BACTERIA AS THE MENACE TO HUMAN LIVE IN SANITARY AMBULANCES

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Abstract

In the study the research findings are described regarding the application of innovative solutions from the field of the material science and medicine to the inside of modern ambulances. The aim of investigation was the most appropriate material and coating choice from the point of view of high sterility level of the inside of an ambulance. Examinations were conducted in the two stage procedure. Firstly, bacteria taken from the ambulance body paneling being in the everyday use were examined. However the second stage included the inoculation and the incubation of identified bacteria on appropriately prepared surfaces of metal sheets of ambulance body paneling with antibacterial paints and coatings. In the course of examinations the topography of the surface, the adhesion and the lifetime of incubated bacteria colony was estimated.

Keywords: bacteria, adhesion, ambulance, melex, varnish coatings, plastic

1. INTRODUCTION

The subject of the hospital infections is constantly discussed. The last data estimates that the hospital infection was the fourth reason of death in the USA and Europe [1, 2]. *Staphylococcus epidermidis*, *Staphylococcus aureus* are the most typical pathogens identified in the nosocomial circle [3]. Moreover the examinations conducted in Chicago indicate the great resistance to antibiotics of *Staphylococcus aureus* detected in ambulances. Despite of application of different techniques of cleaning, the pathogens still stay on surfaces of the ambulance [4]. The very important element of the resistance of some bacteria is the biofilm creation [5, 6]. It is protecting the micro-organisms from disinfectants. By analogy it can be concluded that the discussed problem of infection can concern also the inside of the medical ambulance.

Not every departments of ambulance service have a high standard of the disinfection of the insides. The mobile character of the ambulance functioning, as well as applying strong disinfectants support the phenomenon of the corrosion and the adhesion of micro-organisms on the abiotic surfaces [7-9]. The interior of the ambulance can be the source of the patient infection. Therefore implementing of all procedures making easier keeping the appropriate purity is extremely significant.

Relying on previous experiences and own research findings [7-10] authors decided to widen the investigation spectrum and to concentrate on examining the adhesions of bacteria on surfaces of body paneling of sanitary ambulances.

2. THE AIM AND THE SCOPE OF RESEARCH

The attention was focused on the body paneling of sanitary ambulances: paneling of internal walls made of aluminum sheet (A1 and PA11) and of plastic (three applications). The aluminum surface was coated with a standard and antibacterial varnish layer.

The investigation aim was the most appropriate choice of materials and anticorrosion coating for keeping the sterility of the inside of an ambulance constructed on the HONKER chassis (Fig. 1) used in special conditions (Azerbaijan).

Fig. 1 The view of military ambulance a - outside view of Honker; b, c - inside survival equipment

Research works were conducted is two stage procedure. Firstly, bacteria taken from the ambulance and melex body paneling being in the everyday use were examined. However the second stage included the inoculation and the incubation of identified bacteria on appropriately prepared samples.

3. METHODS OF INVESTIGATIONS

3.1 Material preparation

Material for testing was taken from the inside of sanitary vehicles: ambulance and melex being in the everyday use. Smear tests were made using special transport applicator “Amies” type with agar gel after prior moistening the swab with saline solution. Samples were taken from the following places: ambulance - 1. plastic part – inside cabinet, 2. steering wheel, 3. ceiling, 4. floor, 5. side walls – metallic parts, 6. stretcher-mattress ; melex – 1. plastic part – hand-rail, 2. metal part (aluminum) – patient bed, 3. corroded metal part – patient bad, 4. plastic part – cockpit. Samples were appropriately secured during transport to the bacteriological laboratory where strains of bacteria were isolated.

In the second stage the adhesion of chosen pathogens on tested materials were evaluated. For this purpose samples with dimensions 100 x 100 mm were cut from aluminum sheet and plastic. Investigated material was divided in two groups depending on the kind of the base: **A1** - varnish antibacterial coating RAL 9002, gloss 90%; **A2** – varnish standard coating RAL 9003, gloss 60%; **B1** - plate of plastic - smooth; **B2** – plate of plastic – plexi organic glass; **B3** – plate of plastic – chattered. Farther, on the surface of all samples the measurement of the roughness and surface 3D 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. Fig. 2 illustrates the topography of the samples’ surface.

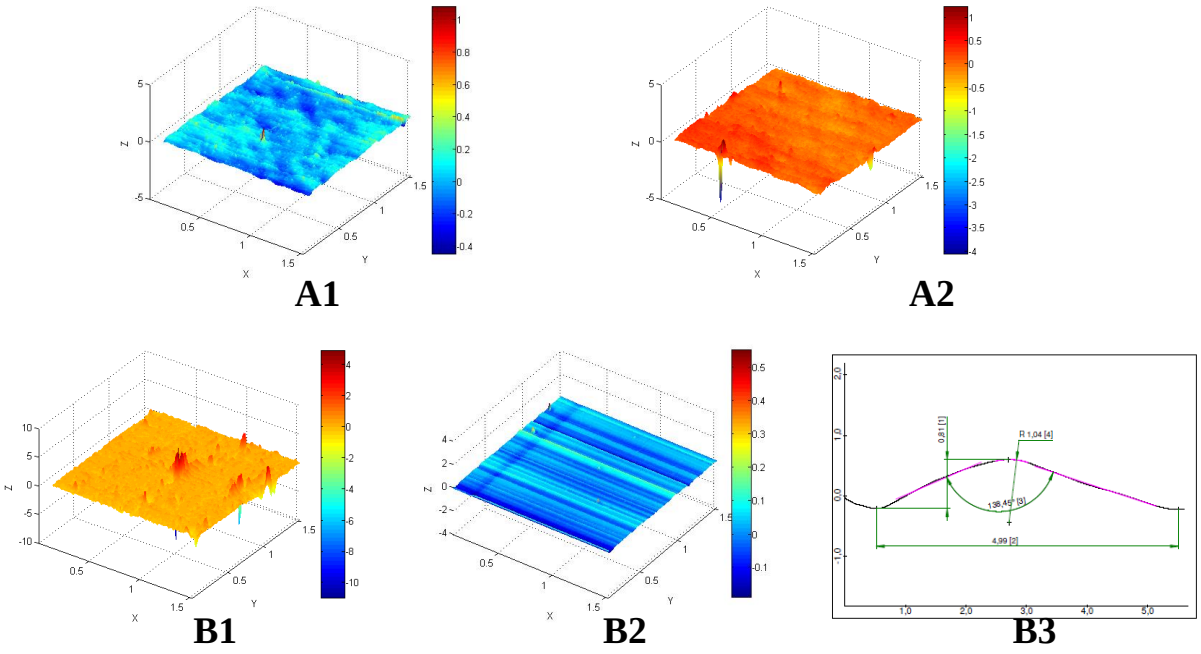

Fig. 2 The surface topography of tested samples

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).

3.2 Methodology of the bacteria identification

Aseptic containers with bacteria taken from ambulance were put in nutritious bouillon in order to multiply the bacterium (37 ^{o}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), Sabouraud base (for breeding Blastomyces from the *Candida* kind and fungus yest similar), selective Decoccosel base (to the isolation of the bacterium from the Enterococcus kind). After an incubation period (48 hrs), 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.

3.3 Methodology of adhesion examinations on model samples

Basic upon identification results to further examination *Pseudomonas aeruginosa* and *Enterococcus faecalis* existing in the inner surfaces of medical vehicle were chosen. 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 hrs) 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 the 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/ cm^2 (colony forming unit per cm^2).

4. ANALYSIS OF RESULTS

The following pathogens were identified inside the medical ambulance: *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Bacillus spp*, *Staphylococcus haemoliticus*, *Pantoea agglomerans*. In the melex vehicle *Pseudomonas fluorescens* and *Pseudomonas oryzihabitans* were isolated. The percentage of contaminated places in the ambulance and melex is given in Table 1 and 2. In no case in isolated species of the bacterium a mechanism of the resistance to antibiotics applied in therapy wasn't stated (MRSE, MRSCN, MBL, HLAR).

Tab. 1 The percentage of contaminated places inside ambulance

Place of analysis	% of surface	Identified bacterium kind
Stretcher mattress (n=4)	20	*Bacillus spp*
metal part of stretcher (n=9)	30	*Enterococcus faecalis* *Bacillus spp*
Steering wheel (n=4)	50	*Pseudomonas aeruginosa*
Floor (n=10)	50	*Enterococcus faecalis* *Staphylococcus epidermidis* *Staphylococcus haemoliticus* *Pantoea agglomerans*
Ceiling (n=10)	10	*Staphylococcus epidermidis*
Metal part of ambulance wall (n=4)	40	*Bacillus spp*

Tab. 2 The percentage of contaminated places inside melex vehicle

Place of analysis	% of surface	Identified bacterium kind
Handrail of the patient's bed (n=9)	20	*Bacillus spp* *Pseudomonas fluorescens* *Pseudomonas oryzihabitans*
Cockpit (n=9)	30	*Enterococcus faecalis* *Pseudomonas stutzeri* *Bacillus spp*
Corroded metallic part of patient's bed (n=10)	50	*Enterococcus faecalis* *Staphylococcus epidermidis* *Bacillus spp*

Results of adhesion research of *Pseudomonas aeruginosa* and *Enterococcus faecalis* on tested aluminum and plastic samples are presented in Tables 3-4. The modern sterilization techniques and the disinfection procedures of medical interiors are insufficient to eliminate the risk of the appearance of infection. The problem regards both of the hospital circle and the inside of sanitary vehicles. 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. 2). However the level of the adhesion on plastic samples is considerable higher than on varnish samples.

Tab. 3 Bacteria colonies number on the tested surfaces - **A1)** antibacterial varnish (RAL 9003); **A2)** standard varnish (RAL 9002)

Samples number	Bacterial strain	Density of bacterial cells [McF]	A1	A2
			Amount of bacterial colony [CFU / cm^2]	
n=6	*Pseudomonas aeruginosa*	0.5	-	0
		1	0	0
		3	0	-
		5	0	> 150
		7	1	-
n= 6	*Enterococcus faecalis*	0.5	-	0
		1	0	0
		3	0	-
		5	1	> 180
		7	1	-

Tab. 4 Bacteria colonies number on the tested surfaces - **B1**) Plastic - plate (smooth surface); **B2**) Plastic - plexi plate; **B3**) Plate of plastic – chattered

Samples number	Bacterial strain	Density of bacterial cells [McF]	B1	B2	B3
			Amount of bacterial colony [CFU / cm^2]		
n=6	*Pseudomonas aeruginosa*	0.05	> 10	>10	-
		0.5	> 250	>100	0
		1	-	-	0
		5	> 300	>150	>500
n= 6	*Enterococcus faecalis*	0.05	>10	>10	-
		0.5	> 150	>100	0
		1	-	-	0
		5	>250	>150	>600

Bacteria isolated from the surface of internal ambulance paneling can be a certain threat to patients health with lowered immunity and/or during immune-suppressing treatment, particularly *Enterococcus faecalis*, *Staphylococcus haemoliticus*. However a *Staphylococcus aureus* bacterium responsible for hospital infection dangerous to the life of patients wasn't isolated including MRSA. *Staphylococcus epidermidis* and *Enterococcus faecalis*, *Pseudomonas aeruginosa* are the most frequent isolated pathogens from the interior of the sanitary ambulance (Tab. 1). In the melex vehicle *Pseudomonas spp.* and *Enterococcus faecalis* bacteria were identified (Tab. 2). Authors aren't able to state, whether tested vehicles are pointing at the permissible level of bacterial pollutant. However, undoubtedly pathogens can be a threat to patients health with the lowered immunity or during immune-suppressing treatment. As a result of conducted adhesion analyses of the chosen bacteria strains (*Psudomonas aeruginosa*, *Enterococcus faecalis*) authors stated, that varnish antibacterial coating (RAL 9003) on A1 samples effectively protects from adjoining of tested pathogens. In tested densities of the bacteria culture (1-5 McF) on A1 (RAL 9003) plates, we didn't state the

presence of the bacteria after 24 hours of the incubation (Tab. 3). Adjoining and survival rate of tested bacteria on standard A2 (RAL 9002) plates is higher in comparison to A1 (RAL9003) plates. After 24 hours of the incubation of bacteria cultured on plates A2 (RAL 9003) in density 5 McF, over 150 bacterial colonies at the cm^2 of surface were observed that confirms the high level of the surface infection. However standard plates were sterile after the bacteria culture in lower concentrations (0.5-1 McF). It was stated that tested bacteria adjoin easily to plastic surface. After 24 hours of the incubation, depending on micro-organism kind and the density of the culture, different contamination of the tested surface was observed >300 CFU/cm^2 (*Pseudomonas aeruginosa*), >250 CFU/cm^2 (*Enterococcus faecalis*) at the density of culture 5 McF. The analogous density of the culture on chattered plates results in the even higher percentage of adjoining bacterial colonies after 24 hours of the incubation >500 CFU/cm^2 (*Pseudomonas aeruginosa*), < 600 CFU/cm^2 (*Enterococcus faecalis*).

5. CONCLUSIONS

On the basis of conducted laboratory examinations and results discussion the following conclusion can be formulated:

1. Bacteria isolated from the surface of internal ambulance paneling can be a certain threat to patients health with lowered immunity and/or during immune-suppressing treatment, particularly *Enterococcus faecalis*, *Staphylococcus haemoliticus*. However a *Staphylococcus aureus* bacterium responsible for hospital infection dangerous to the life of patients including MRSA wasn't isolated.

2. The varnish coating RAL 9003 covers the surface effectively and protects against adhesion of tested micro-organisms in a wider range of culture in comparison to standard plates RAL 9002.

3. It was stated that with the surface roughness increase the level of bacteria adhesion increases, but the colonization on the surface is independent of this factor. The most easily run the adhesion of bacteria cultured on plastics particularly on chattered surface in comparison to the smooth one.

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