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Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Reduction of bacterial surface contamination in the hospital environment by application of a new product with persistent effect

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ARTICLE INFO

Article history:

Received 11 September 2009

Accepted 5 February 2010

Available online xxx

Keywords:

Appeartex®

Contact plate

Nylon flocked swab

Persistent effect

Sample

Surface disinfection

SUMMARY

The benefit of routine surface disinfection in hospitals has been discussed. In this study we have investigated a new product, Appeartex®. After application on surfaces a remnant effect is achieved due to the positive charge of the active molecule. We studied the persistent effect of Appeartex one day after application in both an experimental study in the laboratory and a field study in a hospital ward. Surfaces of bedside tables were investigated. In the experimental study, large inocula of $\geq 10^6$ cfu of *S. aureus* or enterococci were inoculated on to well-defined areas which had been treated/not treated with Appeartex. One hour later, samples were taken with a swab rinse technique. A reduction in the number of viable bacteria in the magnitude $10-10^3$ cfu was seen due to the effect of Appeartex. In the field study the effect on naturally occurring low level contamination was studied. Defined surfaces on bedside tables used by patients were treated/not treated with Appeartex. One day later, samples were taken with contact agar plates and with a new swab method using two sequential nylon flocked swabs. Significantly fewer bacteria were found on Appeartex-treated surfaces compared with untreated surfaces. The median counts on Appeartex-treated surfaces were 10 cfu/50 cm², and on untreated surfaces 20 cfu/50 cm². There was no significant difference in the number of bacteria found by culture of samples taken with the contact agar method compared with samples taken using the nylon flocked swab method.

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Introduction

Environmental surfaces near infected or colonised patients in hospitals are frequently contaminated by potentially pathogenic micro-organisms such as vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus*.^{1,2} The contamination of environmental surfaces may play a role in disease transmission.³⁻⁷ The role of routine surface disinfection in the prevention of infectious diseases has been discussed.^{8,9}

Appeartex® (Appeartex AB, Göteborg, Sweden) is a new product for treatment of surfaces. It is applied on a surface as a solution that dries in the air. The product is composed of the active polymer A-200, polyhexamethylene biguanide (PHMB), and a surfactant solution to achieve adequate moistening of the

treated surface. A-200 acts by trapping negatively charged moieties on a surface. Micro-organisms have a net negative surface charge and will be immobilised by the Appeartex-coated surface. Due to the adherence the access will be facilitated for PHMB to exert its biocidal activity. In addition it is known that cationic molecules enhance the effect of PHMB, hence a synergistic effect to the biocide can be expected. According to the manufacturer, A-200 adsorbs to virtually any kind of surface – hard surfaces, textiles or paper/non-woven fibres. Even if the biocide is rinsed off, the physically active entrapment of micro-organisms due to the charge of A-200 may protect the surface at least partly from contamination.

The aim of this study was to evaluate the persistent antibacterial effect of Appeartex. This was done in an experimental laboratory study as well as in a field study in a hospital ward. Since there is no standardised method to take a sample from a surface for quantitative culture, different sampling methods were used including contact agar plates and a new swabbing method (G. Hedin, J. Rynbäck, B. Loré, unpublished manuscript).

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Methods

Nylon flocked swabs manufactured by MicroRheologics, Italy, and rayon-tipped swabs, Copan (Nordic Biolabs AB, Täby, Sweden) were used.

Experimental laboratory study

The surface of an EVAB bedside table with plastic laminated MDF-board-surface (Proton Caretec AB, Skillingaryd, Sweden) was thoroughly cleaned and disinfected by the use of an alcohol-based disinfectant with a non-ionic detergent (Dax ytdesinfektion plus, Opus Health Care AB, Malmö, Sweden). This detergent has no antibacterial effect and the alcohol has no effect after evaporation. The surface was allowed to air-dry for about 1 h. Part of the table was then wiped for 1 min with a non-woven polypropylene fibre web (Fiberweb Sweden AB, Norrköping, Sweden) moistened with Appeartex (Appeartex AB, Göteborg, Sweden), while another part of the table was left untreated. The table was placed in an empty room for 24 h and thereafter test bacteria were applied.

The test surfaces were inoculated with (1) suspensions of reference strains, (2) a swab taken from a wound infected by *S. aureus*, and (3) a urine sample containing $>10^5$ cfu/mL *Enterococcus faecalis*.

Well-defined surfaces on a bedside table were either treated or not with Appeartex. Reference strains *Enterococcus hirae* ATCC 10541 and *Staphylococcus aureus* ATCC 6538 were suspended in PBS, and the turbidity was visually adjusted to McFarland 0.5. New bacterial suspensions were prepared for each experiment and viable counts were performed. A rayon-tipped swab was moistened by immersion into the bacterial suspension. A square cardboard frame 5 × 5 cm was used to define the test area and the rayon swab was rotated and rubbed over the whole surface. The weight of the swab was determined before and after inoculation. By comparing the weight before and after inoculation (1 mg corresponding to 1 µL) and by knowing the viable count (cfu/mL) it was possible to calculate the number of bacteria applied on the test surface.

A clinical sample taken with a rayon swab from a wound infected by *S. aureus* was used to inoculate two surfaces on the bedside table, treated or not with Appeartex, using the same method as when the reference strains were inoculated (see above). A clinical urine specimen with $>10^5$ cfu/mL *E. faecalis* was also used. The tip of a rayon swab was immersed into the urine and then rubbed against two surfaces on the bedside table, treated or not with Appeartex, as before.

Sampling was commenced 1 h after inoculation of the surfaces, when the inocula were dry. Two types of sampling solutions were compared in the first experiment, phosphate-buffered saline (PBS), pH 7.4, and neutraliser (polysorbate 80, 30 g/L; saponin, 30 g/L; and lecithin, 3 g/L; pH 7.0). In all subsequent experiments the sampling solution was neutraliser. Nylon flocked swabs were used for sampling, and each sample was taken with two sequential swabs from the same site, as described elsewhere (G. Hedin, J. Rynbäck, B. Loré, unpublished manuscript). The first swab was moistened by immersion into the sampling solution and then rotated and rubbed in a zigzag pattern over the whole surface. This process was repeated at an angle of 90° to the first. The second swab was moistened only by the fluid that remained on the sampling surface after completion of the first swabbing. As much fluid as possible was absorbed by gentle application. The second swab was put into the same tube of sampling solution as the first. The swabs were then shaken to dislodge bacteria, left in the tube for 5 min and then, after vortexing the tube, discarded. Following mixing of the sampling solution, serial 10-fold dilutions were prepared in PBS, and 100 µL from each dilution was inoculated on to blood agar

plates. After incubation at 36 °C for 24 h the numbers of cfu on each plate were counted. Counts in the range 15–300 cfu were used for further computation. If suitable counts were obtained from two adjacent dilution steps the weighted arithmetic mean of both was calculated.

For the reference strains the ratio between the number of bacteria found by culture of the sample and the number of bacteria inoculated on the surface 1 h before sampling was determined. The ratio was expressed as the percentage recovery.

Field study in a hospital ward

The study was performed in a clinical ward at the Department for Infectious Diseases, Falun Hospital. The surfaces of bedside tables were investigated while they were used by the patients. It was decided to choose 12 bedside tables and to repeat the investigation three times during three consecutive weeks. At the start of each investigation the tables were cleaned and disinfected by the use of an alcohol-based disinfectant with a non-ionic detergent (Dax ytdesinfektion plus). The surface was then allowed to dry in the air for 1 h. Thereafter one half of the surface of the table was wiped for 1 min with a fibre web moistened with Appeartex, while the other half of the table was left untreated. Six tables were treated with Appeartex on the left side, while six tables were treated on the right side. The staff on the ward were unaware which tables had been treated. The normal routine in the ward was to clean the bedside tables every day or whenever needed with an alcohol-based disinfectant. It was decided that during the investigation the tables must not be cleaned with any disinfectant or detergent. If deemed necessary, only tap water was allowed for wiping. Before the start of the study all staff in the department were informed. Patients were not informed about the aim of the study.

Samples were taken one day after the treatment of the bedside tables with Appeartex. Three different methods, A, B and C, were used to take samples. The sampling area for each was 25 cm², but double samples were taken, giving a sampling area of 50 cm² for each method on each side of the table.

Method A (contact agar plates)

Tryptic Soy Contact Agar plates with lecithin and polysorbate 80 (Heipha Diagnostika, Eppelheim, Germany) were pressed against the surface for 5 s. The plates were incubated at 32 °C for one day and the numbers of cfu were counted.

Method B (direct agar inoculation using swabs)

A sterile square cardboard frame 5 × 5 cm was used to define the test area. Nylon flocked swabs were used for sampling. Each sample was taken with two sequential swabs from the same site, using the same technique as when the samples were taken in the experimental study with reference strains. Both swabs were rotated and rubbed over the whole surface of one blood agar plate per swab. The plates were incubated at 36 °C for one day and the numbers of cfu on each plate were counted. The count from the first agar plate was added to the count from the second.

Method C (swab rinse technique)

A sterile square cardboard frame 5 × 5 cm was used to define the test area. Nylon flocked swabs were used for sampling, which was performed using the same technique as in method B. Both swabs, however, were put in the same tube with 1 mL neutraliser, and left in the tube for 5 min. After mixing, 100 µL was inoculated on to blood agar, and 100 µL was inoculated on to Enterococcosel agar (BBL). After incubation at 36 °C for one day the numbers of cfu on each plate were counted. The counts on the agar plates were multiplied by 10 to compensate for the 10-fold decrease in number

which occurred because only 100 µL of the 1 mL sampling solution was applied to the plates. Bacteria growing on Enterococcosel agar were confirmed to be *Enterococcus* by conventional methods.

For each method, A–C, the bacterial counts obtained by culture of the two samples taken from the same half of the table were added to give the number of cfu/50 cm². For statistical comparison Wilcoxon matched-pairs signed-ranked test was used. The matched-pairs were both halves of the same table, treated or not with Appeartex, respectively. Similarly, the result obtained with method A, the contact agar plate method, was compared with the results obtained with methods B and C by using Wilcoxon matched-pairs signed-ranked test. To further describe the results the median and range of all results (cfu/50 cm²) obtained with each method from Appeartex-treated or untreated surfaces was determined.

Results

Experimental study

Cultures of samples taken with PBS as sampling solution from surfaces not treated with Appeartex yielded 2.7×10^5 cfu *E. hirae* (26% recovery) and 3.5×10^5 cfu *S. aureus* (28% recovery). The corresponding values when neutraliser was used as sampling solution were 3.3×10^5 cfu *E. hirae* (34% recovery) and 4.9×10^5 cfu *S. aureus* (56% recovery). The higher recovery of *S. aureus* with neutraliser may be explained by polysorbate 80, which breaks up clumps of cells.¹¹ When Appeartex-treated surfaces were sampled it was necessary to use neutraliser as sampling solution because, when PBS was used, no viable bacteria were identified upon culture. When neutraliser was used as sampling solution, cultures revealed 9.7×10^2 cfu *E. hirae* (0.1% recovery) and 5.9×10^2 cfu *S. aureus* (0.5% recovery).

When a swab taken from an infected wound was used for inoculation, the sample taken from the untreated surface showed growth of 1.0×10^8 cfu *S. aureus*, whereas the sample taken from the surface treated with Appeartex showed growth of 1.9×10^7 cfu *S. aureus*. When the surfaces were contaminated with urine, the sample taken from the untreated surface showed growth of 2.0×10^7 cfu *E. faecalis* whereas the sample taken from the surface treated with Appeartex showed growth of 2.9×10^6 cfu *E. faecalis*.

Field study

Of the 36 bedside tables that were included in the study, samples were taken from 31. In one case a table was mistakenly disinfected with an alcohol-based surface disinfectant, and therefore excluded from the study. In four cases sampling was not undertaken because patients had become unwell, therefore admission to the room was not possible.

The median number cfu/50 cm² as well as the range obtained by each sampling method is shown in Table I. With methods A and C, a statistically significant difference ($P < 0.05$) between results from Appeartex-treated and untreated surfaces was seen, with higher

counts obtained from surfaces not treated with Appeartex. With method B, however, the observed difference was not statistically significant.

Results obtained with methods A and C were not significantly different, as regards samples taken from both Appeartex-treated and untreated surfaces. Method B showed a significantly higher count compared with method A when treated surfaces were sampled ($P < 0.05$), but when untreated surfaces were sampled no significant difference was seen.

E. faecalis was found in cultures from five of the 31 tables investigated. The number was low, in four cases 10 cfu/50 cm² and in one case 220 cfu/50 cm². In two of the cases with 10 cfu/50 cm² the positive cultures were taken from Appeartex-treated surfaces.

Discussion

The hospital environment may play a role in the epidemiology of multiresistant bacteria.⁴ Heavy contamination of the environment occurs from spillage of human secretions and excretions. Such spillage must be cleaned promptly and the surface disinfected. The routine use of disinfectants on surfaces with low level contamination, however, is controversial, as some experts point out that routine cleaning with a detergent is sufficient.¹¹ Such surfaces include frequent hand touch surfaces near the patients, for example the surfaces of bedside tables.

In this study the persistent effect of a new product, Appeartex, on the number of viable bacteria on bedside tables was studied. A series of experiments was performed. Large inocula of enterococci and *S. aureus* were inoculated on to surfaces that had been either treated or not with Appeartex one day before the inoculation. Samples were taken with the swab rinse method 1 h after bacterial inoculation.¹² Nylon flocked swabs were used, thereby enhancing the efficiency compared with rayon swab use.^{10,13} The neutraliser used as sampling solution has been shown to completely counteract the effect of Appeartex and itself not to have any toxic effect on *Staphylococcus aureus* ATCC 8739 (K. Granath, Appeartex AB, Gothenburg; personal communication). It was necessary to neutralise the effect of Appeartex when a sample was taken from a treated surface; otherwise Appeartex had an effect on the bacteria after the sample was taken, probably due to the continuous activity of PHMB. When neutraliser was used as sampling solution, a reduction in the number of viable reference bacterial strains of *S. aureus* and *E. hirae* from approximately 10^5 – 10^3 cfu was observed. This means that Appeartex one day after application was able either to exert a biocidal effect or to physically bind a substantial number of bacteria to the surface.

The effect, however, was not as pronounced when *S. aureus* from a wound infection or *E. faecalis* from urine were inoculated on to the surface. Only a 10-fold reduction of viable bacteria was observed, and as many as 10^6 cfu were detected by samples taken from surfaces treated with Appeartex. It is concluded that if a surface becomes contaminated by wound secretions or urine containing large numbers of bacteria, the inactivating effect of Appeartex is not sufficient. Since organic debris/body fluids seem to compromise the efficacy of Appeartex, this could significantly limit its usefulness within a hospital environment.

A field study was conducted in a hospital ward. The aim was to investigate the persistent effect of Appeartex on the relatively low level of bacterial contamination of bedside tables which occurs, for example, from touching hands and from airborne particles. The results showed a statistically significant reduction in the total number of aerobic bacteria on surfaces treated with Appeartex compared with surfaces not treated with Appeartex, when samples were taken using the swab rinse method and with contact plates. The median counts obtained with these methods

Table I

Total aerobic count (cfu/50 cm²) obtained with different sampling and culture methods from surfaces treated or not treated with Appeartex® one day before sampling

Method	Treated	Untreated
(A) Contact agar	9 (0–58)	22 (0–348)
(B) Two swabs, direct inoculation on to agar	13 (0–243)	16 (0–698)
(C) Two swabs, swab rinse	10 (0–90)	20 (0–960)

Values are median (range).

were 9 and 10 cfu/50 cm² respectively on Appeartex-treated surfaces compared with 22 and 20 cfu/50 cm² on surfaces remaining untreated. There are no legislation, rules or agreements concerning the level of surface bacterial contamination deemed acceptable in hospitals, but a total aerobic count <5 cfu/cm² on different sorts of hand contact surfaces has been proposed in the UK.¹⁴ By comparison with what may be achieved on the surface of a bedside table, this limit appears to be very high. Surface cleaning and disinfection is considered to be of importance to control the spread of VRE during outbreaks.¹⁵ In our study we looked for enterococci on the bedside tables and found low numbers of enterococci on five of the 31 tables investigated, in two cases on Appeartex-treated surfaces. A previous study has stressed the importance of using broth enrichment cultures for detection of enterococci in the environment.¹⁶ If we had used a major part of the sampling solution for broth enrichment culture of enterococci we might have detected some additional tables with a low level of contamination. If used alone, however, broth enrichment culture provides no information concerning the level of surface contamination, which may be of importance for the risk of transmission.

Previous studies have shown that contact agar plates are more suitable than swab methods to take samples from low level contaminated surfaces.¹⁷ In our study we compared the results using contact plates with samples taken using a new swabbing technique, the use of two sequential nylon flocked swabs (G. Hedin, J. Rynbäck, B. Loré, unpublished manuscript). When the two swabs were put into the same tube with sampling solution, which was subsequently subcultured on agar, the total aerobic counts obtained were not significantly different from the corresponding counts obtained with the contact plate method. An advantage with the swab rinse method is that the sampling solution can be aliquoted, if required, and be spread on different kinds of selective agar plates, e.g. in this study where part of the solution was spread on Enterococcosel agar.

If bacterial contamination in the environment is of importance in the transmission of multiresistant bacteria, it would be prudent to reduce the microbial load in the environment as much as possible. According to the results of the present study, a significant persistent effect on the number of bacteria on the surfaces of bedside tables in a hospital ward was achieved by the application of Appeartex to the surfaces. The impact of the observed reduction on reducing transmission of multiresistant bacteria between patients,

however, is unknown. More studies are needed in this field, in general, as well as in relation to the effect of Appeartex.⁸

Conflict of interest statement

None declared.

Funding sources

None.

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