmful biological agents.

While monitoring the microbiological status of health-care institutions, e.g., hospitals has become routine [1–5], the analysis of microbiological contamination of ambulances is not subject to specific supervision. According to the 2009 State Sanitary Inspection that assessed the overall state of ambulances, 5.3% out of the 375 institutions that provided the records did not meet the sanitary criteria. This resulted primarily from deficiency of sanitary equipment, contamination of walls, ceilings and sanitation instruments and mismanagement of medical waste [6]. Moreover, in the 2010 report in 29 (10.5%) out of 277 inspected ambulances inadequate sanitary conditions were found. In most cases the failures also resulted from deficiencies in sanitary equipment [7]. In 2011, the observations resembled those from 2009, where in the case of 18 (6.2%) out of 291 Ambulance Services checked, defective sanitary engineering was found [7].

The most frequently found neglect arose from damaged interior surfaces, mainly floors and walls, which limited the capacity to perform effective disinfection of the ambulance space. In addition, failures were observed in complying with the medical facility mandatory procedures concerning washing and disinfection of hands, tools and medical equipment as well as small and hard to reach surfaces (air ventilation grating, medical equipment buttons, etc.) [6–8]. Moreover, the mobile nature of ambulance work promotes acceleration of corrosion processes and adhesion and growth of bacteria and; the latter is amplified by the usage of strong disinfectants [9]. Residual potential infection resulting from the bacterial growth may accumulate and persist in the microenvironment of an ambulance and thus be available for transmission to a new host [10]. According to Alves and

Bissell [10], a number of objects in the health care setting carry infectious bacteria: scissors, ballpoint pens, stethoscopes, coats, adhesive tapes, computer keyboards, water faucets or pagers or cell phones, air vent grates. It is known that presence of bacteria on these objects contributes to new infections for patients. On the other hand, a patient may also transmit infectious microorganisms (opportunistic or pathogenic) by coughing, vomiting, excreting, urinating in an ambulance during transport [10].

Therefore, the sanitary and hygienic status of ambulances requires assessments of the status of microbiological contamination. Persistence of high levels of contamination may result in microbial transmission from patients to medical personnel and to other patients. A careful analysis of microbial quantity and identification of potentially pathogenic agents may determine occupational risks of emergency medical services.

## Objective

Assessment of microbial air quality and surface contamination in ambulances and office spaces without occupational exposure to biological agents; based on quantitative and qualitative analysis of bacteria, yeast and filamentous fungi found in collected samples.

## MATERIAL AND METHODS

### Sampling, culturing, quantitative and qualitative analysis

The bioaerosol and work surface sampling was done by wet technology, imprint and swab methods, respectively. The sampling was carried out during winter period in ambulances and office buildings without air conditions. The sampling of work surfaces was done by swab and imprint methods; air samples were collected by means of the cyclone wet technology method. In total, 280 samples were collected from 28 ambulances and 10 offices in Warszawa. The material for analysis from one examined workplace was gathered according to the following scheme: 1 air

sample, 9 surface samples included 4 swabs (door handles, medical instrument buttons, ballpoint pens and air conditioner/ventilation grate) and 5 imprints (3 for bacteria, 2 for fungi included floors, walls, couches/tables). Similar sampling was used in the offices (10 workplaces) as a control not exposed to harmful biological agents.

Air samples were collected with the assistance of a Coriolis Recon apparatus (Bertin, France) placed on the high of 1.5 m which collects biological particles from 0.5–10.0  $\mu\text{m}$  in a liquid sample (volume 25 ml) at a flow rate of 6  $\text{m}^3$  for 10 min. The liquid material was filtered through 0.22  $\mu\text{m}$  filter (Merck Millipore, USA) using a Vacuum Pump P504 Millipore apparatus (Merck Millipore, USA). In the next step, the filter was placed onto the surface of the agar medium with 5% sheep blood (Columbia Agar, Graso Biotech, Poland) and Sabouraud medium (Sabouraud Dextrose Agar, Graso Biotech, Poland). The culture plates were incubated for 24 h, respectively, at 37°C followed by 3 days at 22°C for the bacteria and for 96 h at 28°C for the fungi. Next the number of bacterial and fungal colonies were counted and recalculated as colony forming units per  $\text{m}^3$  (CFU/ $\text{m}^3$ ).

The biological material from irregular or hard to access surfaces (doorknobs, air conditioning units) was collected by swabbing, using wet cotton swabs placed in sterile saline solution. Flat work surfaces were sampled using: Count-Tact® 3P™ Agar – CT3P (bioMérieux, France) for bacteria and Count-Tact® CTSI (bioMérieux, France) for yeasts and filamentous fungi determination, respectively. In order to ensure reproducibility of the collection conditions, a special applicator was used (Count-Tact® Applicator, bioMérieux, France). The biological material collected on Count-Tact plates (CT3P, CTSI) was culturing according to the manufacturer's recommendations (bioMérieux, France): 3 days at 30°C±1°C (CT3P for bacteria) and 5 days at 22.5°C±2.5°C (CTSI for fungi). Material from swabs was transferred onto agar medium with 5% sheep blood (Columbia Agar, Graso Biotech,

Poland) and Sabouraud medium (Sabouraud Dextrose Agar, Graso Biotech, Poland). Biological material was incubated at 37°C for 1 day followed by 3 days at 22°C for bacteria and at 28°C for 5 to 7 days for fungi. Afterwards, the bacterial and fungal colonies were counted and calculated as CFU/25  $\text{cm}^2$ .

Subsequently, the microbial isolates on respective agar media were classified at the genus and/or species using generally accepted diagnostic procedures. Identification of bacteria strains and testing their antibiotic susceptibility was carried out using an automated system Vitek2 Compact system (bioMérieux, France) following the manufacturer's instructions.

Species identification was carried out using the appropriate bar-coded cards for Gram-negative (GN), Gram-positive (GP) and anaerobic bacteria (ANC) (bioMérieux, France); suitable antibiogram cards were also used for determining isolates antibiotic susceptibility: AST-N258 (amikacin, amoxicillin/clav. acid, ampicillin, cefepime, cefotaxime, cefalexin, ceftazidime, cefuroxime, ciprofloxacin, ertapenem, gentamicin, imipenem, nitrofurantoin, norfloxacin, piperacillin/tazobactam, trimethoprim/sulfamethoxazol), AST-N260 (amikacin, ampicillin/sulbactam, aztreonam, cefepime, cetazidime, cirpofloxacin, colistin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, netilmycin, ticarcillin/clav. acid, tobramycin, trimethoprim/sulfamethoxazol), AST-GP67 (ampicillin, benzylopicillin, cefoxitin screen, ciprofloxacin, clindamycin, erythromycin, gentamicin, inducible clindamycin resistance, levofloxacin, linezolid, moxifloxacin, nitrofurantoin, oxacillin, quinopristin/dalfopristin, rifampicin, tetracycline, trimethoprim/sulfamethoxazol, vancomycin) (bioMérieux, France).

Phenotypic methods were used for confirming specific antimicrobial resistance (disc diffusion method). Interpretation of results was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

recommendation [11]. The following strains were used as the control references: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Streptococcus pneumoniae* ATCC 49619.

The identification of fungi was carried out in microcultures on potato dextrose agar (PDA) medium and was based on the morphological characteristics of the mycelium, e.g., the type of filaments and reproductive structure. Classification of genera and species of the isolated fungi was carried out according to the monographs of Domsch et al. [12], Barnett and Hunter [13] using a microscope Olympus BX-41 with a digital camera and software CVIII4 Cell-A. In accordance with the latest nomenclature currently in use the species name of the fungi have been introduced [14].

## RESULTS

The microbial air and surface contamination in ambulances and administrative offices is shown in the Table 1. In terms of the air quality assessment, most of isolated bacteria in ambulances (8.4%) were coagulase-negative *Staphylococci* (CoNS) (*Staphylococcus hominis* (methicillin-resistant coagulase-negative *Staphylococci* – MRCNS) – 2.8%, *Staphylococcus warnerii* – 2.8%, *Staphylococcus capitis* – 2.8%). Moreover, *Bacillus* spp., *Sphingomonas paucimobilis*, *Kocuria roseae*, *Rhizobium* spp., *Serratia plymuthica*, *Globicatella sulfidifaciens*, *Leclercia adecarboxylata* and *Aerococcus viridans* were identified. There were no potentially pathogenic bacteria in indoor office air (Table 2).

In microbiological quality of imprint samples from ambulances, 5.6% of potentially pathogens were found. Most of them belong to *Acinetobacter baumannii* cplx. – 0.9%, *A. radioresistens* – 0.9%, methicillin-resistant *S. aureus* (MRSA) (alert-patogen) – 0.95%, *S. sciuri* – 0.95%, *S. warnerii* – 0.95%, *S. xylosus* – 0.95%. In offices 14.3% microorganisms

include non-fermentive Gram-negative bacilli (NFGNB): *Acinetobacter lwoffii* – 11.4%, *A. baumannii* cplx. – 2.9%, and 8.56% – *Staphylococcus* spp. *S. hominis* – 2.86%, *Staphylococcus haemolyticus* – 2.86%, *S. warnerii* – 2.86%. Main species of culturable bacteria identified from swabs in ambulances were *A. haemolyticus* – 0.8%, *A. lwoffii* – 0.8%, *Micrococcus luteus* – 0.8%, *S. warnerii* – 1.6%, *S. saprophyticus* – 0.8%, *S. haemolyticus* – 0.8% and *S. epidermidis* – 0.8%. In offices 18.5% potentially pathogenic bacteria included mainly coagulase-negative *Staphylococci* (CoNS) – 33.3%. Alert pathogens were not detected (Table 2).

In the distribution analysis, the proportion of individual fungal groups in relation to total mycobiota isolated from the indoor air and surfaces was similar and showed 92% and 94% of filamentous fungi and 8% and 6% of yeasts in ambulances and offices, respectively.

The mycological analysis of air samples present in ambulances as well as in offices revealed similar distribution of fungi: *Aspergillus* spp. (25%), *A. versicolor* (12.5%), *Penicillium* spp. (12.5%), *P. expansum* (12.5%), *P. citrinum* (12.5%) and *Trichoderma koningii*, *Chaetomium globosum* and *Ulocladium atrum* (37.5%). As far as imprints and swab samples from ambulances as well from offices are concerned, the quality assessment results mainly revealed the presence of *Aspergillus* (42%) and *Penicillium* (42%) genera, widely thought of as toxin-producing. The isolates included the following species in ambulances: *A. niger* (18%), *A. fumigatus* (9%), *Aspergillus* spp. (18%), *P. expansum* (27%), *Penicillium* spp. (18%) and *T. koningii* (9%), and in offices: *A. niger* (6.6%), *A. fumigatus* (6.6%), *A. versicolor* (6.6%), *Aspergillus* spp. (33%), *P. expansum* (25%), *Penicillium* spp. (25%) and *T. koningii* (6.6%). These species were found on surface environments. However, *A. niger*, *A. fumigatus* and *P. expansum* were mainly found in ambulances and office ventilation systems and on office computer keyboards.

**Table 1.** Air and surface imprint and swab counts of bacteria and fungi collected from the studied ambulances and offices

| Tested place and area                    | Bacteria              |                            | Fungi                 |                           |
|--|-----------------------|----------------------------|-----------------------|---------------------------|
|  | min.-max              | M                          | min.-max              | M                         |
| <b>Ambulance</b>                         |                       |                            |                       |                           |
| air (N = 28) [CFU/m <sup>3</sup> ]       | 0-2.3×10 <sup>1</sup> | <b>6.2×10<sup>0</sup></b>  | 0-1.8×10 <sup>1</sup> | 4.6×10 <sup>0</sup>       |
| floor (N = 28) [CFU/25 cm <sup>2</sup> ] | 0-3.0×10 <sup>2</sup> | 1.3×10 <sup>2</sup>        | 0-3.0×10 <sup>2</sup> | 4.27×10 <sup>1</sup>      |
| wall (N = 28) [CFU/25 cm <sup>2</sup> ]  | 0-3.0×10 <sup>2</sup> | <b>1.0×10<sup>1</sup></b>  | 0-3.0×10 <sup>2</sup> | <b>2.8×10<sup>0</sup></b> |
| couch (N = 28) [CFU/25 cm <sup>2</sup> ] | 0-3.0×10 <sup>2</sup> | 3.6×10 <sup>1</sup>        | n.t.                  | n.t.                      |
| <b>Office</b>                            |                       |                            |                       |                           |
| air (N = 10) [CFU/m <sup>3</sup> ]       | 2-4.2×10 <sup>1</sup> | <b>1.35×10<sup>1</sup></b> | 0-1.9×10 <sup>1</sup> | 6.8×10 <sup>0</sup>       |
| floor (N = 10) [CFU/25 cm <sup>2</sup> ] | 2-3.0×10 <sup>2</sup> | 8.5×10 <sup>1</sup>        | 2-3.0×10 <sup>2</sup> | 5.8×10 <sup>1</sup>       |
| wall (N = 10) [CFU/25 cm <sup>2</sup> ]  | 0-7.0×10 <sup>1</sup> | <b>1.1×10<sup>1</sup></b>  | 0-3.8×10 <sup>1</sup> | <b>1.3×10<sup>1</sup></b> |
| table (N = 10) [CFU/25 cm <sup>2</sup> ] | 2-2×10 <sup>2</sup>   | 2.6×10 <sup>1</sup>        | n.t.                  | n.t.                      |

N – samples; CFU – colony-forming unit; min. – minimal value; max – maximal value; M – mean; n.t. – not tested. The results that are statistically significant ( $p < 0.05$ ) are bolded; Kruskal-Wallis test ( $\alpha = 0.05$ ). Statistically significant differences between ambulances and offices were observed only for walls (bacteria:  $p = 0.0033$ , fungi:  $p = 0.0041$ ;  $\alpha = 0.05$ ). In the air quality analysis the predominant isolated bacteria and fungi were: *Staphylococcus* spp. and *Aspergillus* spp., respectively. Statistically significant differences were observed only in the case of bacteria ( $p = 0.0436$ ,  $\alpha = 0.05$ ).

**Table 2.** Bacterial groups isolated from air and surface samples collected from the studied ambulances and offices

| Tested place and area              | Sample   | Bacterial group [n (%)]      |                              |          |  | Environmental contaminants [n (%)] |
|------------------------------------|----------|------------------------------|------------------------------|----------|--|------------------------------------|
|                                    |          | <i>Staphylococcus</i> (CoNS) | <i>Staphylococcus aureus</i> | NFGNB    | <i>Bacillus</i> spp. <i>Enterobacteriaceae</i> |                                    |
| <b>Ambulance</b>                   |          |                              |                              |          |  |                                    |
| floor (N = 28)                     | imprints | 2 (7.7)                      | 0                            | 2 (7.7)  | 7 (27.0)                                       | 2 (15.4)                           |
| wall (N = 28)                      | imprints | 2 (7.7)                      | 1 (3.8) MRSA <sup>a</sup>    | 0        | 0  | 0                                  |
| couch (N = 28)                     | imprints | 9 (25.0)                     | 1 (3.8)                      | 3 (11.5) | 8 (30.7)                                       | 1 (3.8)                            |
| air (N = 28)                       | air      | 8 (30.8)                     | 0                            | 0        | 10 (38.5)                                      | 0                                  |
|                                    |          | include 1 MRCNS <sup>b</sup> |                              |          |  |                                    |
| door handle (N = 28)               | swabs    | 4 (30.7)                     | 0                            | 1 (3.9)  | 2 (7.7)  | 0                                  |
| medical instrument button (N = 28) | swabs    | 10 (37.0)                    | 0                            | 1 (3.7)  | 1 (7.7)  | 0                                  |
|                                    |          | include 1 MRCNS <sup>c</sup> |                              |          |  | 3 (11.1)                           |

**Table 2.** Bacterial groups isolated from air and surface samples collected from the studied ambulances and offices – cont.

| Tested place and area                      | Sample   | Bacterial group<br>[n (%)]              |  |          |   | Environmental<br>contaminants<br>[n (%)] |
|--|----------|---|--|----------|---|--|
|  |          | <i>Staphylococcus</i><br>spp.<br>(CoNS) | <i>Staphylococcus</i><br><i>aureus</i> | NFGNB    | <i>Bacillus</i> spp.<br><i>Enterobacteriaceae</i> |  |
| Ambulance – cont.                          |          |   |  |          |   |  |
| ballpoint pen (N = 28)                     | swabs    | 7 (24.2)                                | 1 (3.5)                                | 0        | 2 (6.9)   | 2 (6.9)                                  |
| air conditioner/ventilation grate (N = 28) | swabs    | 8 (30.7)                                | 1 (3.8)                                | 0        | 6 (23.1)  | 3 (11.5)                                 |
| Office                                     |          |   |  |          |   |  |
| floor (N = 10)                             | imprints | 3 (23.0)                                | 0                                      | 2 (15.4) | 4 (30.8)  | 13 (100.0)                               |
| wall (N = 10)                              | imprints | 3 (23.0)                                | 0                                      | 1 (7.7)  | 2 (15.4)  | 5 (38.5)                                 |
| table (N = 10)                             | imprints | 6 (46.2)                                | 1 (7.7)                                | 3 (23.1) | 2 (15.4)  | 6 (46.2)                                 |
| air (N = 10)                               | air      | 3 (23.0)                                | 0                                      | 0        | 3 (23.0)  | 8 (66.7)                                 |
| door handle (N = 10)                       | swabs    | 4 (30.7)                                | 0                                      | 0        | 0   | 0  |
| computer keyboard (N = 10)                 | swabs    | 10 (37.0)                               | 0                                      | 0        | 0   | 0  |
| ballpoint pen (N = 10)                     | swabs    | 3 (24.2)                                | 0                                      | 0        | 0   | 0  |
| air conditioner/ventilation grate (N = 10) | swabs    | 3 (24.2)                                | 0                                      | 0        | 0   | 0  |

N – samples; CoNS – coagulase-negative *Staphylococci*; NFGNB – non-fermentive Gram-negative bacilli [38]; MRSA – methicillin-resistant *Staphylococcus aureus*; MRCNS – methicillin-resistant coagulase-negative *Staphylococci*.

<sup>a</sup> Cefoxitin (FOX) – positive; clindamycin (CD) – resistance; constitutive macrolide, lincosamide and streptogramin B (MLS<sub>B</sub>), tetracycline (TE) – resistance.

<sup>b</sup> *Staphylococcus haemolyticus*; FOX – positive; erythromycin (E) – resistance; CD – sensitive; macrolides and streptogramin B (MS<sub>B</sub>) – resistance (efflux pump system).

<sup>c</sup> *Staphylococcus hominis*; FOX – positive; E – resistance; CD – resistance; constitutive MLS<sub>B</sub>.

## DISCUSSION

The conditions in ambulances may increase the risk of transmission among patient, to the healthcare personnel and the environment. Particularly air and surfaces of surrounding areas may be a source of contamination [15–17]. The study of microbial counts in indoor air samples collected from ambulances found relatively low level (mean (M) =  $6.2 \times 10^0$  CFU/m<sup>3</sup>). Moreover, the values were lower in comparison to offices (M =  $1.36 \times 10^1$  CFU/m<sup>3</sup>). The levels of yeast and filamentous fungi in the air samples also showed lower values than in office spaces. These microbiological levels contrast with the results of Luksami-jarulkul et al. [18] where the bacterial and fungal counts in ambulance air showed the presence of several hundreds of colony forming units in 1 m<sup>3</sup>;  $3.18 \times 10^2$ – $4.85 \times 10^2$  CFU/m<sup>3</sup> for bacteria and  $5.22 \times 10^2$ – $5.81 \times 10^2$  CFU/m<sup>3</sup> for yeast and filamentous fungi.

The quantity of fungi in the air of office demonstrates a satisfying level from  $3.0 \times 10^0$  to  $4.2 \times 10^1$  CFU/m<sup>3</sup> at the mean value  $6.8 \times 10^0$  CFU/m<sup>3</sup>. It may be explained by good quality indoor air (the humidity above 50%, no indications of moisture, no smell of mildew). Similar values were found by other researchers; while inspecting offices with evident traces of moisture facilitating to fungal growth, Buczyńska et al. received values 10 times higher ( $2.7 \times 10^2$ – $3.22 \times 10^2$  CFU/m<sup>3</sup>) in comparison with a control panel of locations complying with the requirements for Environment, Health and Safety (EHS) (low humidity, presence of an air-conditioning units) [19]. In similar studies designed to evaluate microbiological air evaluation on university premises, Skowron et al. showed the presence of yeasts and filamentous fungi at the level of  $10^1$ – $10^2$  CFU/m<sup>3</sup> [20].

Due to the lack of globally accepted criteria of evaluation of biological agents exposure in the workplace, as well as commonly implemented standard values, the criteria proposed by Górny (2004) ( $> 2 \times 10^2$ – $5 \times 10^2$  CFU/m<sup>3</sup>) may be adopted in interpreting the obtained results, including

the total fungi counts in enclosed spaces [20,21]. In the light of these criteria the status of microbiological contamination in examined ambulances as well as in the office spaces has been highly satisfactory. In normal conditions the main source of fungal bioaerosol in the air of confined spaces (offices, etc.) is aerosol migrating from the external environment. The phenomenon becomes particularly relevant in summer and autumn, when the concentrations of fungal spores in the atmospheric air increase. As this study was conducted in winter period, the level of fungal contamination did not reach critical values [22].

In the study, detected non-fermenting Gram-negative bacilli – *Acinetobacter* spp. and filamentous fungi (according to the list of harmful biological agents) [23], as well as a wide spectrum of coagulase-negative *Staphylococci* (CoNS), presented various antimicrobial resistance phenotypes.

The level of bacterial and fungal contamination in ambulances varies widely from 0 to  $3.0 \times 10^2$  CFU/25 cm<sup>2</sup>, which is similar to the level of microbiological contamination of office surfaces. The exceptions are ambulance floors where much higher concentrations of microorganisms have been observed. This situation may signal the need to implement appropriate procedures of disinfection, decontamination and cleaning of ambulance floors. Nigam and Cutter demonstrated the effectiveness of routine washing and disinfection of the ambulance interiors and equipment used by medical services [24,25]. In several related studies [18,25,26] similar levels of microbiological contamination of internal ambulance surfaces were found. In contrast to these results, in the study of Alves and Bisell, the contamination of ambulance equipment, instruments and accessories in ambulances was 100 times higher ( $0$ – $1.0 \times 10^2$  CFU/mm<sup>2</sup>) [10].

The quality analysis of imprints and swab samples collected from ambulances showed the presence of environmental flora such as *Bacillus* spp., *S. paucimobilis*, *K. roseae*, *Rhizobium* spp., *S. plymuthica*, *G. sulfidifaciens*,

*L. adecarboxylata*, *A. viridans* and also potentially pathogenic coagulase-negative *Staphylococci* (3.8%) presenting efflux pump system (macrolides and streptogramin B resistance – MS<sub>B</sub>), constitutive macrolide, lincosamide and streptogramin B (MLS<sub>B</sub>) and methiciline resistance coagulase-negative *Staphylococci* (MRCNS) – *S. haemolyticus* (a swab from a medical equipment) and 1.8% non-fermentative Gram-negative bacilli – *Acinetobacter* spp. with multidrug resistance. In one location (a wall imprint) the MRSA (alert-pathogen), additionally resistant to erythromycin, clindamycin (constitutive MLS<sub>B</sub>) and tetracycline was also identified.

The microbiological qualitative analysis of ambulance surfaces demonstrated similarity to the results of other authors who isolated *P. aeruginosa*, *E. faecalis*, *S. epidermidis*, *Bacillus* spp., *S. haemolyticus*, *Pantoea agglomerans* [10,27]. Other studies of microbiological content of work surfaces, accessories and medical equipment in ambulances showed a wide spectrum of microorganisms, from environmental flora to potentially pathogenic non-fermentative Gram-negative bacilli (15.1%) with the prevalence of *Pseudomonas* spp. and *Acinetobacter* spp.; MRSA and MRCNS 0.9% and *K. pneumoniae* (0.5%) were also found [28]. Vikke and Giebner conducted an interesting examination of ambulance crew uniforms; the study showed contamination with potentially pathogenic bacteria, such as *Bacillus cereus* (27.78%), *Clostridium* spp., *Enterococcus* spp. (2.22%) and *S. aureus* (21.11%) [25]. Similar profile of microorganisms was revealed by Nigam and Cutter in the swab analysis of work spaces and medical instruments. In this case, the most widespread microorganisms were: coliforms (31.5%), *S. epidermidis* (29.4%) and *Bacillus* spp. (21.9%) [24]. Luksamijarulkul and Pipitsangjan presented a similar microbiological profile in the analysis of surfaces in ambulances *Staphylococcus* spp. (47%), Gram-negative bacilli (32%), Gram-positive bacilli (12%) [18].

The mycological analysis revealed the presence of various species of pathogenic as well as non-pathogenic fungi. Existing *Aspergillus* spp. included following species: *A. niger*, *A. fumigatus*, *A. versicolor* and *Penicillium* spp. presented by *P. expansum* and *P. citrinum*. A similar mycological profile was observed by Noh et al. and Luksamijarulkul and Pipitsangjan [18,28].

Apart from ubiquitous saprophytic species such as *T. koningii*, *C. globosum* and *U. atrum* air samples from ambulances and offices contained other, potentially dangerous, species which may cause allergies, infections or toxic reactions to mycotoxins [19,29,30]. The mycological analysis of air and surface samples from ambulances and offices mainly showed the presence of *Aspergillus* and *Penicillium* genera. However, these genera occurred commonly in atmospheric air, the presence of them at the workplaces may constitute potential hazards to employees (group 2 according to their level of risk of infection and allergy) [23]. Fungal quality assessment of examined workplaces is similar to results obtained by other researchers [18,31–33].

Pathogenic *A. fumigatus* was found in swab samples from an office keyboard and an ambulance ventilation system. This species is the main etiologic agent of pulmonary and systemic aspergillosis in immunocompromised persons. It may occur as an opportunistic pathogen or as an allergen, moreover, it is found on the list of harmful biological agents found in the workplace [23,34–36].

However, only exposure to high levels of airborne filamentous fungi (e.g., 10<sup>10</sup> spores/m<sup>3</sup>) associated with bioaerosols may result in sickness partly caused by mycotoxins [37]. Further to this, the number of fungi in our research has not exceeded the designated level.

In this study, the level of microbiological contamination in the ambulance workplaces does not create a potential health threat to medical personnel and patients, as well. However, the presence of potentially pathogenic biological agents in the ambulances may be the source of



transmission to patients and ambulance personnel. Due to exposure to potentially dangerous biological agents, the personnel of the Emergency Medical Services requires special health and safety procedures. The implementation of procedures preventing microbial transmission and interrupting transmission routes may increase occupational safety in ambulance environment.

## CONCLUSIONS

The level of microbiological contamination in ambulances was satisfactory and within accepted range as well as comparable with office spaces without putative occupational exposure to biological agents. This may be an evidence of the effectiveness of existing cleaning and disinfection procedures. However, the presence of potentially pathogenic microorganisms, including alert-pathogens (MRSA, *Aspergillus* spp.) and toxynogenic fungi, in ambulances is a threat to the health of medical personnel and patients if their infectious doses would be reached. The predominant isolated bacteria and fungi in air samples were *Staphylococcus* spp. and *Aspergillus* spp., respectively.

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