Introduction

VRE (vancomycin-resistant enterococci) colonization is a serious problem in hospitals. Because of their resistance to environmental conditions and their natural resistance to antibiotics, VRE is easily spread in the environment. The contamination of pathogenic microorganisms on various surfaces and their direct or indirect contact with these surfaces contribute to the formation and spread of nosocomial infections. Therefore, the cleaning and disinfection of these surfaces and devices are admitted as a major issue [1,2]. The proper selection and application of disinfectants and antiseptics make it possible to obtain much more effective results than the use of antibiotics in combating hospital infections [3]. Bacoban® (Adexano, Germany) produced with nanotechnology has a Nano sponge layer which prevents microorganisms from building surfaces and also has antimicrobial effect with biocidal deposits.

Material and Methods

A prospective study was conducted at Istanbul University Cerrahpasa Medical School, a 1,300-bed tertiary care teaching hospital in Turkey. The long-acting disinfectant, Bacoban®, was examined on various surfaces/materials in a laboratory setting. Bacoban®’s efficiency was also investigated on VRE contamination with environmental samples (n = 969), and on colonization from the hospitalized patient samples (n = 447). Data were analyzed using Fischer’s exact test.

Results:

Bacoban® has significantly decreased the rates of VRE contamination and new VRE colonization in the hospital environment and hospitalized patients, respectively (p < 0.001, p = 0.028). The in vitro study showed that Bacoban® has a bactericidal effect on VRE, especially during the 22 days on the tile and 18 days on plastic and metal surfaces.

Conclusion:

This study demonstrates that Bacoban® has a permanent antimicrobial effect, especially on flat and smooth surfaces, also reduces VRE contamination on the hospital environment, and new VRE colonization in patients.

Keywords: Vancomycin-resistant enterococci; Surveillance; Disinfection; Bacoban®; Contamination; Colonization

Abstract

Background/Aim: Vancomycin-resistant Enterococci (VRE) cause outbreaks and infections by easily spreading in hospitals. Effective cleaning and disinfection play an important role to control and prevent VRE infections. We aim to investigate the clinical and in vitro efficacy of a nanotechnology-based surface disinfectant on VRE contamination and colonization.

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Preventing Spread of Vancomycin-Resistant Enterococci in a Hospital by Using a Nanotechnology-Based Disinfectant

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The Fresenius Institute in Germany has approved that Bacoban® showed the disinfectant effect in five minutes and continued for ten days. Bacoban® contains ethanol, benzalkonium chloride, isopropanol, pyridine-2-thiol-1-oxide sodium salt (sodium pyrite), inorganic/organic polymer and distilled water [4,5]. In the study, firstly, the bactericidal effect of Bacoban® on VRE was investigated in laboratory conditions, then the disinfectant effect of Bacoban® on VRE contamination and colonization were studied with environmental and rectal swab samples, respectively. As far as we know, it is the first study to demonstrate the efficacy of Bacoban® in the clinical setting.
hospital. First, the long-acting disinfectant (Bacoban®, Cheshire, UK) was examined on various surfaces/materials in a laboratory setting. Next, its efficiency on contamination and colonization with VRE was investigated in several intensive care units (ICUs), as well as other units in which vancomycin-resistant enterococcal outbreaks were reported by the Hospital Infection Control Committee.

Anesthesiology and Reanimation ICU, General Surgery, Infectious Diseases, and Bone Marrow Transplantation Hematology Units were identified as the main units in the study. Neurosurgical and Emergency ICUs, Hematology, Neurosurgery, and Internal Medicine Units with the similar patient demographics and VRE contamination and colonization rates were also identified as the control groups.

**Experimental Laboratory Studies**

Two series of materials/surfaces were used for the main and the control groups, including tile, wood, fabric, plastic (flat, slight and rough surfaces), metal, and Formica in sizes of 10 x 10 cm. After these surfaces were cleaned with alcohol and dried, 200μl of 1Mc Farland VRE suspension was spread over the surfaces/materials with sterile swabs. After drying the surfaces, Bacoban® solution was sprayed on them (for the main units) at a distance of 30 cm. The same amount of sterile saline solutions was sprayed on the surfaces for the control groups. Five minutes later, swab samples from the surface series were cultured onto the VRE agar plates (Oxoid, Ottawa, Canada). Along with the continuation of the study, these surfaces received equal amounts of VRE suspension, and in 15 minutes, swab samples were taken with sterile saline solution and cultured onto the VRE agar plates. This practice was continued until all material surfaces were reproduced with VRE. The first days of VRE reproduction on the surfaces/materials were recorded.

**Environmental Disinfection Applications**

Diluted household bleach contains ~5-6% sodium hypochlorite (1:100 or 1/4 cup:1 gallon, 525-615ppm chlorine) was used as a surface disinfectant in the pre-Bacoban® period of the study [6]. During this period, a total of three times a week, environmental samples were collected from the main units. In General Surgery, Infectious Diseases and Bone Marrow Transplantation Hematology Units, the peripheral swab samples were taken only from the rooms of VRE-positive patients, while the samples were taken all environmental surroundings in Anesthesiology and Reanimation ICU. In the Bacoban® period, practical information of the Bacoban® application was given to the staff, and the other disinfectants were not used. Bacoban® was applied once a week at Anesthesiology and Reanimation ICU, twice a week at Bone Marrow Transplantation Hematology Unit, and once a week for the other two units. Disinfection was repeated in case of contamination with patient wastes. During this period, the environmental swab samples were taken on average six times per week from the previously sampled areas and sent to the laboratory as soon as possible.

**Rectal Swab Samples**

During the environmental disinfection applications, patients admitted to the units were included in the study for VRE colonization. Rectal swab samples from the patients were taken during the initial hospital admission and then once a week. The samples were sent to the laboratory as soon as possible. Patients with no colonization in the first admission, but were VRE positive in their later samples were identified as “new VRE colonization”.

**Microbiological Methods**

In the experimental study, swab samples from the surfaces were cultured on VRE agar plates (Oxoid, Ottawa, Canada) and incubated at 37 °C for 24-48 hours. The environmental and rectal swabs samples were firstly cultured with VRE Broth (Oxoid, Ottawa, Canada) and incubated at 35-37 °C for 24 hours. After the incubation, passages were taken on VRE agar and incubated at 35-37 °C for 24-48 hours. VRE strains isolated from the agars were identified by conventional microbiological methods [7,8]. Susceptibility of the isolates to glycopeptide was determined by disc diffusion method, and the confirmation was performed by E-test strips (Liofilchem, Italy). All breakpoints were applied according to the CLSI (Clinical and Laboratory Standards Institute) guidelines [9,10]. Quality control was performed by using Enterococcus faecalis ATCC 29212 reference strain.

**Statistical Analysis**

Biostatistical evaluation of the study results was conducted. Fischer’s exact test was used to compare frequency and percentages for the pre- and post- Bacoban® periods with control groups, and the Pearson Chi-Square test was used for group comparisons of continuous data and appropriate criteria of the normal distribution. All analyses were performed using SPSS 16.0 package program (SPSS, Chicago, IL, USO). The significance value was considered as p < 0.05.

**Results**

**Table 1:** Reproduction dates of VRE from the surfaces/materials after Bacoban® application.

<table>
<thead>
<tr>
<th>The surfaces/materials</th>
<th>First reproduction of VRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood</td>
<td>2nd day</td>
</tr>
<tr>
<td>Fabric</td>
<td>2nd day</td>
</tr>
<tr>
<td>Plastic (Apparent rough)</td>
<td>3rd day</td>
</tr>
<tr>
<td>Plastic (Light rough)</td>
<td>8th day</td>
</tr>
<tr>
<td>Formica</td>
<td>11th day</td>
</tr>
<tr>
<td>Plastic (Flat)</td>
<td>18th day</td>
</tr>
<tr>
<td>Metal</td>
<td>18th day</td>
</tr>
<tr>
<td>Tiles</td>
<td>22nd day</td>
</tr>
</tbody>
</table>

*: Vancomycin-resistant enterococci

In the experimental study with Bacoban®, VRE strains were isolated from the first-day samples of the control surface/materials. After the Bacoban® application, VRE growth was not detected during the 22 days on tile, 18 days on metal and flat plastic, 11 days on Formica, 8 days on slightly roughened plastic, 3 days on roughened plastic, and 2 days on wood and fabric surfaces. The experimental study showed that Bacoban® had a bactericidal effect on VRE, and was long-acting on preventing VRE growth, especially on tile, metal and flat plastic surfaces. The first day of the VRE growth on different surface/materials after the application of Bacoban®
are shown in Table 1. A total of 969 environmental samples, 362 and 607 from the pre-and post-Bacoban® periods were studied, respectively. All samples were taken from the main units (Anesthesiology and Reanimation ICU, General Surgery, Infectious Diseases, and Bone Marrow Transplantation Hematology Units). 38 (10.5%) of the 362 and 13 (2.1%) of the 607 environmental samples were VRE positive in the pre- and post-Bacoban® periods, respectively.

In the pre-Bacoban® period, the highest VRE contamination rates were seen on the edges of the bedside (23.2%) and tables near the patients (13.6%). Compared with pre- and post-Bacoban® periods, the decrease in VRE isolation rates was found statistically significant (p ≤ 0.001). VRE positivity and negativity in peripheral swab samples in the pre- and post- Bacoban® periods are shown in Table 2. In the rectal swab samples from the patients, VRE contamination rates were 25.3% and 19.1% during the pre- and post- Bacoban® periods, respectively. Although the difference was not statistically significant (p = 0.139), there was a significant difference between the newly acquired VRE colonization rates in pre-Bacoban® (12.8%) and post-Bacoban® (6.5%) periods (p = 0.028). The results of the total and newly acquired VRE colonization in the pre- and post-Bacoban® periods are shown in (Table 3). There wasn’t a significant difference between the rates of total VRE colonization (29.4% and 23.1%) and newly acquired VRE colonization (7.4% and 8%) at control units without Bacoban® application. In this study, it was reported that the application of Bacoban® was easy, it dried quickly and did not leave any residues after the application. On the other hand, some side effects such as burning sensation, shortness of breath, and slight itching were reported by three staff members within direct contact with Bacoban®.

### Table 2: VRE* contaminations in pre- and post- Bacoban® periods

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pre-Bacoban® period</th>
<th>Post-Bacoban® period</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRE (-)</td>
<td>VRE (+)</td>
<td>Total</td>
</tr>
<tr>
<td>Bed</td>
<td>66</td>
<td>20</td>
<td>86</td>
</tr>
<tr>
<td>Table</td>
<td>57</td>
<td>9</td>
<td>66</td>
</tr>
<tr>
<td>Monitor keys</td>
<td>51</td>
<td>3</td>
<td>54</td>
</tr>
<tr>
<td>Etagere</td>
<td>20</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Sink and surroundings</td>
<td>40</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Door handles</td>
<td>28</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>Computer and phone keys</td>
<td>29</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Desk</td>
<td>18</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>
| Total                    | 324     | 38 (10.5%) | 362 | 594     | 13 (2.1%) | 607 | <0.001

*: Vancomycin - resistant enterococci
1: p<0.05

### Table 3: VRE* colonizations in pre- and post- Bacoban® periods

<table>
<thead>
<tr>
<th>Main Units</th>
<th>Pre-Bacoban® Period</th>
<th>Post-Bacoban® Period</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surveillance Samples</td>
<td>Total VRE colonization</td>
<td>New VRE colonization</td>
</tr>
<tr>
<td>Infectious Diseases</td>
<td>8</td>
<td>65</td>
<td>17</td>
</tr>
<tr>
<td>General Surgery</td>
<td>6</td>
<td>81</td>
<td>17</td>
</tr>
<tr>
<td>ICU**</td>
<td>10</td>
<td>83</td>
<td>20</td>
</tr>
<tr>
<td>Hematology***</td>
<td>2</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>249</td>
<td>63 (25.3%)</td>
</tr>
<tr>
<td>Control Units</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>4</td>
<td>51</td>
<td>16</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>6</td>
<td>91</td>
<td>41</td>
</tr>
<tr>
<td>Neurosurgery ICU</td>
<td>5</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>General Internal Medicine</td>
<td>7</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Emergency ICU</td>
<td>15</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>241</td>
<td>71 (29.4%)</td>
</tr>
</tbody>
</table>

*: Vancomycin - resistant enterococci
**: Anesthesiology and Reanimation Intensive Care Units
***: Bone Marrow Transplantation Hematology Unit
1: p<0.05

Discussion

Hospital-acquired infections (HAIs) are one of the major patient safety problems in hospitals, especially in ICUs [11]. Approximately, 20-40% of hospital-acquired pathogens are spread with the hands of healthcare professionals or by colonized patients or contaminated environmental surfaces [12,13]. The risk of infectious disease of environmental microorganisms depends on the pathogenicity as well as the ability to survive on the surface [14]. Among environmental pathogens, VRE pathogenicity isn’t a high factor, but it is confused as a causative agent that can lead to outbreaks that are difficult to control [15]. VRE can stay alive for days, weeks, even on dry surfaces, and this can cause them to be particularly persistent in a hospital environment [16]. Hands of healthcare professional play a major role in the spread of VRE among patients [17,18]. Even if the handwashing is effective, the hand can be recontaminated with the contact of the surface. Therefore, cleaning is very important for environmental surfaces with VRE [19,20]. However, cleaning with water and detergent is not sufficient for reducing of VRE colonization or contamination. Frequently touched surfaces and patient surroundings must be disinfected in addition to cleanliness. An effective method of disinfection leads to a significant reduction in environmental contamination [21,22].

In hospitals, hypochlorites and phenolic compounds are highly effective, and widely used in environmental and surface disinfection [23-25]. In a study by Eryilmaz and colleagues, 2% glutaraldehyde, 4% chlorhexidine gluconate, 7.5% povidone-iodine, 10% povidone-iodine and 70% 2-propanol were found to be effective on VRE, while 3% hydrogen peroxide was not effective even in 10 minutes [26]. In another study, Sakagami et al. investigated the effect of 35 commonly used disinfectants on VRE by microtiter plate method and reported that alcoholic preparations were the most effective compounds [27]. The areas where VRE contamination is intensive, is generally determined that there is a deficiency in cleaning/disinfection processes. Even if the process is appropriate, new contamination can occur until the next disinfection. Long-acting disinfectants can be an alternative solution to this problem [28]. In this study, Bacoban® containing ethanol, isopropanol, and benzalkonium chloride was used as a long-acting disinfectant, and its efficacy was studied for VRE contamination and colonization.

Our experimental study on various surfaces/materials showed that Bacoban® has a bactericidal activity on VRE especially during the 22 days on the tile, 18 days on plastic and metal surfaces. Even the most competitive products have a bacteriostatic effect during nine to ten days, but bactericidal effect maximum two days [27]. Our study showed that long-term bactericidal effect of Bacoban® can be used to prevent VRE contamination in hospitals. Bacoban® establishes an easy-to-clean, Nano-ultra-thin layer with a lasting effect which prevents the adhesion of pollutants. Antimicrobial biocides are used to allow the thin layer to actively destroy germs for a prolonged period. On the other hand, traditional disinfectants are used to kill the bacteria/viruses/mold/fungi during the cleaning phase or contact time. Once dry, that surface is exposed to new contaminants until the next cleaning period [4,5]. Our study showed that Bacoban® protects those surfaces until the next cleaning period, diminishing the risk of new contamination and thus allows a continuous protection.

The state of continuous protection has overwhelming importance in diminishing the gross infections. The results of this study with Bacoban® have been consistent with other studies. Sultan and his colleagues have found that glass and plastic surfaces applied with Bacoban® are effective for 10 days to contaminate with new microorganisms [4]. Akgül and colleagues were compared the Bacoban® with the disinfectant named Actosept®, and found that a single Bacoban® application was equivalent in efficacy to the daily application of Actosept® for 15 days in anesthesia devices in the operating room [29]. In another study, Ayoubi and colleagues found that the duration of action of Bacoban® on Pseudomonas aeruginosa was 10 days for the alcoholic formulation, and 6 and 7 days for the water based 0.5% and 1% formulations, respectively [30]. Colonized and/or infected patients with VRE are important reservoirs for contaminating surrounding surfaces and medical devices. Several studies have reported that VRE is isolated from a wide variety of devices and surfaces such as patient uniforms, bedside, sphygmanometers, electronic thermometers, electrocardiography devices, intravenous fluid pumps, and table tops [31,32].

The most frequently contaminating surfaces are reported to be closely related to the colonized body part with VRE [33,34]. In our study, the highest VRE positivity was detected in samples taken from patients’ beds and tables. In the study, 38 (10.5%) of the 362 peripheral swabs were found VRE positive during the pre-Bacoban® period in the main units. Twenty (57.1%) and 9 (23.6%) of them were isolated from the bedsides and tables near the patients, respectively. In the post-Bacoban® period, 13 (2.1%) of the 607 peripheral swabs were found VRE positive. The rates of VRE contamination in the bedsides and tables were 46.1% and 30.72%, respectively. The total number of environmental samples was higher than the pre-Bacoban® period, and it was considered to affect percentages. The decrease in VRE contamination between the pre- and post-Bacoban® periods was found statistically significant (p ≤ 0.001). Considering this study with others to reduce VRE contamination in hospitals, Bacoban® was found more effective than many disinfectants used to prevent VRE contamination [23-26].

In this study, during the period of Bacoban® application, a significant decrease of VRE colonization (p = 0.001), and a decrease in the number of patients with new VRE colonization were detected in Infectious Diseases Unit (p = 0.005). On the other hand, there was no significant difference in VRE colonization rates in Anesthesiology and Reanimation ICU, General Surgery, and Bone Marrow Transplantation Hematology Units in the pre- and post-Bacoban® periods. Overall, Bacoban® was found to be effective against the colonization, and new infections of VRE (p = 0.028). This study showed that using of Bacoban® remarkable reduced the VRE contamination, compared with levels of VRE contamination detected during the use of sodium hypochlorite (bleach) for disinfection in the control units. These findings have important implications to prevent VRE in endemic health care settings.
There are some limitations of this study. First of all, significant risk factors for colonization with VRE (using antibiotics or the procedures of invasive) could not be excluded from the study. Also, the situations such as illness or adaptation of the staff members to the study have not been followed adequately. Lastly, the Bacoban® application was not implemented by a specific team, each unit used own staff, and the compliance of the staff cannot be controlled. In conclusion, this study demonstrated that Bacoban®, a long-acting disinfectant, has a permanent antimicrobial effect, especially on flat and smooth surfaces, and reduces the acquisition of new VRE in patients with a marked decrease in VRE contamination. However, more studies are needed to make a definite judgment on the clinical effectiveness of Bacoban®.

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