

Survival of *Staphylococcus* and other bacteria in skin prick test antigens solutions

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Background: Skin prick tests are commonly used for human allergy testing and determination. Because solutions are kept at 4°C and patient's skin is cleaned, the same solutions are used on many patients with the same applicators without sterilization.

Objectives: Our objectives were to determine whether a model bacteria could survive in antigen solutions and whether bacteria from patient's skin could be found in clinical antigen solutions.

Methods: Unused antigen solutions were spiked using *Staphylococcus epidermidis*. Concentration was followed from time = 0 to time = 21 days. Clinical samples consisted of antigen solutions used on a daily basis, from different hospital centers (n = 146). These were tested for total bacterial counts and detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci. Isolates were identified by 16S rDNA sequencing.

Results: The concentration of *S epidermidis* remained stable over the 21 days. Twenty-three percent of the clinical antigen solutions contained bacteria. As expected, species like *S aureus*, *S hominis*, *S epidermidis*, and *Micrococcus luteus* were highly represented (62.5%) within a total of 20 different species.

Conclusion: Skin bacteria can survive in antigen solutions used in skin prick tests. Moreover, even if no MRSA were observed in field samples, nosocomial infections could be a concern if pathogens were present on the skin of some tested patients.

Key Words: Nosocomial infection; skin bacteria; allergy testing.

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Nosocomial infections are major concerns in the medical environment. Good practices in hospitals and care centers can reduce the spread of pathogens.¹ Some communal medical equipment are potential sources of cross infections. Stethoscopes² and marker pens used in surgical practices^{3,4} have been identified as vector of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Skin prick tests or scratch tests are commonly used for allergy testing and determination.⁵ In current practices, skin prick testing is most commonly performed on the forearm, although the back is sometimes used. In the standard procedure, the arm is first cleaned with alcohol and then a drop of commercially produced allergen extract is placed onto a marked area of skin using a glass dipstick. Using a

sterile lancet (usually a 27-gauge needle), a small prick through the drop is made. This allows a small amount of allergen to enter the skin. Because solutions are kept at 4°C and patient's skin is cleaned with alcohol, the same solutions are used on many patients with the same dipstick without being sterilized. Generally, these tests are done without knowing the patient's infections and/or colonization status. The aim of this study was to determine whether a model *Staphylococcus* can survive in antigen solutions and to verify whether viable and culturable bacteria from patient's skin can be recovered from clinical antigen solutions suggesting that these solutions can potentially act as reservoirs for cross contamination of pathogens such as MRSA.

METHODS

Growth and survival of model *Staphylococcus* in antigen solution

Commercial antigen solutions (n = 6) containing histamine (1 mg/mL), dog hair danders, horse hair danders, and cat hair were inoculated with approximately 1000 colony-forming units (CFU) of *Staphylococcus epidermidis* in logarithmic growth phase (based on growth curve). The concentration of bacteria was followed by plating 50 µL in triplicate on Blood Agar (Difco, BD Canada, Oakville, ON, Canada) at various times (before inoculation [control], time [t] = 0; t = 3

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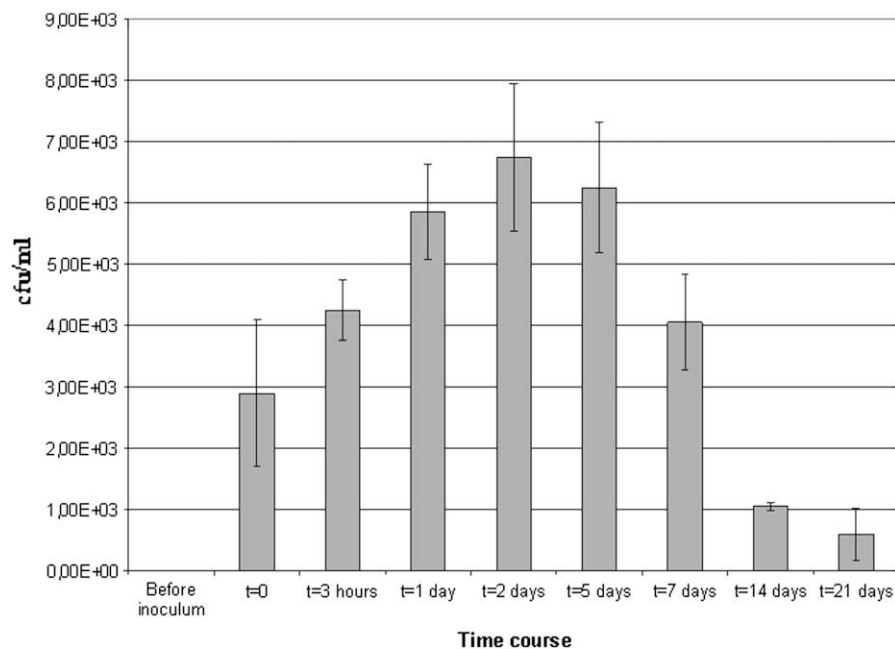


Fig 1. Follow-up by culture on blood agar of *Staphylococcus epidermidis* spiked in commercial antigen solutions.

hours; and $t = 1, 2, 5, 7, 14$, and 21 days). Plates were incubated 48 hours at 37°C before counting.

Bacteria from clinical antigen solutions

A total of 146 antigen solutions used on a daily basis from 4 different hospitals in the Quebec City area were collected for testing. These solutions were all kept at 4°C . For plate counts and bacteria isolation, $50\ \mu\text{L}$ of each sample solutions were plated in duplicate on Blood agar, Oxacillin Resistance Screening Agar Base (ORSAB) (Oxoid, Nepean, ON, Canada), and vancomycin-resistant Enterococci agar (VREA) that consist of m Enterococcus agar (Difco, BD Canada) supplemented with $8\ \text{mg/L}$ vancomycin. These culture media were used for the detection of MRSA and vancomycin-resistant enterococci, respectively. Each plate was incubated at 37°C for 48 hours before counting and isolation of the species found. Isolation was done on triplic soy agar, and each isolate from blood agar was also plated on ORSAB and VREA to verify the presence of MRSA and vancomycin-resistant enterococci. Isolates were archived on FTA Cards (Whatman, United Kingdom) according to the manufacturer's instructions.

Identification of the isolated bacteria

To achieve the identification of the bacterial strains found in clinical antigens solutions, the partial 16S rRNA gene (nucleotide 63-1387; *Escherichia coli* numbering) was amplified from isolates' DNA extracts prepared from FTA Cards using primers 63forward

(5'-CAGGCCTAACACATGCAAGTC-3') and 1387reverse (5'-GGGCGGWGTGTACAAGGC-3') previously described by Marchesi et al.⁶ The polymerase chain reaction (PCR) mix was the same as described by the authors, and the PCR conditions were as follows: 1 hold at 95°C for 5 minute, then 30 cycles at 95°C for 60 seconds, 55°C for 60 seconds, 72°C for 90 seconds, followed by a final extension step of 5 minutes at 72°C . The PCR products (1324 base pairs) were visualized by agarose gel electrophoresis prior to sequencing. Sequencing was done at the Centre Hospitalier de l'Université Laval sequencing service in Quebec City using the 63forward primer on a ABI 3730/XL. Each DNA sequence was compared with sequences available in databases, using BLASTN from the National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>).⁷

RESULTS

As shown in Fig 1, the average inoculum at $t = 0$ was 2900 CFU/mL. As the bacteria were in logarithmic phase, we observed an increase of the population up to 4250 CFU/mL after 3 hours and 5850 after 1 day. The maximum concentration of *S epidermidis* was after 2 days at 6750 CFU/mL. After that point, the population declined to reach 600 CFU/mL at day 21.

Of the 146 clinical antigens solutions tested, 23.3% (36/146) were positive for the presence of culturable bacteria. The concentrations were between 3 to 40 CFUs/mL. From the 36 positive solutions, 39 isolates were obtained. As expected, the majority of the isolated strains was gram-positive bacteria, generally associated with human

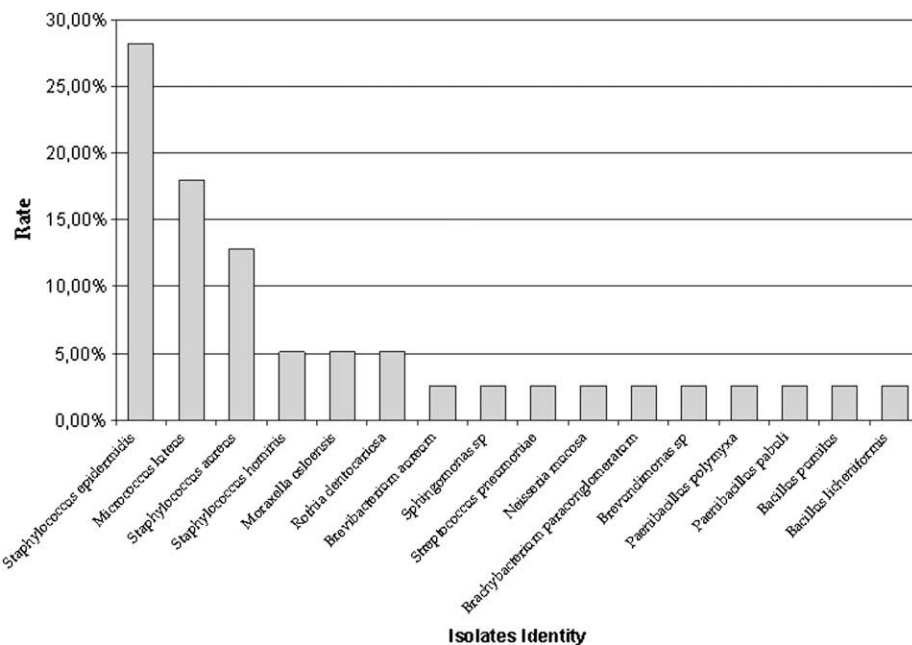


Fig 2. Identity and recovery frequency of the 39 identified isolates found in used clinical antigens solutions.

skin and/or mucosa. The most frequent isolated species were *S epidermidis* (11/39), *Micrococcus luteus* (7/39), and *S aureus* (5/39). *Staphylococcus hominis*, *Moraxella osloensis*, and *Rothia dentocariosa* were found twice and *Brevibacterium aureum*, *Streptococcus pneumoniae*, *Neisseria mucosa*, *Brachy bacterium paraconglomeratum*, *Paenibacillus polymyxa*, *Paenibacillus pabuli*, *Bacillus pumilus*, and *Bacillus licheniformis* were all found once. Two isolates—*Sphingomonas* spp and *Brevundimonas* spp—could not be identified to the species level. Figure 2 presents an overview of the isolates identity. None of the isolated *S aureus* were resistant to methicillin.

DISCUSSION

Results show that *S epidermidis* can grow and survive in antigens solutions and remain culturable for as long as 21 days. This suggests that an antigen solution contaminated by a patient's skin could act as a reservoir of pathogenic bacteria over a long period of time. The survival of bacteria in these solutions generally composed of glycerin (50%), sterile distilled water, albumin, and antigen is not surprising.

The frequency of culture-positive used clinical antigens solutions demonstrates that the contact of the dipstick with patient's skin, even if the skin is cleaned with ethanol prior to the testing, can contaminate the antigens solution. Moreover, the origin of the isolated bacteria that is normally associated with human skin flora confirms the carry over potential and the risk of cross contamination possibly encountered by the use of a

single dipstick over a wide number of patients. Even if no official reports or case study has been made based on nosocomial infections because of skin prick test procedure, the augmentation of antibiotic-resistant infections acquired in hospitals and care centers incite us to evaluate potential sources of cross infections.

No MRSA or vancomycin-resistant Enterococci was observed in our samples. However, because the contamination and survival of bacteria occur in antigens solutions and *S aureus* was found in 12.8% of the isolates, nosocomial infection could be a concern if these tests were performed on subjects who are pathogen carriers.

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