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Major article

Influence of biological fluids in bacterial viability on different hospital surfaces and fomites

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Background: The hospital environment is susceptible to bacterial contamination along with survival in fomites and surfaces, allowing dissemination of potential pathogenic strains. The present research aimed to evaluate the influence of biological fluids in bacterial viability on fomites and surfaces commonly present in nosocomial environment.

Methods: Four different fomites and surfaces (ceramic floor, cotton fabric fragments and synthetic fibers, and eggcrate foam mattress) were contaminated with potential pathogens (*Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*), then submitted to influence of biological fluids (blood, urine, artificial saliva). The viability of strains was evaluated at 24 hours after contamination and then in intervals of 7 days, by the colony-forming unit count technique.

Results: *S aureus* presented viability (>70 days) in all conditions tested, *E faecalis* and *K pneumoniae* had decreased viability over time, and *E coli* did not exhibit a growth relationship with surfaces or fluids. Persistence and adaptability capacity of potential pathogens in fomites and surfaces exposed to the patient are important for guidance, planning, and outlining of protocols for microorganism dissemination control and prevention in the health care environment.

Infections in hospitals are related to the hospitalization period, irrational use of antibiotics, poor hygiene, and lack of education in the general population, which results in high morbidity and mortality. Another problem associated with hospital-acquired infections is the high costs for the patient, family, and health care institutions.[1,2]

In general, opportunistic bacteria can contaminate the nosocomial environment, and sometimes can colonize the human body through invasion of injured tissue, leading to proliferation and development of disease symptoms.[3,4] The vast majority of microorganisms are able to grow and survive on fomites and surfaces such as ceramic, stainless steel, glass, and polyethylene.[5] Some studies have suggested that this behavior may be related to the ability to adhere and form biofilms on different materials even in the presence of antibiotic or biocide exposure.[5] In terms of the hospital environment, the persistence of those potential pathogens on surfaces and fomites poses another challenge, given that these pathogens can cause severe infections in patients during or after admission. Moreover, the dissemination of multidrug strains, such as heteroresistant vancomycin-intermediate *S aureus*, further underscores the importance of this kind of monitoring.[6]

Considering the potential of pathogenic bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* to survive in different environments and serve as a source of contamination, in the present study we aimed to evaluate the influence of biological fluids in bacterial viability on fomites and surfaces commonly present in the nosocomial environment.

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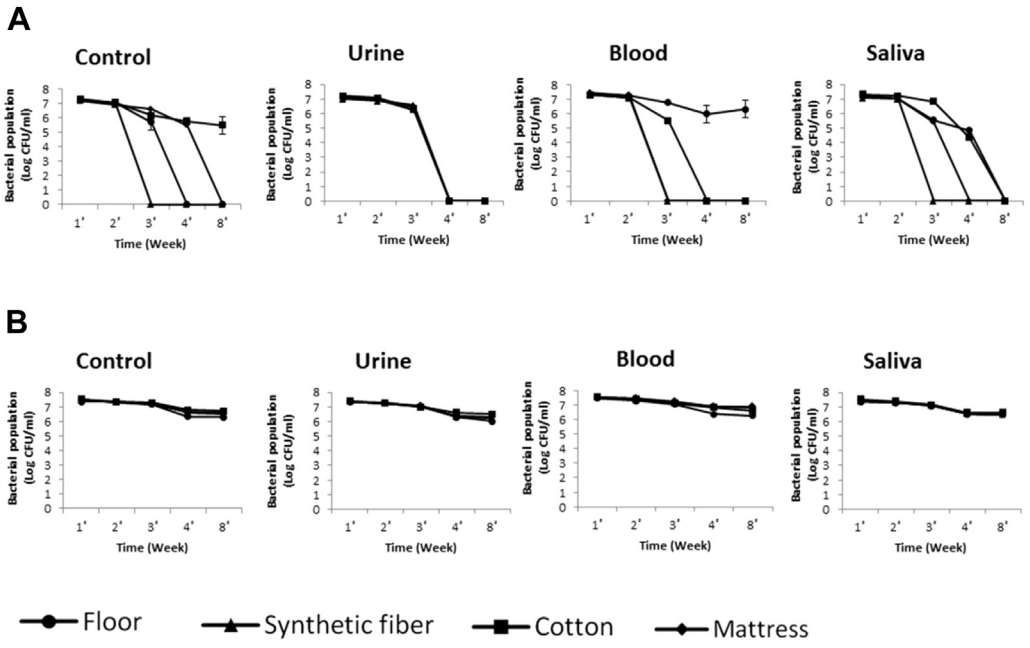

Fig 1. Populations of *E faecalis* (A) and *S aureus* (B) grown in different combinations of conditions using biological fluids (control, blood, urine, and saliva) and surfaces (floor, synthetic fibers, cotton, and mattress). Results are mean of triplicates; vertical bars represent standard deviation.

MATERIALS AND METHODS

Bacterial strains

We tested various bacterial strains, including *S aureus* (American Type Culture Collection [ATCC] 25923), *E faecalis* (ATCC 29212), *E coli* (ATCC 35218), *K pneumoniae* (ATCC 700603), and *P aeruginosa* (ATCC 27583). All strains were part of the bacterial collection of the microbiology laboratory at Universidade do Oeste Paulista. The strains were maintained at −80°C in brain heart infusion broth (BD GmbH, Heidelberg, Germany) containing 20% (vol/vol) glycerol.

Inoculum preparation and surface contamination

The inocula were prepared from bacterial culture grown in brain heart infusion broth (BD GmbH). The culture was incubated at 37°C for 24 hours until it reached the exponential phase, followed by centrifugation for 10 minutes at 2500 rpm. The pellet was washed and resuspended in sterile saline at a concentration corresponding to 1.0 on the McFarland scale (3.0×10^8 CFU/mL).[7] Subsequently, the volumetric concentration (1:1) was added to the suspension composed of blood, urine, artificial saliva (dose manipulation of Salivan; Apsen Farmacêutica, São Paulo, Brazil), and sterile distilled water. Bacterial viability was also assessed on various surfaces, including ceramic floor (model 3020, PEI 4, quality A, 7.7 mm thick; LEF Cerâmica, Paracicaba, Brazil), fragments of cotton fabric (white) and synthetic fibers (blue), and eggcrate foam mattress (blue, 7 mm thick), previously cut in dimensions of 3.0 cm^2 each, washed, and autoclaved. An aliquot of 20 μL obtained with the mixture of the inoculum and biological fluids was applied to different surfaces, and each was incubated in a sterile Petri dish plate at room temperature.

Viability of bacterial cells

The growth and survival of the bacterial inoculum was evaluated after 24 hours and in intervals of 7 days until the absence of bacterial growth was noted. The contaminated surfaces were immersed in reservoirs containing tryptic soy broth (HiMedia, Mumbai, India), slowly agitated, and then incubated at 37°C for 24 hours. Afterward, 100 μL of grown bacterial culture were transferred to a tube containing 1.0 mL of sterile saline for serial dilutions (1:10, 1:100, 1:1000, and 1:10,000). An aliquot of 100 μL obtained from the lowest dilution (1:10,000) was pipetted, inoculated in Mueller-Hinton agar (Neogen, Lansing, MI), and then incubated at 37°C for 24 hours. CFU were measured by the pattern counting technique and expressed in CFU/mL.

Statistical analysis

The data were expressed as log base 10 and analyzed using nonparametric tests. The Friedman test was used for dependent variables in randomly paired groups, and the Kruskal-Wallis test was used for analysis of independent variables. Comparisons among the conditions studied were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria), and a P value $<.05$ was considered significant.[8]

RESULTS AND DISCUSSION

Our finding demonstrate that the viability and survival of bacteria can be altered according to the type of surface and the presence of biological fluid. In general, it was observed that the vast majority of bacteria tested could not survive for long periods in the presence of urine (Figs 1-3). This finding can be explained mainly by the composition of urine, which contains up to 294 different metabolites including urea, inorganic salts, creatinine, ammonia, organic acids, various water-soluble toxins, and pigmented products, and has an acidic pH.[9] On the other hand, blood was the best medium for bacterial survival, likely because it provides a nutritionally rich environment.[10]

E faecalis exhibited reduced viability from the 21st day, with no significant difference compared with controls (Fig 1). Comparison of groups showed significant differences between cotton fabric