THREAT TO HUMAN HEALTH GENERATED BY FUNGI FOUND INSIDE THE AMBULANCE

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Abstract

Infections caused by bacteria or fungi is a serious health worldwide problem. In order to disinfect the inside of the ambulance very strong disinfectants are used - they can also promote the growth of fungi and increase the threat of infections induced by them. Furthermore these micro-organisms are becoming more resistant against applied agents. The publication presents firstly the results of research on fungi that were identified in the interior of the ambulance and medical vehicle type melex – corresponding swabs were collected and microorganisms occurring there were identified. In the next step the selected fungi were incubated on the surface of previously prepared samples. For research samples from aluminum sheet (two types) and plastic (two types) were cut. Sample surface was earlier sandblasted and then coated with varnish (standard and anti-bacterial). The experiment enabled the comparison of the survival of the examined fungi on selected surfaces - coatings.

Key words: fungi, adhesion, ambulance, melex, varnish coatings, plastic

1. INTRODUCTION

Infections caused by microorganisms is a serious threat to health, not only in Poland but throughout the world. Etiologic factors of hospital infections are mainly bacteria. First of all: Staphylococcus aureus, Staphylococci coagulase-negative and Pseudomonas aeruginosa. Candida are on fourth place in this hierarchy [1].

Infections caused by fungi constitute about 9-10% of all hospital infections. The most frequent etiological threat are fungi of a kind Candida (85.6%), and in second place - Aspergillus (1.3%). Other pathogens of this group causes about 11% of infections [2,3]. Due to the possibility of biofilm formation, the fungi are more dangerous for the patient [4].

The incidence of nosocomial infections varies between 5-10% and depends on the age, condition of the patient, the immune system and his physiological barriers. From the epidemiological point of view, the possibility of infection should be considered as real not only on the department, but also during change of the patient's stay - hallways, elevators, transportation for diagnostic tests [1]. However, before the patient staying in hospital, the infection source may be also the medical transport – ambulance. Medical vehicles may be contaminated with microorganisms even during a short the transport time [5].

The processes of cleaning and disinfecting the interior of ambulances is executed according defined principles. Normally, after every patient transport, chairs/stretchers that was in contact should be disinfected. There are also a daily routine disinfection of the vehicle and once a week (or if necessary) a general decontamination. In addition, once a month, may be used a dry mist disinfection by using appropriate preparations and devices. Applied measures most frequently combine activity: anti-bactericidal (including with MRSA), anti-fungicidal and anti-virucidal (including with HBV, HCV, HIV, HCV, rotaviruses and adenoviruses) [6,7].
It is well known that the observance of procedures for disinfection and sterilization leads to increase the safety of patients in hospitals. In order to decontaminate the interior of ambulances the strong disinfectants are used. However, they can also promote the growth of fungi and infections caused by them. In addition, these organisms stay more and more resistant [8]. Therefore, it is very important to implement all procedures to facilitate the maintenance of the cleanliness.

2. THE AIM AND THE SCOPE OF RESEARCH

The aim of our study was to identify the fungi occurring inside of medical vehicles - melex (Fig. 1) and ambulances (Fig. 2) and the selection of appropriate materials for the preservation of aseptic interior.

3. METHODS OF INVESTIGATIONS

3.1. Methodology of bacteria and fungi identification

The research material was collected from the interior of medical vehicles: melex and ambulance, which are in daily use. The vehicle is used in hospitals, among others to transport patients between buildings. Smear tests were taken using sticks with cotton pad, after previous wetting physiological saline, and transported to
the laboratory for 24 hours, in a sterile test tube with the substrate AMIES. The conditions of smears downloading were as follows: the air temperature - about 10°C, humidity of the air – about 80%. Smear tests were taken from the following places: melex – patient bad (mattress), handle of the stretcher and armchair; ambulance – stretcher, handle of the stretcher, floor, cupboard and wall.

The material of the smear tests with bacteria taken from melex and ambulance were put in nutritious bouillon in order to multiply the bacterium (37 °C, 24 h). After the incubation period, material from containers was planted (with marbled culture) to the bases type: bloody agar (multiplying basis), gram negative bacilli bacteria bases: McConkey (for breeding gram negative bacilli), Chapman (for the Staphylococcus aureus isolation), selective Decoccosel base (to the isolation of the bacterium from the Enterococcus kind). After an incubation period (48 h), obtained mixed population of bacteria were isolated using streak plate techniques to isolate pure bacterial colonies. Next, to the final bacterial identification biochemical API test was made using VITEK 2 apparatus (BioMerieux). Additionally drug resistance mechanisms were also examined in the identified bacterial strains (i.e. MRSA, HLAR, MBL, KPC). Interpretation was done by the certified board of the division of bacteriology (laboratory medicine) as a routine interpretation.

The material of the smear tests with fungi were plated on the substrate Sabouraud, agar with chloramphenicol. Culture was performed at 36 °C (+/- 1 °) for 7 days and at 30 °C (+/- 1 °) for 14 days.

### 3.2. Material preparation

Samples for evaluation the adhesion of microorganisms were cut from aluminum sheet and plastic of size 100 x 100 mm. For research five kinds of samples were prepared: A1 - varnish antibacterial coating RAL 9003, A2 – varnish standard coating RAL 9002, B1 - plate of plastic – smooth, B2 – plate of plastic – plexi (organic glass), B3 – plate of plastic – chattered (Table 1).

<table>
<thead>
<tr>
<th>Characteristic of tested samples</th>
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<tr>
<td><strong>Group</strong></td>
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<td><strong>View</strong></td>
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<td><strong>Description</strong></td>
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On the surface of all samples the measurement of the roughness and 3D surface topography in a few randomly chosen areas was made. Examinations were conducted using the profile measurement gauge Perthometer Concept (MAHR). Surface roughness was evaluated on the basis of the value of the parameter Ra - arithmetic mean of the profile ordinates. The following Ra values were determined: A1 (0,08 μm), A2 (0,11 μm), B1 (0,17 μm), B2 (0,04 μm), B3 (3,20 μm). (Fig. 3)
Fig. 3 The surface roughness of the tested samples - values factor Ra

3.3. Methodology of adhesion examinations on model samples

Based on the results of microorganisms identification, for further research were chosen: Pseudomonas aeruginosa and Enterococcus faecalis (there were no fungi growth). Adhesion test in metal and plastic plates consisted in incubation of chosen strains of bacteria suspension at the density (0.05 - 7 McF) in the volume of 3 ml of phosphate buffered saline PBS (Sigma-Aldrich, Mo, USA) together with above mentioned plates (metal coated or plastic). Before seeding the bacteria on the plates, plates were also sterilized using UV-radiation (24 h) to avoid accidental contamination. Next, selected bacteria strains were seeded in streak plate technique at the appropriate plates and were incubated 24 hrs in room temperature (25 °C). After incubation period these plates were rinsing 3 times using PBS solution (x1). Afterwards, dry plates were impressed using Coun-Tact® media (BioMerieux, Fr) to elucidated amount of bacteria in tested surfaces (plates). The amounts of obtained bacterial colonies were presented in CFU/cm² (colony forming unit per cm²).

4. ANALYSIS OF RESULTS

In previous studies, inside ambulance has been identified the following pathogens: Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Bacillus spp, Staphylococcus haemoliticus, Pantoea agglomerans. In the vehicle melex were isolated additionally: Pseudomonas fluorescens and Pseudomonas oryzihabitans [9].

Results of adhesion research of Pseudomonas aeruginosa and Enterococcus faecalis on tested aluminum and plastic samples are presented in Table 2. It was stated that with the surface roughness increasing the level of bacteria adhesion also increases, but the colonization on the surface is independent of this factor. It is particularly visible in case of the adhesion of chosen bacteria strains on plastic surfaces. The roughness expressed by Ra coefficient has the highest value in the B3 sample - 3.2 µm, the smallest in B2 - 0.04 µm. In case of varnish coatings the value of the Ra coefficient in samples A2 and A1 is comparable and amount to 0.08 µm and 0.11 µm (Fig. 3). However the level of the adhesion on plastic samples is considerable higher than on varnish samples.
Table 2 Bacteria colonies on the tested surfaces

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Bacteria</th>
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<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<th>Density of bacterial cells [McF]</th>
<th>Amount of bacterial colony [CFU/cm²]</th>
<th>Amount of bacterial colony [CFU/cm²]</th>
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<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>&gt;150</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>0.5</td>
<td>&gt;250</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>&gt;300</td>
<td>&gt;150</td>
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The modern sterilization techniques and the disinfection procedures of medical interiors are insufficient to eliminate the risk of the appearance of infection. As research has shown, despite the use of guidelines for aseptic interiors medical vehicle, bacteria were present on the examined surfaces. Probably the main reasons are: the mobility of vehicles, the strong disinfectants, hardly accessible places for disinfection, bacterial resistance and biofilm produced by bacteria.

The present study showed, that independently of the surface, there was not observed fungi growth on the test surfaces. It can be assumed that these microorganisms react differently to disinfectants and are more sensitive. Probably this situation is caused by a lower adhesion of fungi to the surface than bacteria. Works concerning of hospital infections [1-4,10] confirm that not only bacteria, but also fungi are a common pathogenic factor. Observed divergences suggest the need for further research.

4. CONCLUSIONS
On the basis of the performed studies and discussion of the results we can conclude:
1. From the interior of the medical vehicles were isolated mainly bacteria: *Pseudomonas aeruginosa* and *Enterococcus faecalis*, that can cause infection.
2. No fungal growth from smear tests was stated, independently of the surface, that confirm a high standard of aseptic protection both melex and ambulance.
3. Due to bacteria and fungi are considered as pathogenic factors, as shown in the literature review, we should pay special attention to the selection of appropriate disinfectants and materials in order to achieve aseptic medical vehicle interiors.

ACKNOWLEDGEMENTS
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LITERATURE
[1] MARCHLIK W.D., KURNATOWSKI P. Fungi as pathogens in nosocomial infections. Otoarynolaryngologia 2010, 9(2), s.50-54