Validation of Akacid plus as a Room Disinfectant in the Hospital Setting

Christina Kratzer,[1] Selma Tobudic,[1] Ojan Assadian,[2] Astrid Buxbaum,[1] Wolfgang Graninger,[1] and Apostolos Georgopoulos[1*]

Department of Internal Medicine I, Division of Infectious Diseases and Chemotherapy,[1] and Clinical Institute for Hygiene and Medical Microbiology,[2] Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria

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*Corresponding author: Prof. DDr. A. Georgopoulos
Department of Internal Medicine I, Division of Infectious Diseases and Chemotherapy,
Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria
Phone: +431 40400 5139 Fax: +431 40400 5200.
E-mail:apostolos.georgopoulos@meduniwien.ac.at

ABSTRACT

Akacid plus, a novel polymeric guanidine with broad antimicrobial activity against multi-antibiotic resistant bacterial strains, was used in the present study as a room disinfectant. Disinfection of closed rooms experimentally contaminated with antibiotic-susceptible and multi-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Escherichia coli* was performed using Akacid plus at concentrations of 0.1, 0.25 and 0.5% for 100 minutes. Bacterial suspensions were distributed on plastic and stainless steel plates and placed in a test room. Recovery of the test micro-organisms was determined before nebulizing, 60 and 100 minutes after initiation and 4 hours after the end of room disinfection by a simple swab-rinse technique. The swab-rinse method demonstrated a dose- and time-dependant effectiveness of Akacid plus in eradicating *S. aureus*, *E. coli* and *P. aeruginosa* on plastic and stainless steel plates. Nebulizing 0.5% Akacid plus was successful in eliminating all hospital pathogens within 340 minutes. After the use of 0.25% Akacid plus MRSA was still detectable on microbial carrier plates. The test concentration of 0.1% Akacid plus achieved a significant reduction of *S. aureus* and *P. aeruginosa* on plastic and stainless steel plates, but was only sufficient to eradicate *E. coli*. These results suggest that nebulized Akacid plus at a concentration of 0.5% is a potent substance for eradication of pathogenic organisms in the hospital setting.

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Data of the World Health Organization show that in the United States some 14,000 individuals are infected and die each year as a result of drug-resistant microbes acquired in hospitals. In intensive care units, nosocomial infections increase the total costs by $3306 and the length of stay by 18.2 days per patient (3). Methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-resistant Gram-negative rods are wreaking havoc in hospital wards around the world (24). Herr *et al.* (12) determined additional costs of hygiene measures (barrier precautions, isolation, and decontamination) required for MRSA carriers in German hospitals by averaging 372 euros for one MRSA patient hospital-day and 9,263 euros per MRSA case. Already, MRSA and extended spectrum beta-lactamase producing Enterobacteriaceae have spread outside the hospital. Epidemic spread of resistant bacteria and resistance genes is primarily supported by selection of resistant micro-organisms by frequent application of antimicrobial agents, inadequate or inappropriate therapy, use of broad spectrum antibiotics as growth promoters for animal foods and as pesticides for agriculture (16) and, because of lack of general hygiene measures, transmission of hospital strains to other patients and medical staff (2, 19, 20).

Clearly, preventive measures for the termination of this mode of selection and transmission are of vital importance. In hospital and health care settings antiseptics and disinfectants are an essential tool for infection control and aid in prevention of nosocomial infections (5, 9). By acting rapidly, disinfectants can prevent the spread of antibiotic-resistant pathogens (4). It has been postulated that room disinfection in hospital settings is an important measure in the prevention of colonization and new infections.

The novel polymeric guanidine Akacid plus is a new member of the cationic family of disinfectants. It shows high water solubility with broad *in vitro* activity against Gram-positive and Gram-negative bacteria and fungi. Recently, we have demonstrated bactericidal activity

of Akacid plus in basic and extended quantitative suspension tests against bacterial quality control strains (15). Moreover, similar MIC values were evaluated for antibiotic-sensitive and multi-antibiotic resistant bacterial strains, whereas MIC_{90} of chlorhexidine and mupirocin showed a 4-fold and 32-fold increase for MRSA in comparison to methicillin-sensitive *S. aureus* (A. Buxbaum, C. Kratzer, W. Graninger, and A. Georgopoulos, Antimicrobial profile of the new biocide Akacid plus, Journal of Antimicrobial Chemotherapy, revised manuscript submitted). The aim of the present study was to evaluate the antimicrobial activity of different concentrations of Akacid plus for disinfection of closed rooms, which had been experimentally contaminated by antibiotic-susceptible and multi-resistant *S. aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, by a simple swab-rinse technique.

MATERIALS AND METHODS

Disinfectant and neutralizing solutions. A stock solution of Akacid plus, a 3:1 mixture of poly-(hexamethylen-guanidinium-chloride) and poly-[2-(2-ethoxy)-ethoxyethyl)-guanidinium-chloride] as a 25%/v aqueous solution (Ch. 1007, POC, Vienna, Austria), was used and diluted with tap water to the desired concentrations of 0.1, 0.25 and 0.5%/v (pH=6.5). Sodium tryptone solution (NaT) supplemented with neutralizing substances including 3%/wt saponin (VWR international, Fontenay sous Bois, France), 3%/wt polysorbate 80 (Merck, Hohenbrunn, Germany), 0.1%/wt histidine (Fluka, Buchs, Switzerland) and 0.1%/wt cysteine (Fluka) was used as neutralizer as described previously (15).

Micro-organisms. For activity testing, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 10536 were selected. Multi-antibiotic resistant clinical strains of *S. aureus* 9892 (resistant to oxacillin, amoxicillin/clavulanic acid, cefazolin, gentamicin, erythromycin, clindamycin, ciprofloxacin and mupirocin), *P. aeruginosa* A9726I (resistant to piperacillin/tazobactam, ceftazidime, cefepime, fosfomycin, tobramycin and ciprofloxacin) and *E. coli* 1905 (resistant to mezlocillin, piperacillin, amoxicillin/clavulanic acid, cefazolin, cefotaxime, cefepime, gentamicin, trimethoprime and ciprofloxacin) were utilized. These strains were isolated and identified in 2005 at the Department of Internal Medicine I, Division of Infectious Diseases and Chemotherapy, Medical University of Vienna from wound, respiratory tract and urinary tract infections.

Preparation of test plates. Plastic boards (6.5 × 5 cm) (Fackelmann, Hersbruck, Germany) and stainless steel plates (5 × 5 cm) served as microbial carriers. Viable ATCC and multi-resistant strains of *S. aureus*, *P. aeruginosa* and *E. coli* were used as test organisms.

Following the procedure of the European basic quantitative suspension test (6) bacteria were grown on tryptone soya agar (TSA) (Oxoid, Basingstoke, Hampshire, UK) for 24 hours and transferred to TSA for another 24 hours. Then the test bacteria were suspended in sodium tryptone (NaT) solution to an optical density at 620 nm (1.5-5 $\times$ 10^8 CFU/ml). Hundred microliters of the phase 1 standard test suspension were inoculated onto hard surfaces and evenly distributed with a sterile glass spatula. A single test plate was contaminated with the test suspension of only one test strain. Microbial carriers were dried for 1 hour in a lamina air flow cabinet at a room temperature of 20-22°C and a relative humidity ranging from 45 to 60%.

Room disinfection in the test room. In order to evaluate the activity of Akacid plus as a room disinfectant, a test room of approximately 41 m^3 was chosen. Medical devices and equipment were left in the room. The inlet and outlet vents of the air-conditioning system were sealed with adhesive tape, and the door and windows were closed. For each test strain, microbial carrier plates were placed on the floor corner, below the table, on the work space and on the cupboard. After placing the bacterial carriers, five liters of liquid containing 0.1, 0.25 or 0.5% Akacid plus solution or five liters of liquid alone (Akacid plus-free control) were poured in a FONTAN Portastar ULV aerosol applicator (Swingtec, Isny, Germany) which produces a droplet size of 2-20 microns. Nebulizing with gaseous Akacid plus or water alone was performed for 100 minutes.

Recovery of the test bacteria. To evaluate the antimicrobial activity of Akacid plus, the survival of the test bacteria was determined before nebulizing (timepoint 0), 60 (timepoint 1) and 100 minutes after the initiation (timepoint 2) and 4 hours after the end of room disinfection (timepoint 3) in the test room using a simple swab-rinse technique with neutralizing solution. For this detection method 1.5 ml neutralizing solution were transferred

onto each test surface. With this fluid and a pre-moistened cotton swab, the test area was systematically abraded for 15 s, 0.5 ml-amounts of the neutralizing solution were collected, ten-fold dilutions in neutralizing solution were prepared and plated on TSA containing neutralizing substances. Swab-rinse cultures were incubated for 48-72 hours at 37 C. Bacterial colonies on TSA were distinguished on the basis of different morphology (size and color of the colonies). To confirm the presence of *S. aureus*, *E. coli* and *P. aeruginosa*, bacterial cells were cultured on blood agar and identified by biochemical tests (API Staph, API 20E, API 20NE).

Data and statistical analysis. The reduction of the number of viable bacterial cells (CFU/plate) was described by arithmetic means and standard deviation of three individual experiments for 0.1, 0.25 and 0.5% Akacid plus in comparison to the biocide-free control at three different time points (60 and 100 minutes after the initiation and 4 hours after the end of room disinfection) on plastic and stainless steel plates. Differences between the selected concentrations and the biocide-free control were assessed with Student's t test for independent samples. If significance was achieved, the multi-comparison of means was performed using the Bonferroni-Holm-correction, multi-comparison significance level was ≤ 0.05.

RESULTS

Room disinfection with 0.1, 0.25 and 0.5% Akacid plus. Three room disinfection trials with 0.1, 0.25, 0.5% Akacid plus and with the biocide-free control were performed with antibiotic-sensitive and multi-resistant strains of *S. aureus* (Figure 1), *E. coli* (Figure 2) and *P. aeruginosa* (Figure 3) applied on stainless steel and plastic plates. In the presence and absence of Akacid plus the temperature and relative humidity in the test room increased from 21-23°C and 40-60% humidity to 24-25°C and 85-100% humidity during the nebulizing procedure. Four hours after the end of nebulizing the relative humidity reached the initial value again, while the temperature was still elevated. Recovery of the tested strains from steel and plastic plates was evaluated before nebulizing, 60 and 100 minutes after the initiation and 4 hours after the end of room disinfection by a swab-rinse technique. At time point 0 (before room disinfection) 1.2×10^6-1.0×10^7 CFU of *S. aureus*, 6.0×10^5-2.2×10^6 CFU of *E. coli* and 2.0×10^5-1.5×10^6 CFU of *P. aeruginosa* were detectable on stainless steel plates and 5.0×10^5-4.8×10^6 CFU of *S. aureus*, 2.5×10^5-1.3×10^6 CFU of *E. coli* and 1.5×10^5-1.0×10^6 CFU of *P. aeruginosa* were detectable on plastic plates. In the absence of Akacid plus a moderate reduction of the bacterial count of <1 log10 step was seen for *S. aureus* (Figure 1) 4 hours after the end of nebulizing, whereas a reduction of 1-3 log10 steps was detected for the Gram-negative test organisms (Figure 2 and Figure 3) on plastic and stainless steel plates.

Room disinfection with 0.5% Akacid plus was successful in eliminating all tested pathogens (lower detection limit 3 CFU/plate) on stainless steel and plastic plates within 340 minutes. On plastic plates both strains of *S. aureus* (p=0.006) and *E. coli* (p=0.005-0.008) were killed within 60 minutes, while *P. aeruginosa* required a longer exposure for 340 minutes. On stainless steel plates *E. coli* (p=0.007) was eliminated within 60 minutes. In

contrast, ATCC and antibiotic-resistant strains of *P. aeruginosa* (p=0.005-0.007) and *S. aureus* (p<0.001-0.002) were still detectable on steel plates after nebulizing Akacid plus for 100 minutes (~10^1 CFU/plate), but they were eradicated within 340 minutes.

Four hours after nebulizing 0.25% Akacid plus stainless steel and plastic plates still yielded 6.0×10^1-1.2×10^2 bacterial cells of MRSA 9892 (p=0.002-0.003), whereas the Gram-negative micro-organisms were not detectable on test materials regardless of their antibiotic susceptibility.

The test concentration of 0.1% Akacid plus achieved a significant reduction of *S. aureus* (p=0.002-0.005) and *P. aeruginosa* (p=0.006-0.007) on bacterial carriers, but was only sufficient to eradicate *E. coli* (p=0.003-0.005) within 340 minutes.

DISCUSSION

Akacid plus, a mixture of two different polymeric guanidine compounds (CAS No.:374572-91-5 and CAS No.:57028-96-3), is a new commercial product of an Austrian company and is registered in the European Union. The present study demonstrates the activity of Akacid plus as a room disinfectant of closed rooms contaminated with antibiotic-susceptible and multi-resistant strains of *S. aureus*, *E. coli* and *P. aeruginosa*. Disinfectants currently validated for room disinfection achieve high antimicrobial activity, but also high toxicity. Therefore, safety guidelines have to be considered. At the present time the only accepted method available for decontaminating a biological safety cabinet is to use formaldehyde gas (17). Formaldehyde is highly effective against bacteria, virus, bacterial toxins and spores (21), but also highly toxic (23). Formaldehyde gas has a pungent, irritating odor that is detectable even at very low concentrations (below 1 ppm) and can cause irritation of the eye, skin, and respiratory tract even at low levels for short periods. Its vapor is flammable between 7% and 73% at room temperature and it is explosive in the presence of strong oxidizers (1). Also hydrogen peroxide vapor which is highly active as a room disinfectant against MRSA (8), *Clostridium botulinum* spores (13) and *Mycobacterium tuberculosis* (14) requires exact surveillance of the gas concentration throughout the decontamination cycle due to its corrosive and toxic properties (22). In contrast, the well-known cationic antimicrobials such as benzalkonium chloride, chlorhexidine and polyhexamethylene biguanide combine a broad antimicrobial activity and a low toxicity profile (11). Similarly, low toxicity of Akacid plus was detected in toxicological animal experiments. In the “Acute Oral Toxicity Study” and the “Acute Dermal Toxicity Study” with rats a median lethal dose of Akacid plus >2000 mg/kg body weight was determined. The

"Acute Dermal Irritation/Corrosion Study" did not reveal any irritating or corrosive properties of the novel polymeric guanidine (A. Buxbaum, C. Kratzer, W. Graninger, and A. Georgopoulos, Antimicrobial profile of the new biocide Akacid plus, Journal of Antimicrobial Chemotherapy, revised manuscript submitted). Akacid plus is a safe, not flammable, non-explosive and odorless substance. Patients in hospital side-rooms in direct vicinity to the contaminated room are not disturbed or endangered during the disinfection process. Due to its low toxic and non-corrosive properties, there was no need for preparations such as protection of medical devices and computer monitors or sealing the doors by adhesive tapes, when nebulizing was performed.

To evaluate the antimicrobial activity of Akacid plus quantitative cultures of experimentally contaminated stainless steel and plastic plates were performed by a simple swab-rinse technique with neutralizing solution. The detection method demonstrated a dose-dependant and time-dependant activity of nebulized Akacid plus. All in-door controls of the bacterial pathogens applied on the stainless steel plates tended to reach higher bacterial counts than on plastic plates. Although Neely (19) has shown a short survival time for *E. coli* and *P. aeruginosa* on fabrics and plastics used in hospitals (only 1-7 hours), in the present study viable bacterial cells on test materials were detectable during the whole nebulizing and exposure to the Akacid plus-free control. Nevertheless, the stainless steel and plastic plates yielded higher bacterial counts of *S. aureus* than of the Gram-negative micro-organisms.

0.5% Akacid plus was active in eradicating most tested pathogens within 100 minutes, ~10^1 CFU of *S. aureus* ATCC 6538 and MRSA 9892 were still detectable on stainless steel plates. Further exposure of 4 hours was required to eliminate all bacterial strains. Complete room disinfection takes on average less than 6 hours.

Due to its cationic nature, Akacid plus can be inactivated by the presence of anionic soaps. Therefore, the conventional terminal cleaning must not be performed before the nebulizing, but following the complete disinfection procedure.

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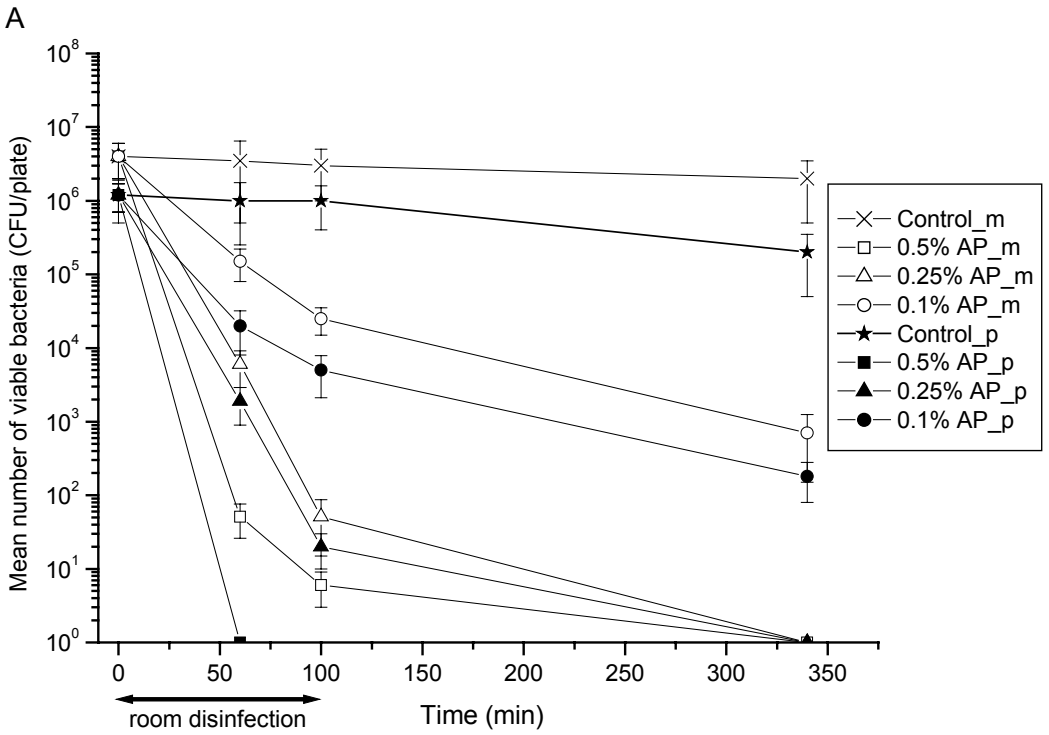
FIGURE 1. Time-dependant bacterial reduction of the ATCC (A) and the multi-antibiotic resistant strain 9892 (B) of *S. aureus* on stainless steel (_m; open symbols) and plastic plates (_p; filled symbols) after the use of 0.5 (square), 0.25 (triangle) and 0.1% (circle) Akacid plus (AP) in comparison to an Akacid plus-free control on steel (cross) and plastic plates (asterisk) determined by the swab-rinse technique. Errors bars represent average of 4 samples ± 1 standard deviation of 3 independent experiments.

FIGURE 2. Time-dependant bacterial reduction of the ATCC (C) and the multi-antibiotic resistant strain 1905 (D) of *E. coli* on stainless steel (_m; open symbols) and plastic plates (_p; filled symbols) after the use of 0.5 (square), 0.25 (triangle) and 0.1% (circle) Akacid plus (AP) in comparison to an Akacid plus-free control on steel (cross) and plastic plates (asterisk) determined by the swab-rinse technique. Errors bars represent average of 4 samples ± 1 standard deviation of 3 independent experiments.

FIGURE 3. Time-dependant bacterial reduction of the ATCC (E) and the multi-antibiotic resistant strain A9726I (F) of *P. aeruginosa* on stainless steel (_m; open symbols) and plastic plates (_p; filled symbols) after the use of 0.5 (square), 0.25 (triangle) and 0.1% (circle) Akacid plus (AP) in comparison to an Akacid plus-free control on steel (cross) and plastic plates (asterisk) determined by the swab-rinse technique. Errors bars represent average of 4 samples ± 1 standard deviation of 3 independent experiments.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0.1% AP_m
Control_p
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0.25% AP_p
0.1% AP_p

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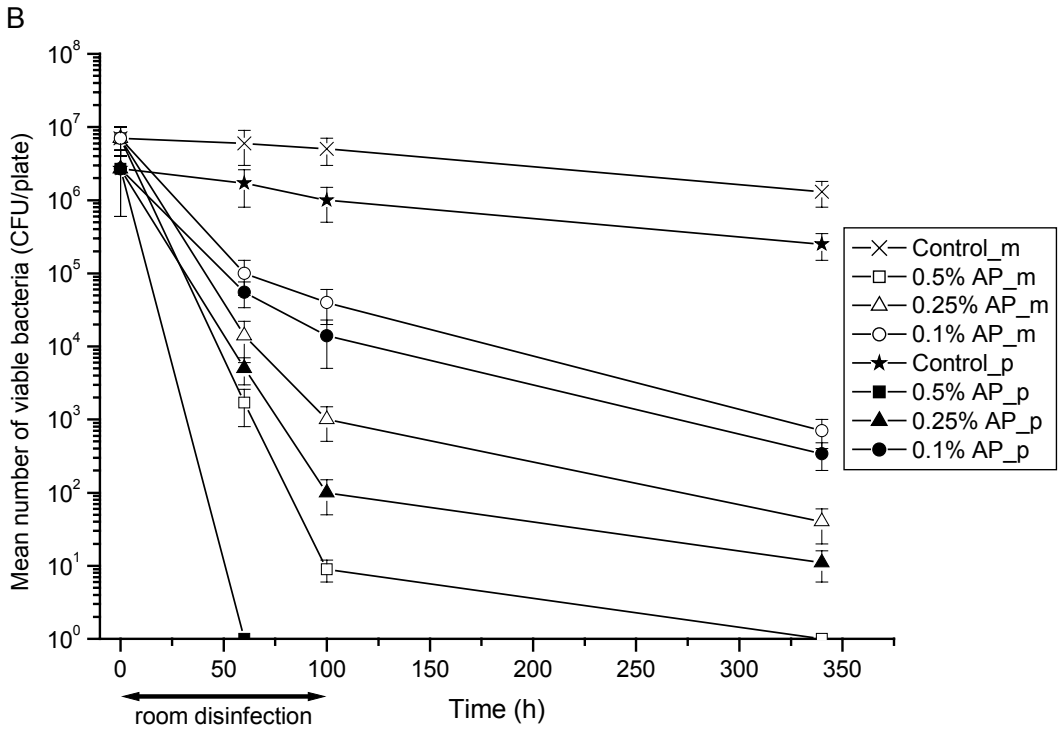
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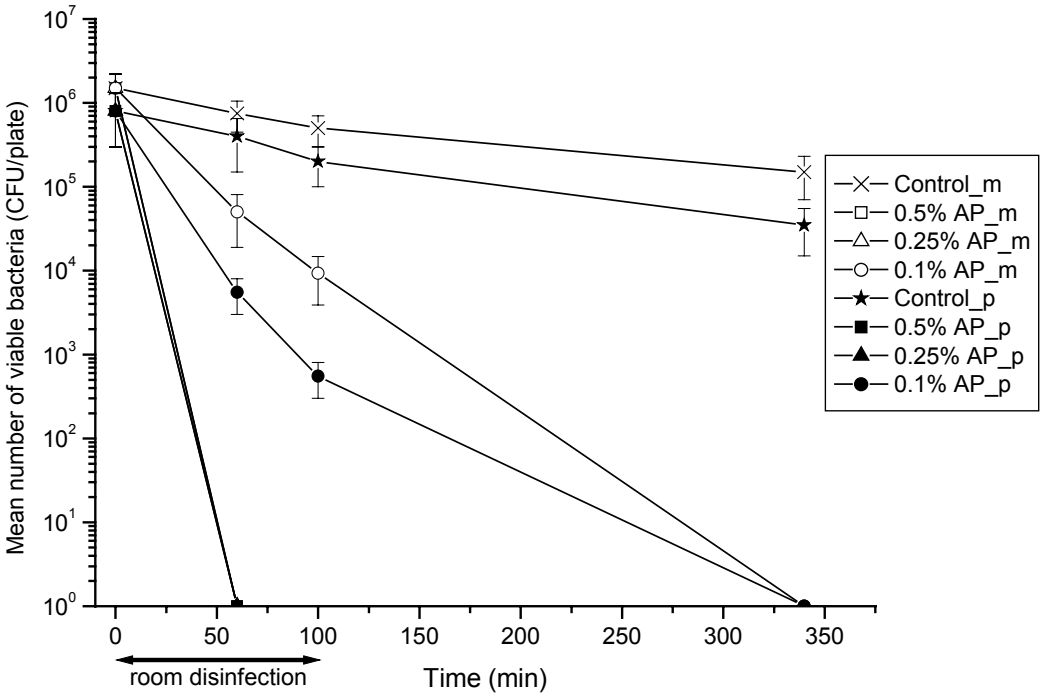
2

3

C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10^6
10^7
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100
150
200
250
300
350
room disinfection
Time (min)
Control_m
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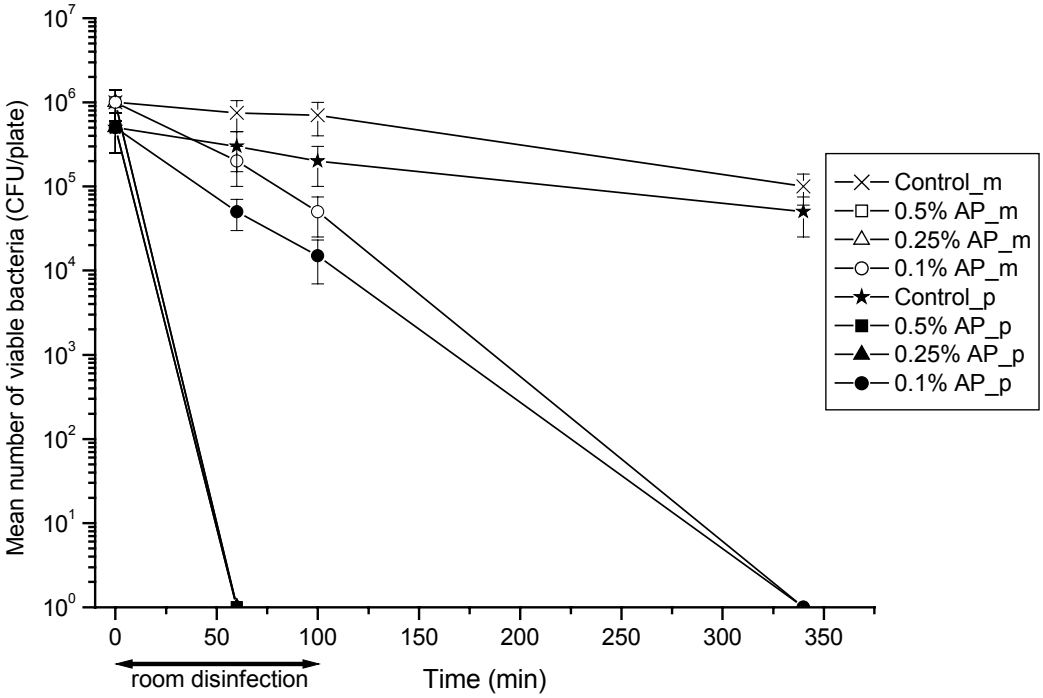
1

D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10^7
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100
150
200
250
300
350
room disinfection
Time (min)
Control_m
0.5% AP_m
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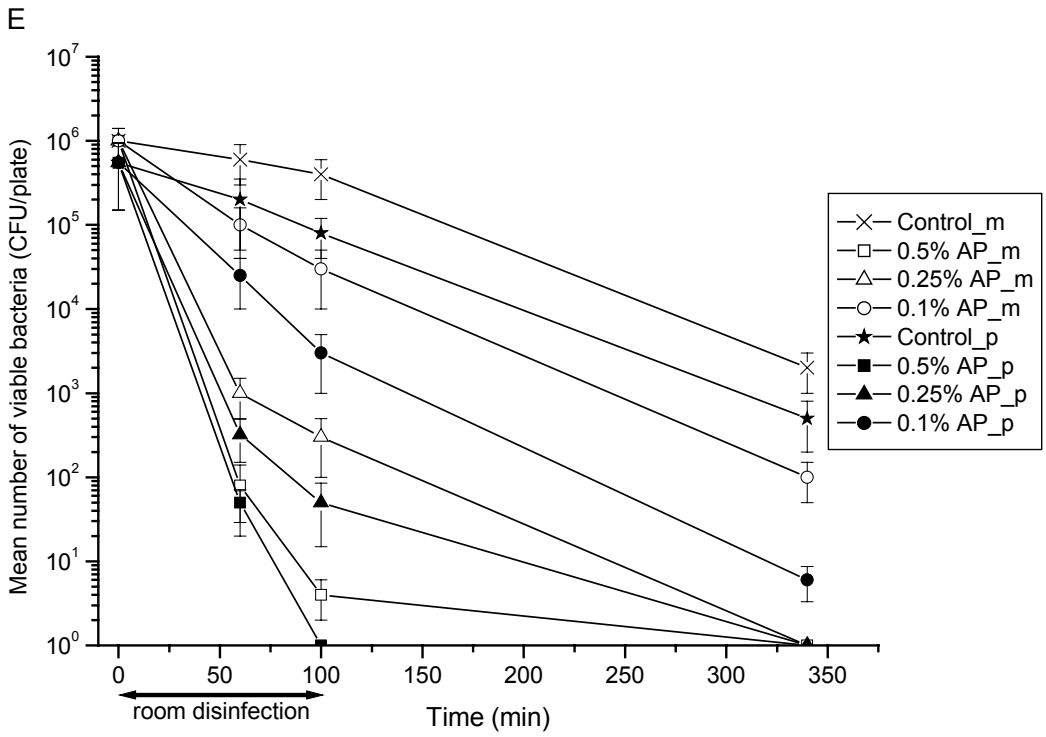
2

3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Time (min)
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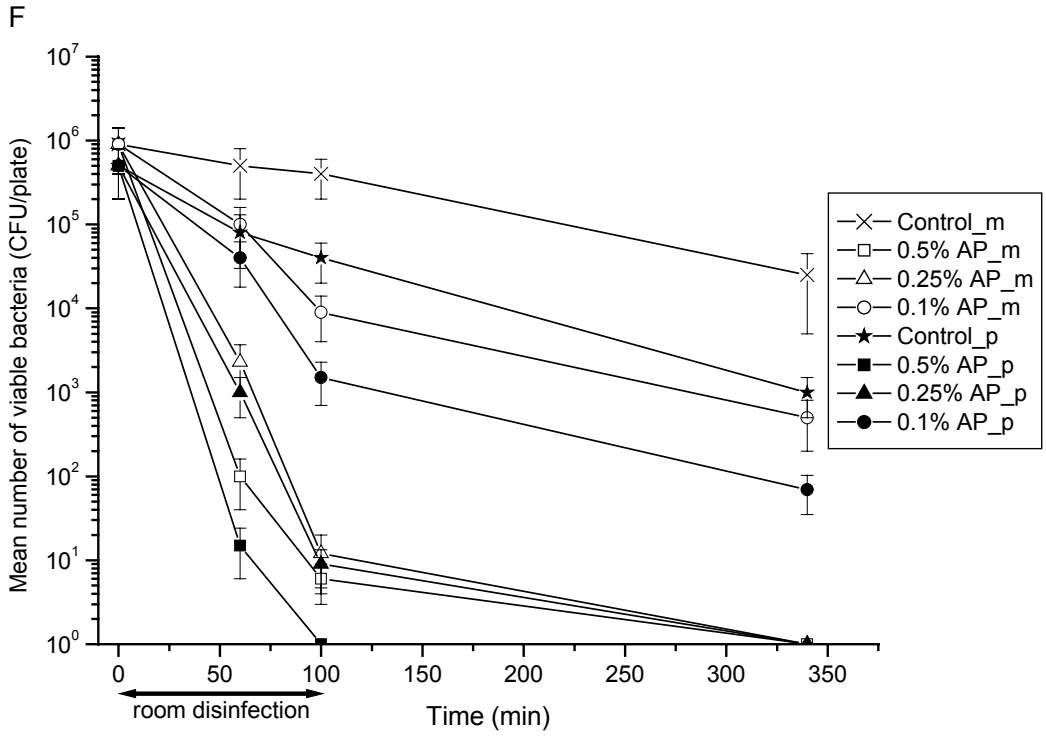
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0.1% AP_m
Control_p
0.5% AP_p
0.25% AP_p
0.1% AP_p

2