

Making the Hospital a Safer Place by Sonochemical Coating of all its Textiles with Antibacterial Nanoparticles.

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Abstract

The ability to scale-up the sonochemical coating of medical textiles with antibacterial nanoparticles is demonstrated in the current paper. A roll-to-roll pilot installation to coat textiles was built taking into consideration the requirements of the sonochemical process. A long-run experiment was conducted in which 2500 m of fabric were coated with antibacterial ZnO nanoparticles (NPs). The metal oxide NPs were deposited from an ethanol:water solution. In this continuous process a uniform concentration of coated NPs over the length/width of the fabric was achieved. The antibacterial efficiency of the sonochemically-coated textiles was validated in a hospital environment by a reduction in the occurrence of nosocomial infections. NP-coated

bed sheets, patient gowns, pillow cover, and bed covers were used by 21 patients. For comparison 16 patients used regular textiles. The clinical data indicated the reduced occurrence of hospital-acquired infections when using the metal oxide NP-coated textiles.

In order to reduce the cost of the coating process and considering safety issues during manufacturing, the solvent (ethanol:water) (9:1 v:v) used for the long-run experiment, was replaced by water. Although lesser amounts of ZnO NPs were deposited on the fabric in the water-based process the antibacterial activity of the textiles was preserved due to the smaller size of the particles.

1. Introduction

Hospital-acquired nosocomial infections are a major health and financial issue worldwide. The problem of bacterial infections in general, and in hospitals in particular, has led to extensive research and to industrial efforts to produce antibacterial textiles. The financial impact of these infections overwhelms the current medical advances by increasing the length of hospitalisation by at least 8 days on the average per affected patient, resulting in more than 10 million patient days in hospitals in Europe per year [1]. The statistics on patient safety in the EU show alarming tendencies: 1 in 10 patients are affected by hospital-acquired infections, 3 million deaths are caused yearly by hospital-acquired infections.

In the last 2 decades, due to the continuous consumption of antibiotics, and the evolution and spread of resistance genetic determinants, multidrug resistant (MDR) and even extremely drug resistant (XDR) bacteria that cause life-threatening infections have emerged [2]. An active infection control program could significantly reduce both the number of infections and hospitalisation costs.

The most common sources of infectious agents causing healthcare problems are: the individual patient, medical equipment, the hospital environment, and the healthcare personnel. Although the person-to-person transmission route is the most likely, the role of the environment should not be ignored and hospital textiles may contribute to the spread of nosocomial infections. Since textiles are a common material in healthcare facilities, it is important that they do not transfer pathogens to patients or hospital workers. Healthcare textiles include bed sheets, blankets, towels, personal clothing, patient apparel, uniforms, gowns, and drapes for surgical procedures [3, 4].

Medical textiles are often contaminated by microorganisms originating from body substances, including blood, skin, stool, urine, vomits, and other body fluids and tissues.

The growing need for antibacterial textiles has resulted in revolutionary progress in the textile industry [5]. In the last decade, the design of new methods of fabric finishing has included the use of metal and metal oxide nanoparticles that have a high surface area and can be finely spread on the surface of the substrates [6-8]. The metal oxides can be deposited as a separate phase or in a combination of composite nanostructured materials. Most of the methods for antibacterial finishing of textiles are based on multistage procedures and require toxic templating and binding agents for the anchoring of the nanoparticles on the substrate. As a result of earlier attempts to sonochemically coat small pieces of cotton, polyester, nylon, and wool with antibacterial nanoparticles (NPs) [9, 10] successfully kill of bacteria, a small machine operating in continuous mode for sonochemical coating of textile was built [11]. The machine was later upgraded to two industrial scale coating machines for continuous deposition of NPs on 40 cm wide fabrics at a speed of 1-3 m/min. ZnO and CuO NPs were coated on cotton and polyester fabric using these machines. Excellent antibacterial properties of the coated fabrics were maintained after 65 washing cycles in hospital washing regimes (75 and 92 °C) [12]. We have also demonstrated the ability of the metal oxide NPs to eradicate resistant bacteria [13].

The present manuscript reports on the improvement of the original sonochemical process for the synthesis and simultaneous deposition of metal oxide NPs on fabrics, making the synthetic route safer and more cost efficiently using water instead of an ethanol:water solution. Previously, the metal oxide NPs were prepared by the basic hydrolysis of the corresponding metal acetates in 9:1 ethanol:water (v:v). The introduction of ignitable ethanol in an industrial plant not only requires special protective equipment, but is also more costly than using water as the only solvent. When water replaced the ethanol:water mixture, the amount of oxides on the textile was smaller, however, the particle size was also reduced and as a result the antibacterial activity was not hampered.

This article also presents clinical results obtained in an experiment conducted in a hospital (Institute for Emergency Medicine "N.I. Pirogov" (IEM), Sofia, Bulgaria) where patients were dressed, and slept on antibacterial (ZnO) coated fabrics and their propensity to bacterial infection was monitored and compared to control patient using

uncoated textiles. Since the mechanism of the sonochemical coating has been explained previously [14, 15], it is not discussed in the current paper.

2. Experimental

2.1 Characterization

The batch experiments were carried out using a Sonics and Materials instrument (Ti-horn, booster, 20kHz, 750 W at 30 % efficiency). The roll-to-roll coating was carried out using a sonochemical coating pilot installation constructed by CEDRAT Technologies, Grenoble, France.

The X-ray diffraction (XRD) patterns of the product were determined using a Bruker D8 diffractometer with Cu K α radiation. The particle morphology and size distribution have been studied with a high-resolution scanning electron microscope (HRSEM), FEI. The Cu and Zn concentrations on the cotton fabric were determined by ICP analysis on the ULTIMA 2 model of after their dissolution from the fabric using 0.5 M HNO₃.

2.2 Water based synthesis of metal oxide NPs and their coating on cotton.

The coating process of ZnO and CuO from an aqueous solution required the preparation of 0.01 M aqueous solutions of the corresponding metal acetates. A 10x10 cm piece of cotton (supplied by Klopman Int., Italy) was introduced in a 120 mL flask filled with 100 mL of the acetate solution. The sonication was conducted at 30 % amplitude of a 750 W booster sonicator for 30 min. When the solution reached a temperature of 60 °C (approx. after 5min of sonication), 25 % aqueous ammonia solution was added drop wise to adjust the pH to 8. During the sonication the flask was placed in a cooling bath maintaining a constant temperature of 30 °C. The coated fabric was washed thoroughly first with water to remove traces of ammonia, then with ethanol, and dried under vacuum at room temperature.

2.3 Roll-to-roll coating of textiles with ZnO NPs using the large scale sonochemical installation

For the hospital experiments large quantities of ZnO-coated cotton fabrics were prepared by employing the sonochemical roll-to-roll pilot installation (Figure 1). The maximum width of fabric that can be treated in this machine is 40 cm. The

capacity of sonochemical tank is about 160 L. 140 L ethanol were added to the sonochemical tank. 350 g of $\text{Zn}(\text{Ac})_2$ or 320 gr of $\text{Cu}(\text{Ac})_2$ were dissolved in 16 L of water and added to the sonochemical tank to reach a final metal acetate concentration of 0.01 M. After raising the temperature to 60 °C, 400 mL of 25 % aqueous ammonia was added to the reaction solution. The pH of the solution changed to 8, and at this stage, the rolling of the fabric has been started. To compensate the depletion of the metal acetate during the hydrolysis, 2.5 % of the initial amount of the corresponding metal acetate was dissolved in 100 mL and added every 10 min to the sonicated solution. In addition 40 mL of original ammonia solution was added at the same time. These 140 mL are added as long as the coating process is carried out.

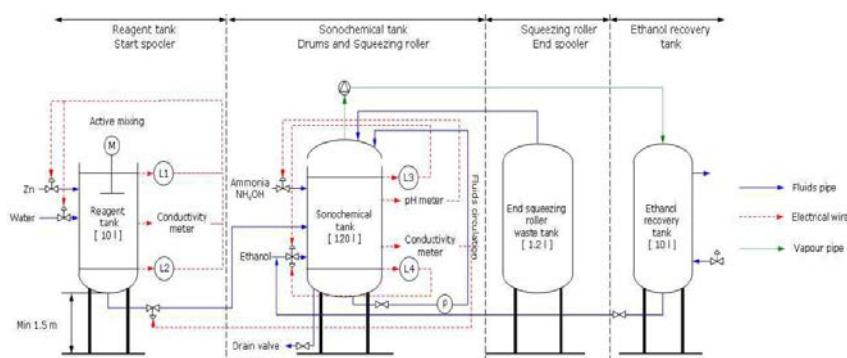


Figure 1. Scheme of Roll-to-roll pilot installation for sonochemical coating

2.4 Antibacterial Tests of ZnO NPs formed by the "new" hydrolytic process

Whether the elimination of ethanol as the solvent in the hydrolysis of the metal acetates can be attractive for industry, depends on the antibacterial performance of the fabric. Therefore the antibacterial activity of metal oxide-coated fabrics

prepared by the new hydrolytic process was tested against *E. coli*. Overnight cultures of the bacteria were transferred into a nutrient broth (NB) ("Difco" Detroit, MI) and grown in at 37 °C with aeration. When the cell number reached 10⁶ CFU, the cells were harvested by centrifugation and washed twice with a 0.85 % NaCl solution at pH 6.5 (saline). The fabric (2x2 cm) was placed in a vial (d = 2.5 cm) containing 2 mL of bacteria in saline. The bacterial suspensions were incubated for up to 60 minutes at 37 °C with agitation (170 rpm). An aliquot (100µL) was taken at different time intervals (0, 7, 15, 30 and 60 min) and plated on nutrient agar plates after 10-fold dilution in saline. The plates were allowed to grow overnight at 37 °C and the viable bacteria were counted.

3. Using antibacterial textiles in the hospital

The study includes 37 patients with burns and different soft tissue lesions spread on areas from 5 to 30 % of the body surface. The patients were divided into two groups: 21 using antibacterial textiles and a control group of 16 using standard type hospital textiles. Each individual set of coated textiles was changed every 12 h. The fabrics were washed for 60 min at 60 °C with a non-ionic detergent. The following parameters were observed and analyzed over a seven-day period – demographic, haemodynamic, haematologic, biochemic, microbiologic more was taken of comfort and side effects. Microbiological samples were collected for both groups from the throat, nose, armpit, perineum and prints from the hospital textile on the 1st, 4th and 7th day. Quantitative and qualitative analyses of all isolated pathogens were performed. The body site's samples were taken using cotton-tipped sterile swabs and transported to the microbiological laboratory within 30 minutes or in Steward's transport medium. Cultures were prepared using standard procedures on blood agar, on the selective medium Endo agar and on Sabouraud agar/ Chromagar Candia (BD). After incubation at 35-37 °C for 18-24 h, the cultures were inspected for microbial growth. The identification of the isolated strains was done using conventional procedures or the automated system Vitek-2 Compact, Bio-Merieux. Contact plates - Contact E, Merck, were used to take samples from the textile, and the samples were incubated for 18-24 h at 35-37 °C and examined for microbial growth. The microbial growth was both qualitatively assessed for the presence of pathogens and quantitatively analyzed for determination of the total microbial number of all growing microorganisms. The following breakpoints were used: no microbial growth; 1

CFU/cm² – very weak growth; 3.5 CFU/cm² – weak growth; 17 CFU/cm² – moderate growth; 58 CFU/cm² – heavy growth; above 140 CFU/cm² – very heavy growth. The target pathogens included: *Staphylococcus aureus*, beta-hemolytic streptococci of various serological groups, *Enterococcus spp.*, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, other non-fermentative Gram-negative bacteria and fungi.

Data were processed with statistical package SPSS 13.0. The statistic significant level for rejecting the null hypothesis was chosen as $p < 0.05$. Various statistical methods were employed in the analysis of the results.

4. Results and Discussions

4.1 Water based synthesis of metal oxide NPs and their coating on cotton.

The crystallinity of the nano-oxides prepared sonochemically by aqueous hydrolysis was studied using XRD measurements. The XRD patterns of sonochemically prepared CuO and ZnO NPs are shown in Figure 2a and 2b, respectively. The CuO NPs are crystallized in a base-centered monoclinic tenorite phase (PDF: 01-089-2529). The peaks at $2\theta = 35.56$, 38.74 , and 48.74 are assigned to (-111), (111) and (-202) reflection planes. The crystallite size was estimated by the Debye-Scherrer equation and found to be around 10 nm.

The XRD patterns of the sonochemically prepared ZnO NPs correspond to hexagonal phase of zincite (Figure 2b). The peaks at $2\theta = 31.772$, 34.420 , 36.256 , 56.602 , and 62.858 , are assigned to the (100), (002), (101), (110), and (103) reflection planes, respectively (PDF: 01-089-1397). The crystallite size estimated by the Debye-Scherrer equation is about 19 nm. No characteristic peaks of impurities were detected. The crystalline structure of metal oxides obtained from water-based synthesis is identical to that from the ethanol-water synthesis.

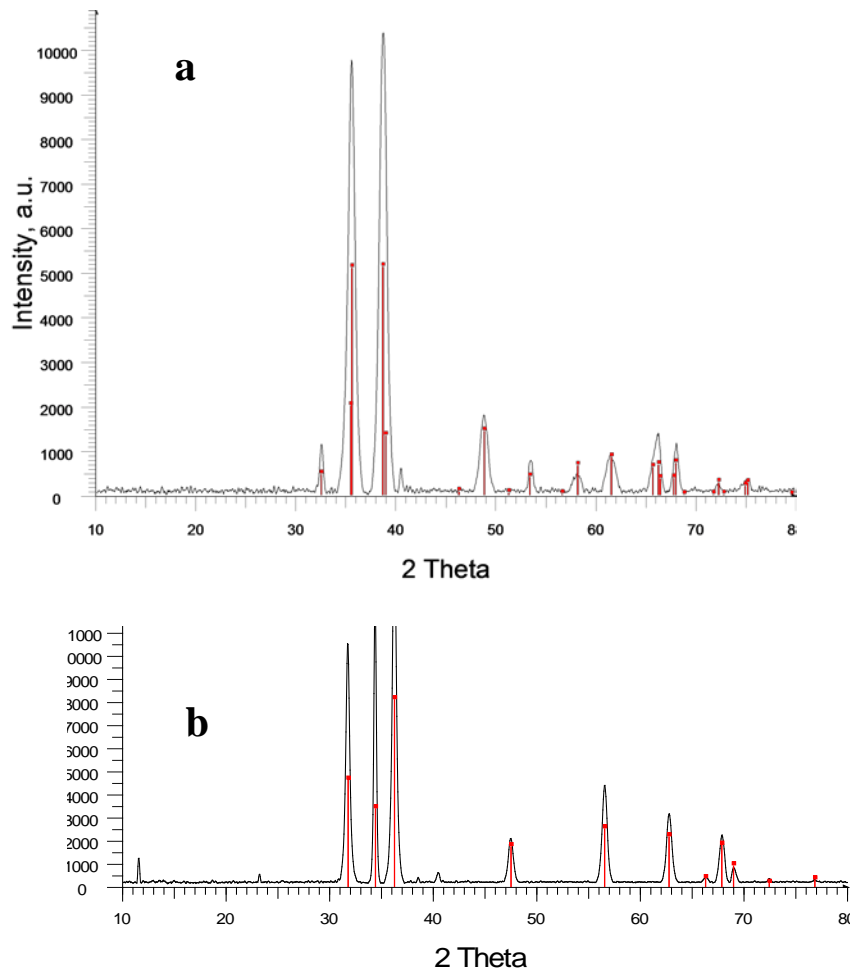


Figure 2. XRD of CuO (a) and ZnO (b) NP's

The morphology of the coated CuO and ZnO NPs was studied by HRSEM and is presented in Figures 3a and 3b, respectively. The average size of CuO and ZnO NPs on cotton is ~30 nm. The same value obtained from the width of the XRD diffraction lines is smaller than 30 nm. We believe that due to resolution limitation of the SEM instrument the particle size obtained from SEM measurements exceed that obtained from the Debey-Scherrer calculations. The amount of the metal oxide on the cotton fabrics was calculated by ICP and estimated to be 0.44 % wt of CuO and 0.48

% wt of ZnO. Our initial studies on coating metal oxides were performed with 9:1 ethanol:water solution, yielding 0.8-0.9 % wt of coated metal oxide. However, in the latter approach the particles were aggregated resulting in 100 to 150 nm aggregates. In case of water-based synthesis, we observed that the coated amount was lower but the particles' size was also smaller. The aqueous coating leads to a uniformly deposited layer of NPs on the fibres. It is suggested that the smaller particle size will compensate for the lower amount of coating and the activity of metal oxides prepared from water will not be reduced compared to the ethanol synthesis. A number of research works were published indicating that smaller nanoparticles are more biocidal [16, 17].

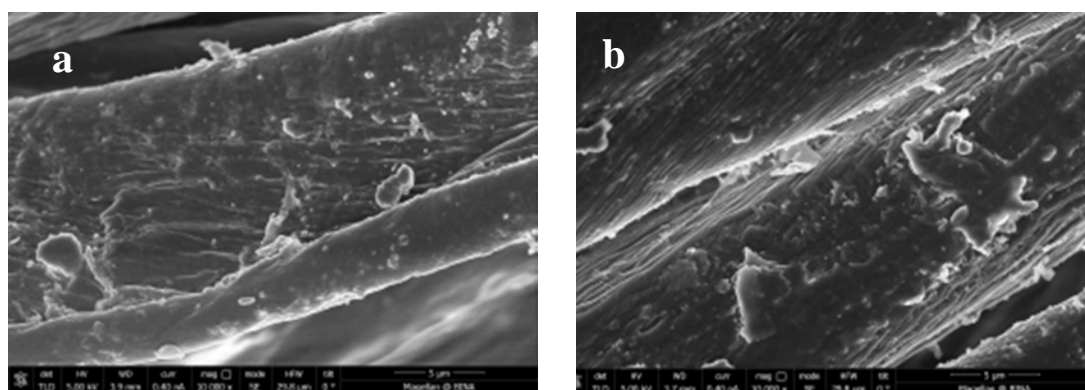


Figure 3. HRSEM of cotton fabric coated with CuO (a) and ZnO (b) NP's.

The antibacterial properties of the ZnO and CuO coating produced from water-based synthesis were evaluated against *E. coli* (Figure 4). Both coatings showed a time dependent antibacterial activity eliminating more than 97 % of bacteria for less than an hour. Two and a half log reduction was obtained for the ZnO coated fabric after 1 h. The activity of CuO fabric was higher - after 30 min a 4.5 log reduction was achieved.

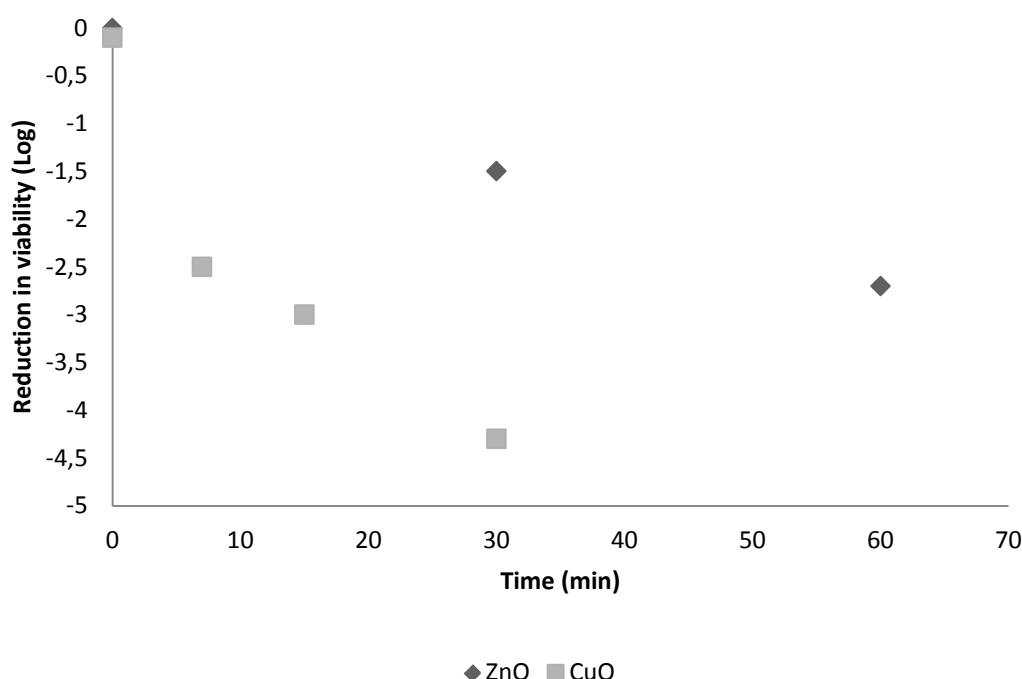


Figure 4. Antibacterial activity of ZnO and CuO coated bandage against *E.coli*

4.2 Roll-to-roll coating of ZnO on textiles to be used in hospital

This manuscript reports experiments conducted in a hospital where 21 patients slept on antibacterial bed sheets, used antibacterial pillow covers, and were dressed with antibacterial pyjamas. The preparation of the NPs-coated cotton textiles was performed on the machine capable of coating of ZnO NPs in a continuous mode. One of the important aspects in the continuous coating process is the ability to maintain a constant amount of coating along the running fabric. The depletion of the metallic ion in the reaction tank leads to a decrease in the amount of coating. A compensation solution of 2.5 % of the initial amount of the precursor was added to the reaction tank at defined time intervals. After a series of trial and error experiments, it was found that the amount of coating is kept constant if the compensation solution is added every 10 min. The speed of the rolling of fabric during the coating process was 0.5 m/min. In order to evaluate the quality of the continuous process, the coated amount was probed over 50 m of fabric. Every 10 m a piece of fabric was cut off for ICP analysis and the results are shown in Table 1. About 0.3 % wt of ZnO was coated on textile. The results indicate that the addition of compensation solution at defined time intervals (every 10 min) leads to a constant amount of coating along the fabric.

Table 1. Concentration of ZnO along 50 m of coated cotton fabric

Sample	wt % ZnO
1	0.26
2	0.28
3	0.3
4	0.29
5	0.3

The morphology of the treated fabric was studied as well showing a homogeneous coating with nanoparticles (Figure 5). The results are in a good agreement with the data obtained from lab-scale coating experiments.

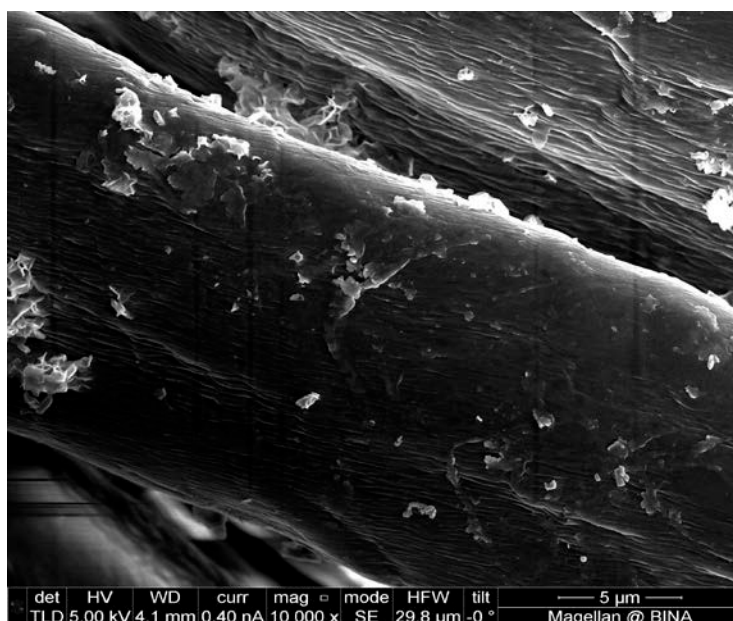


Figure 5. HRSEM image of textiles coated by the sonochemical pilot installation

5. Validation of the ZnO coated textiles in a hospital environment

Although many results regarding the antibacterial properties of the metal oxide coated fabrics have been already obtained, in what can be considered as *in vitro* studies, the examination whether they will function the same way *in vivo* remained

unclear. For this reason we have designed an experiment in a Hospital to probe whether the use of the antibacterial textiles can indeed reduce the level of nosocomial infections. The Hospital chosen for these *in vivo* experiment was Pigorov Hospital Sofia, Bulgaria. For this purpose 2500 m of ZnO-coated fabric was used for bed sheets, bed covers, pillow covers and patients gowns (Figure 6) and such were used in a clinical trial conducted in the Pigorov Hospital. The results are presented in the section below.



Figure 6. Dress gown confected from the ZnO-coated fabric

During the study two main nosocomial pathogens – *S. aureus* and *Acinetobacter baumannii* were isolated from various body sites and from the hospital textile [18-20]. In Figures 7 and 8 the throat and nose colonization with *S. aureus* and dynamic rates during the study are presented. Higher percentage of controls was colonized with *S. aureus* in comparison with the patients throughout the study duration. The patients' throat contamination remained at stable levels, while in the control the bacterial level increased from day 0 to day 4 and 7. Nose colonization in controls, increased from day 1 to day 7, while it decreased in the patients group.

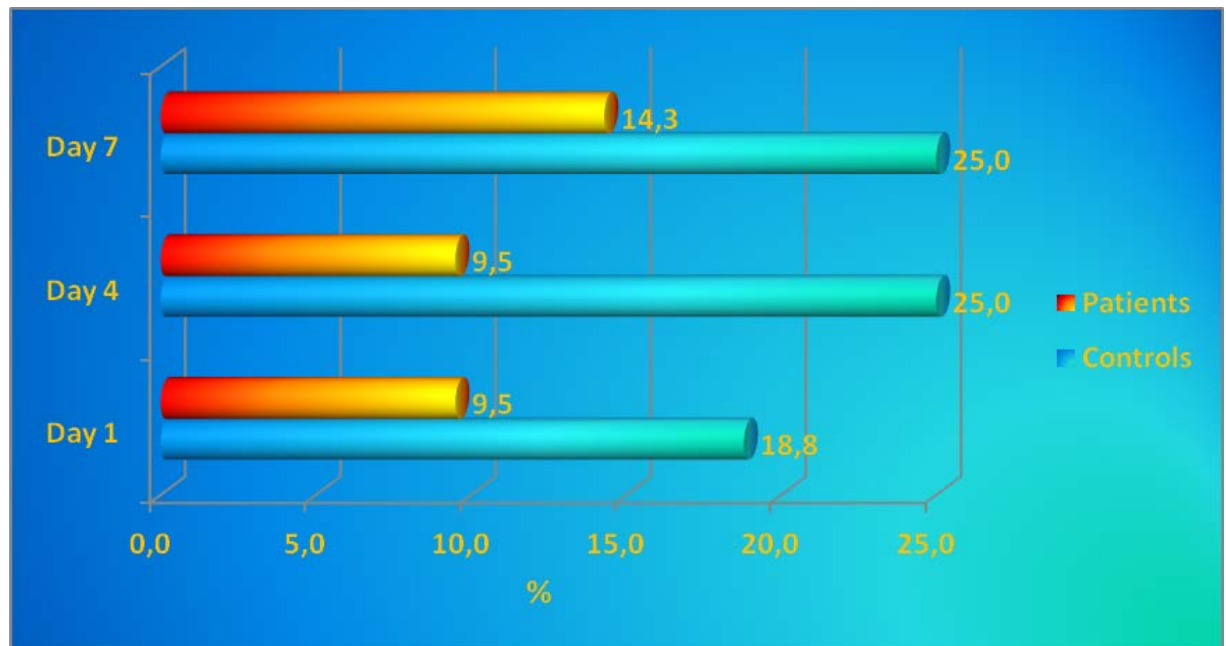


Figure 7. Throat *S. aureus* rate dynamics during the study.

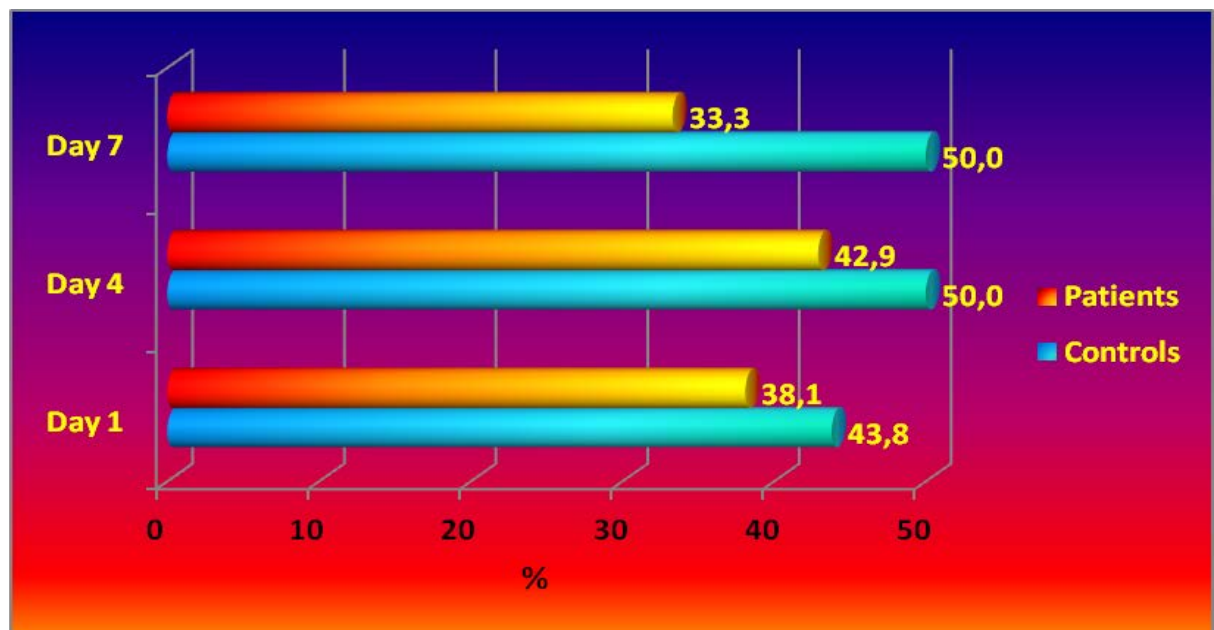


Figure 8. Nose *S. aureus* rate dynamics during the study

A. baumannii is one of the most problematic nosocomial pathogens in hospitals with typical multidrug antibiotic resistance (MDR) and ability to survive for long time in the hospital environment even under unfavourable conditions [21-23]. As presented in Fig. 9, perineum colonization of the controls with *A. baumannii*

increased from day 1 to day 7 from 0 to 18.8 %, while in the patients it remained comparatively stable.

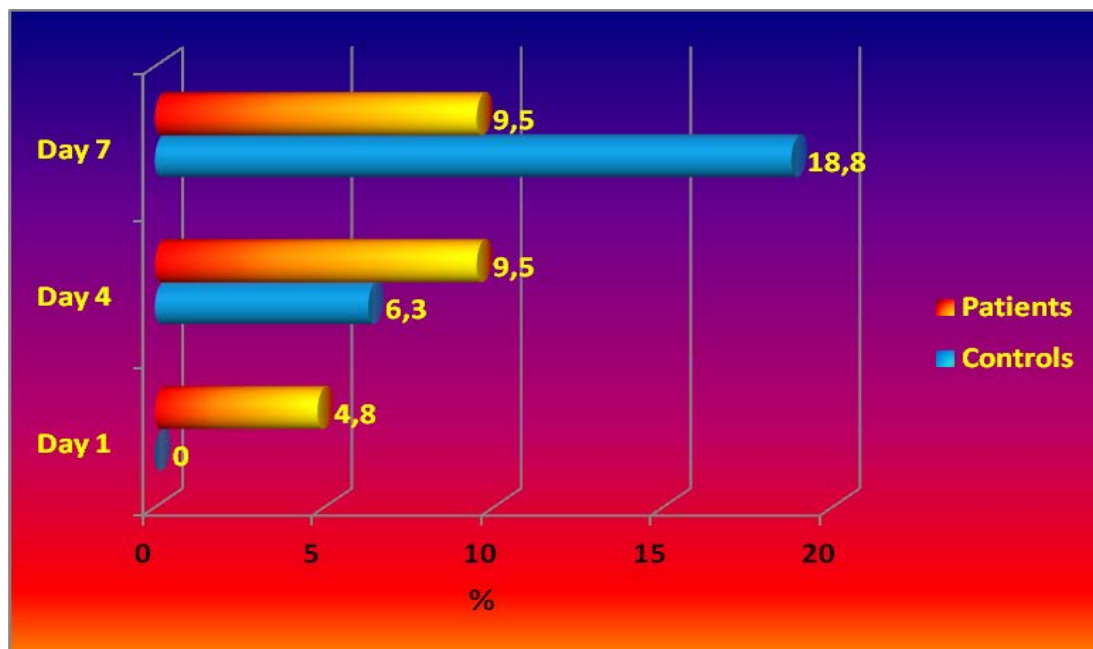


Figure 9. Perineum *A. baumannii* rate dynamics during the study

These three experiments and their statistical analysis demonstrate that indeed the “antibacterial” patients show a lower level of bacterial infection than the “regular” patients. Thus both *in vitro* and *in vivo* experiments are leading us to conclude that the step to be taken for making the hospital a safer place is using only textiles coated by antibacterial NPs. We recommend that the coating should be done sonochemically because it will guarantee the ability to maintain the antimicrobial properties for a long time and many washing cycles.

Conclusions

The current work presents the results obtained during a European project aimed at building two large sonochemical machines capable of coating hospital textiles with antibacterial NPs. The results demonstrate that the patients using the coated textiles were less contaminated with nosocomial pathogens. In addition, the coated textiles do not show toxicity. Side effects such as itching, erythema and rash were observed in both groups without significant difference. The nano-coated textiles possess noticeable antibacterial effect, as they maintained lower contamination level

and better microbiological characteristics both on the patients and the bed linen in comparison with conventional linen even regard multidrug-resistant *A. baumannii* (MDR-AB) - one of the nosocomial pathogens, which are extremely difficult to eradicate.

The current paper reports also results that were obtained after the completion of the project answering a problem raised by the industrial partners, namely, the use of ethanol as a solvent. Ethanol is an expensive solvent on the one hand side, and an ignitable compound that also makes the process more expensive raising safety issues. We have demonstrated that the use of ethanol can be avoided without deteriorating the antibacterial properties of coated textiles. The water-based process yields less metal oxides on the fabric but having also smaller size.

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