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Experimental Studies of the Remnant Effect of Detergent/Disinfectant on *Staphylococcus aureus* on Surfaces

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Overview

We propose three complementary, original and reproducible methods for studying the antibacterial remnant effect of a detergent/disinfectant for surfaces which has recently been commercialised on the French healthcare provider market.

We study the growth curves of a strain of *Staphylococcus aureus* placed in the wells of a microplate which has been pre-soaked for several days (from D minus 10 to D 0) in the detergent/disinfectants to be tested. In addition, the surfaces treated from D -10 to D 0 by one of the detergents are artificially contaminated by a suspension of titrated *E. coli* in a standardised fashion via velvet pad imprint. The surviving bacteria are counted after being transferred to a Rodac® agar plate. Finally, doorknobs are cleaned and disinfected with the product being tested and agar prints are performed over the course of one week in order to study their progressive recolonisation under use.

These studies show that Bacoban exhibits bactericidal activity upon *S. aureus* with a remnant effect of a minimum of 10 days. In parallel we also show that all the products tested exhibit bacteriostatic activity of 10 days but only remain bactericidal, at best, for 7 to 4 days. Some products have already ceased bactericidal activity one day after application in our experimental model.

This remnant bactericidal effect is particularly useful in hospital hygiene for biocleaning the most regularly touched surfaces and should limit the role as bacterial reservoirs of some surfaces used in patient care or which are otherwise in close proximity to patients.

Keywords : bactericidal, detergent/disinfectant, surface, hospital hygiene, remnance, biocleaning

Introduction

Hospital surfaces constitute microbial reservoirs liable to contaminate caregivers' hands or patients directly[1]. It is difficult to determine precisely how much surfaces and the hospital environment contribute to nosocomial infections but hygiene guidelines take this risk into account and all hospital surfaces are cleaned and disinfected at least once a day. For instance, contamination of the staff's hands by the environment is likely in certain areas of hospital activity and for certain species of bacteria such as *S. aureus* and Enterococcus strains especially during epidemic periods [2, 3, 4]. Biocleaning guidelines recommend alternating the three-step process (cleaning, rinsing, disinfecting) with a simplified process using off-the-shelf products which clean and disinfect in one step without requiring rinsing.

For biocleaning hospital surfaces, one manufacturer offers a detergent/disinfectant (dD) with a remnant effect (Bacoban®, Ropimex, France). This feature allows, for certain hospital surfaces which are regularly touched and therefore likely contaminated throughout the day, to ensure low recontamination and thus limit these surfaces' role as microbial reservoirs. To our knowledge, this effect is not currently claimed by other products on the market for hospital biocleaning nor is it taken into account in the specifications of the "positive list of detergent/disinfectants" published regularly by the French Society for Hospital Hygiene (SFHH) [5].

We propose several original experimental protocols to highlight this remnant effect and to compare it to potential unclaimed effects of other surface cleaning and disinfectant products on the French market.

Material and Method

Three complementary methods were implemented in order to verify remnant antibacterial efficacy of the different forms of Bacoban and to compare it to any such activity found in other dD's on the market which are referenced by the SFHH positive list [5].

The dD's tested are graded dD1 to dD4 and are used in concentrations recommended by their respective manufacturers. The primary active ingredients in the tested products are for dD1 to dD4 respectively: quaternary ammonium; quaternary ammonium and tertiary amine; amine acid and quaternary ammonium; and quaternary ammonium and alkylamine.

Bacoban® can be used in a ready-to-use alcohol form or in a water-based form, diluted to 1% (concentration currently recommended by Ropimex France) or to 0.5%.

1. Study of the products' remnant effect by micromethod

From D-10 to D0, the products to be tested are placed in the wells of a microplate (n=3 per product and per day). The product is left in contact with the well walls for 10 minutes and is then removed. The microplate is then dried in a contamination-free environment under the laminar flow of a tissue culture hood for 20 minutes.

At D0, the microplate is turned over and tapped on a flat surface in order to remove any crystals of the antibacterial agents which may have formed, then each well is inoculated with 300 µL of a *Staphylococcus aureus* suspension CIP 106415 (10^5 UFC/mL) .

The microplate is then covered with polyethylene film in order to prevent evaporation of the culture medium during the experiment, which lasts 18 hours at 37°C. Bacterial growth in the wells is detected by a microplate reader (Thermomax, MOLECULAR DEVICES®) and a spectrophotometer (Spectromax, MOLECULAR DEVICES®) which takes a 490 nm

absorption in each well every 10 minutes over the course of 18 hours. MOLECULAR DEVICES®'s SoftMaxPro software is used to process the resulting data.

For each well, the reader renders a graph of absorbency as a function of time. Any significant increase in optical density as a function of time will be interpreted as bacterial growth and thus the inefficacy of the dD tested in the conditions of exposure in the analysed well [6].

Inversely, stable and null optical density will be considered an absence of bacterial growth related to the effect of the dD tested.

To distinguish a bacteriostatic effect of the dD from a bactericidal one, after 18 hours' incubation, the 300 µL of bacterial suspension in each well are placed in 2 mL of brain-heart infusion broth and incubated for 24 hours at 37°C. The product used will be considered as having a bactericidal activity if the broth remains clear whereas it will be considered as only having a bacteriostatic one under the conditions of the experiment if the broth becomes cloudy.

2. Imprint transfer method

26x76 mm microscope slide were treated, either with different formulations of Bacoban® (Bacoban® ready-to-use alcohol, Bacoban® 1% water-based, Bacoban® 0,5% water-based), or with dD1 to dD4 or with sterile water every day from D -10 to D0. Three trials are performed per product per day and the experiment was performed three times. The slides were then left at room temperature away from any contamination. On D0, all the slides were artificially contaminated in a reproducible manner by a suspension of *Staphylococcus aureus* CIP 106415 (10^5 UFC/mL) applied by velvet pad (two 10x20mm prints per slide). After drying (5minutes at room temperature), the survival of the bacteria in the presence of the tested

products was measured by the application of a Rodac® blood agar plate which was then incubated for 24 hours at 37°C.

A numeration was performed for each print made to the slide. During one experiment, three slides per product per day are used. The experiment was performed three times, which, which enabled the obtention of 18 bacterial numerations. For each day and each product, the mean number of colonies was calculated. These findings were analysed by two-factor variance analysis (night and day) . When the variance analysis showed a significant overall difference, Dunnet or Bonferroni comparison tests were performed *a posteriori*.

3. Doorknob method

Twenty-one metal doorknobs were found in our laboratory. The study was conducted three times a week. All the doorknobs were treated once on D0, either with Bacoban® ready-to-use alcohol (week 1), Bacoban® 1% water-based (week 2), or with dD1 (week 3). On D0 after drying (20 minutes), the survival of environmental bacteria in the presence of the tested product is measured by the application of a Rodac® blood agar plate incubated 48 hours at 37°C. The sampling operation is repeated on D1, D2, D3, D4 and D7. This method allows us to observe the survival of any bacteria deposited by users' hands at different times of the study despite the presence of dD applied to the doorknobs at the beginning of the study.

Findings

1. Study of the products' remnant effect by micromethod

The analyses for each plate are validated by a bacterial growth curve in the wells of the control (sterile water).

In our study, there is an absence of bacterial growth with all products up to D -10 (figure 1). All the products, no matter how long they are used seem to be able to inhibit the growth of *S. aureus* in our experimental protocol.

Samples of the different suspensions from the wells were placed in test tubes containing heart brain broth which enabled us to determine the remnance of bactericidal activity in the different dD's tested. It was found that in the presence of *S. aureus,* only Bacoban® ready-to-use remains bactericidal for 10 days. Bacoban WB 1% and WB 0.5% remain bactericidal for 9 days. Products dD2 and dD3 are not bactericidal for more than a day while products dD1 and dD4 are bactericidal for 7 and 4 days respectively.

2. Imprint transfer method

The results of the mean numerations obtained (n=18) for each pair of product imprints/application times are found in figure 1.

Statistical analysis shows a significant difference ($p<0,001$) in activity between Bacoban® alcohol and the four other detergent/disinfectants tested (dD1 to dD4) over the 10 days of the study. It also shows that the Bacoban® alcohol and Bacoban® WB 1% are equivalent but significantly different from Bacoban® WB 0,5% and that results obtained with dD1 to dD4 do not significantly differ between themselves.

We can thus split the tested dD's into three groups which are, from least active to most active, group 1 (dD1 to dD4), group 2 (Bacoban® WB 0,5%) and group 3 (Bacoban® alcohol ready to use and Bacoban® WB 1%).

Bacterial growth between D -1 and D -10 is significant for detergent/disinfectants dD1 to dD4, which tends to show a progressive loss of disinfectant activity over time.

Regarding the different Bacoban® formulations, there is no difference between D -1 and D – 10 with regard to bacterial growth, which means that even after 10 days, Bacoban's disinfectant activity is still as effective according to our experimental protocol.

3. Doorknob method

After cleaning the doorknobs with one or another of the products on D0 of each week of the study, for each day of the experiment, a Rodac® sample was taken from each doorknob. From these samples, bacterial numeration enabled us to evaluate the survival of bacteria deposited by users' hands over time, despite the presence of the dD.

For the statistical study, the mean of the numerations of the day was performed and the doorknobs were paired. The statistic study showed a difference which is not significant. However Bacoban® alcohol ready-to-use seems to be the product with the best activity. DD1 and Bacoban WB® 1% have the same remnant disinfectant activity in our experimental model.

Discussion

The remnant effect of the detergent/disinfectant products is to our knowledge an innovation in the field of hospital hygiene. This criterion does not appear in the specifications for products on the list of detergent/disinfectants published by the SFHH [5] for instance. This particularity announced by the Ropimex® company for its Bacoban® product is appealing for hospitals. Indeed, surfaces in contact with hospital staff or in direct contact with patients likely constitute microbial reservoirs which could contaminate the hands of said persons [1, 2, 3, 4].

Even surfaces that are regularly cleaned and disinfected play a role as microbial vectors for desiccation-resistant species like *Staphylococcus aureus* and other Gram-positive bacteria[2, 3]. These species are widely spread throughout the environment by human activity and if surfaces only undergo one daily biocleaning, between two applications and without a remnant disinfectant effect of the product, the surface becomes a reservoir again [7]. Currently, awareness campaigns for healthcare professionals for the prevention of nosocomial infections focus on hand hygiene especially and the use of hydroalcoholic products. This must not omit the necessary biocleaning of surfaces [7] which contribute to the microbial recolonisation of the hands. Disinfection of surfaces with an extended effect acts as a precious complement for good control of bacterial contamination of the near-patient environment [8] and thus of the maintenance of hand hygiene for everyone.

In our work we have chosen to study the remnant efficacy of products on a strain of *S. aureus* insofar as this is one of the main species responsible for nosocomial infections in our healthcare facilities [9]. It is a particularly desiccation-resistant bacterium which is long-lived in the environment and thus constitutes a reservoir likely to contaminate healthcare providers' hands or the patient directly [survival of SA]. Other species could be used according to our experimental protocols to further study the remnant efficacy of disinfectants. *Pseudomonas aeruginosa* which is the third most frequently responsible bacterium for nosocomial infections and which is particularly feared when treating patients suffering from cystic fibrosis who may become contaminated by their environment [11].

To verify the remnant bactericidal effect claimed by Bacoban and to compare it to a potential remnant bactericidal effect present in other products, we had to invent several additional experimental protocols. We have come up with three original and easy to implement methods.

The first method, using microplates, allows us to verify the activity of the tested products with regard to *S. aureus* by studying bacterial survival and growth (18-hour growth curve) according to the time between the treatment of the plate's wells and their inoculation with the bacterial suspension. This method is easy to implement and interpreting the results after reading the plate is simple [6]. Our findings show excellent activity in all products tested, including Bacoban alcohol ready-to-use and Bacoban water-based (1% and 0.5% dilution) over all 10 days of the study. But we actually show that only Bacoban alcohol possesses real bactericidal efficacy over the 10 days of the study. Bacoban water-based formulations (1 and 0.5 %) are bactericidal for 9 days. The other products do not present this long-lasting activity, the best of which (dD1) remaining bactericidal on the *S. aureus* strain for only 7 days according to our protocol. Indeed, by placing a sample from each well in an appropriate nutrient broth, we were able to distinguish bactericidal activity from mere bacteriostatic activity in all products tested after the application time even though the bacterial growth curve remains flat for the corresponding wells. While all the dD's tested inhibit bacterial growth until the tenth day, they possess only a limited bactericidal effect.

To our knowledge, this remnant activity of surface disinfectants has never been studied before. It seems important however to know the duration of the bactericidal effect of detergent/disinfectants used in hospitals. When applied to regularly handled surfaces like door handles, switches, night tables, closets and patients' bedsides for instance [1], a product possessing a remnant bactericidal effect would limit the recolonisation of these surfaces between two daily biocleanings. Surfaces are contaminated by being touched throughout the day, while biocleaning procedures often recommend, for want of time, only one application per day. A product without a remnant bactericidal effect allows the recolonisation of these surfaces throughout the day and the expected benefit of disinfection quickly fades. The remnant effect of a dD could be considered a sort of permanent "self-disinfection" of surfaces

and thus limit the environment's role as a microbial reservoir. On other surfaces which would appear to play less of a role a microbial reservoirs which might contaminate hands of the patient directly like floors and walls, this product could be applied at less regular intervals.

Our findings appear to confirm the remnant effect of Bacoban® in its alcohol formulation as well as its water-based one. While the optimum concentration of water-based Bacoban is 1%, the 0.5% concentration features a greater bactericidal activity than the other products tested (dD1 à dD4). Our microplate method allows the verification of this effect but can also be used for the more classical study of bactericidal effect of products in general, as we have already shown [6]. Those in charge of hospital hygiene certainly need this information to be able to choose truly effective products which meet healthcare facility requirements for the prevention of nosocomial infections. Our method allows these activities to be tested but above enables their remnance to be tested. Such information should be of interest to hygienists and could be taken into consideration in the hospital's choice of products.

The velvet pad method allows one to verify the remnant activity of the products tested with regard to reproducible artificial contamination with *S. aurues*. The German standard (ASTM Standard E2180) is difficult to implement on a large scale and in our laboratory. Moreover, results obtained via this method have shown the remnant activity of Bacoban (upon different germs and fungi : *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus niger*) over the course of 10 days [Bacoban technical manual]. We therefore wished to develop a new method of artificially contaminating surfaces which would be both simple to implement and to reproduce and which would enable the testing of the remnant effect of detergent/disinfectant no matter what type of surface (plastic, glass, metal) and finally, that would be applicable to numerous species of bacteria. We checked that the velvet allowed for an even contamination of surfaces by depositing a

bacterial film that may then be studied simply by the application of a Rodac® agar plate. Indeed this technique allows one to obtain a homogeneous carpet of bacteria for every imprint. The first results after contamination by *S. aureus* of a glass surface which had been treated beforehand by one of the detergent/disinfectants tested allowed us to show significantly superior efficacy of Bacoban® alcohol ready-to-use and of Bacoban® WB 1% compared to those of dD1 to dD4. In our technique, the study of bacterial survival on the surface is performed by imprints using Rodac type agar plates containing a growth medium enriched with fresh sheep's blood. Preliminary trials were performed with a growth medium containing the usual neutralising agents of the principal active disinfectant compounds (tween, lecithin, histidine and thiosulfate) but the results were identical. The addition of neutralising agents was found to be unnecessary for our experiments. This means of artificial contamination of a surface by a bacterial suspension is simple and should allow us to test and compare the bactericidal effects of many products in the framework of comparative activity studies for example.

Finally, the doorknob study is the closest to the real-world behaviour of the product. It allowed us to observe the survival of bacteria deposited by users' hands at different times throughout the study and despite the presence of dD applied to the doorknobs at the beginning of the study. This method appears simple to implement, however statistical interpretation turns out to be complex. This study introduces 2 variables: the "day" factor , i.e. the duration of action of the product and the "product" factory of the product being tested. These results do not allow however to show a significant difference in remnant efficacy of the products, but rather they confirm a tendency: Bacoban® alcohol ready-to-use possesses activity greater than that of the other detergent/disinfectants tested. To conclude definitively on the remnant

effect in this experimental situation, we will have to study longer periods and include more doorknobs.

Conclusion

We have studied the remnance of activity of Bacoban®, a detergent/disinfectant recently commercialised for biocleaning of surfaces in hospitals and we have compared this activity to that of other detergent/disinfectant products already on the market which are listed on the SFHH positive list. We have shown the action of Bacoban alcohol ready-to-use to be superior as it is the only product which remains bactericidal for at least 10 days whereas currently used detergent/disinfectants have, at best, a bactericidal activity of 7 days on *S. aureus*. The water-based formulation of Bacoban® at a concentration of 1 % has an almost equivalent activity to that of Bacoban alcohol. Diluted to 0.5 %, the remnant activity is naturally weaker but remains much superior to that of other dD's tested.

Other tests including other products and other species of bacteria could be performed according to our experimental methods which are both easy to implement and reproducible. Multiplying the number of such studies would help develop applied research in hospital hygiene to improve the effectiveness of products and better meet the expectations of hygiene teams with regard to disinfecting surfaces. It remains to be seen what real impact this remnant activity of detergent/disinfectants will have upon hospital hygiene in the effective prevention of certain nosocomial or healthcare-related infections. As a complement to washing and disinfecting hands, biocleaning and strict compliance with standard and complementary precautions, the remnant effect of dD on surfaces is suggested as a new tool at the disposal of healthcare professionals to control environment-related infection risks. It

would certainly be beneficial to generalise the use of this tool for the treatment of the most-touched surfaces which should ideally be disinfected after every use.

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