Effect of humidity and nutrient content on the survival of *Staphylococcus aureus* on a glass surface

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Undergraduate
Research Project

Premise

Staphylococcus aureus is an opportunistic pathogen common to the microflora of the upper respiratory tract and epidermis of individuals.[1] Infection rates of this hearty microorganism are on the rise.[2] *S. aureus* is able to survive desiccation on surfaces for long periods of time, thus increasing transmission of this pathogen in communities and hospitals.[3, 4]

Objectives

The objectives of this study was to determine:

1. Effect of nutrient content on survival
2. Survival times of *S. aureus* at low cell concentrations
3. Effect of relative humidity on survival
4. Effectiveness of the swab technique for recovery of cells

Methods

Relative Humidity Chambers

- Airtight Rubbermaid™ 1.6 L blue Servin' Saver containers used as chambers.
- Specified humidities obtained using respective saturated solutions:
  - 27 %; $MgCl_2{*}6H_2O$
  - 58 %; $Mg(NO_3)_2{*}6H_2O$
  - 80 %; NaCl

Fig. 1: Relative humidity chamber with slide boxes for slide incubation

Fig. 2: Chambers with specified saturated solutions in 25 ˚C incubator

Microorganism

- Strain ATCC 25923 cultured in tryptic soy broth (TSB)
- Cells washed and re-suspended in:
  - 1/10 or 1/100 strength TSB
  - Dilute saline (0.23 %)

Inoculation

- Two to three perimeters were made on sterile glass microscope slides with paraffin wax (1-cm^2).
- Perimeters were inoculated with 50 to 150 cells, verified with viable cell counts.
- Slides were incubated at 25 ˚C in chambers, followed by removal of triplicate slides per treatment at each time point.

Fig. 3: Microscope slide following overlay and incubation at 37 ˚C. Each black colony represents a single viable cell.

Recovery

- Squares overlain with 150 μl of molten 45 ˚C Baird Parker Agar.
- Slides incubated at 37 ˚C for 36 hours in a petri dish containing a sterile Kim Wipe® moistened with 2 ml autoclaved water.
- Resulting black colonies were counted using a grid and dissecting scope.
- A flocked swab was used to recover cells from an additional set of slides at time zero in trial two.

Analysis

- Colony counts were averaged for each treatment, and the standard error was determined.
- The natural log of the averages was graphed and used to determine the decay rates from the slope of the linear regression line.

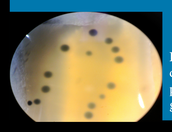

Fig. 4: Overlain square under a dissecting scope for counting possibly exhibiting cryptic cell growth.

Fig. 5: Each square was counted using a grid to ensure accurate colony counts were obtained.

Results

- Overall, humidity did not show a clear difference in survival times when studied over 43 to 99 hours (Figs. 6 and 7).
- After 196 hours, approximately 2 % of the 150 cells suspended in 1/10 strength TSB were still viable when incubated at 27 and 58 % relative humidity, compared to 0.4 % for 80 % relative humidity (Fig. 8).
- The swab technique left 35 and 41 % of the viable cells for the 1/100 and 1/10 strength TSB treatments respectively (Fig. 9).
- Decay rates increase with a decrease in organic nutrients: -0.02 and -0.05 hr^{-1} for 1/10 and 1/100 strength TSB, and -0.06 hr^{-1} for dilute (0.23 %) saline solution (Table 1).

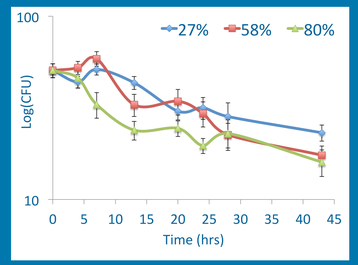

Fig. 6: Trial one using 1/10 strength TSB over 43 hours. Each square was inoculated with an average of 56 cells, verified by viable cell counts. Error bars represent the standard error at each time point for the replicates (n=9). R^2 values for the natural log of the 27, 58, and 80 % treatments was 0.912, 0.904, and 0.844 respectively.

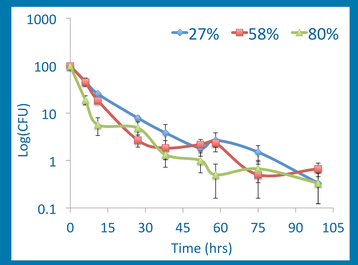

Fig. 7: Trial two using 1/100 strength TSB over 93 hours. Each square was inoculated with an average of 137 cells, verified by viable cell counts. Error bars represent the standard error at each time point for the replicates (n=6). R^2 values for the natural log of the 27, 58, and 80 % treatments was 0.940, 0.807, and 0.810 respectively.

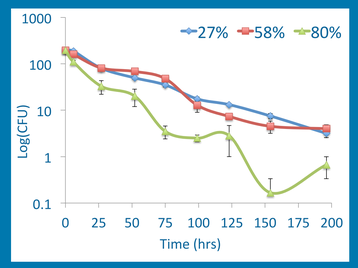

Fig. 8: Trial two using 1/10 strength TSB over 196 hours. Each square was inoculated with an average of 147 cells, verified by viable cell counts. Error bars represent the standard error at each time point for the replicates (n=6). R^2 values for the natural log of the 27, 58, and 80 % treatments was 0.988, 0.947, and 0.872 respectively.

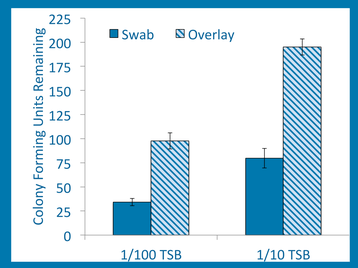

Fig. 9: Cells remaining post-swab for 1/100 and 1/10 strength TSB treatments at time zero. The 1/100 and 1/10 strength TSB squares were inoculated with an average of 137 and 147 cells respectively, both verified with viable cell counts. Remaining cells averaged 34 and 80 for the 1/100 and 1/10 strength TSB treatments respectively. Error bars represent the standard error for the replicates (n=4).

Table 1: Exponential decay rates for each treatment in both trials. The natural log of the mean cell counts for each treatment at each time point obtained was plotted to obtain a linear regression. The slope of the linear regression is *k*, the exponential decay rate.

Trial	Treatment	Relative Humidity *k* Values (hr^{-1})		
		27 %	58 %	80 %
1	0.23 % NaCl	-0.07	-0.04	-0.07
	1/10 TSB	-0.02	-0.03	-0.03
2	1/100 TSB	-0.05	-0.05	-0.05
	1/10 TSB	-0.02	-0.02	-0.03

Discussion

A great deal of research conducted on the survival rates of *S. aureus* on surfaces has used high (10^5 to 10^7 CFU/mL) inoculation levels, greater than the typical nasal carriage rates (10^2 to 10^4 CFU/mL).[5,6] This study utilized lower levels of inocula in order to represent what may be deposited following a contamination event, and to prevent cryptic cell growth which may occur at higher inocula levels leading to increased survival rates (Fig. 4).

The results suggest *Staphylococcus aureus* decays at faster rates in periods of desiccation when experiencing decreased levels of available nutrients (Figs. 6, 7, 8, and Table 1). For example, the dilute saline treatment in trial one had an average of 94 cells per square, but had no viable cells after 4 - 7 hours (data not shown). To contrast, the 1/10 strength TSB treatment had approximately 56 cells per square, and viable cells remaining over 43 hours (Fig. 6).

The effect of humidity on the survival of this microorganism was varied, though the strongest data came from the trial two 1/10 strength TSB treatment over 196 hours (Fig. 8). More trials should be completed over longer periods of time in order to support the data which suggests higher decay rates occur at higher relative humidities (Fig. 8).

The use of swabs in the recovery of bacteria is standard practice, though the results suggest actual cell numbers can be greatly underestimated (Fig. 9). For this reason, using *in vitro* incubation methods to monitor the survival of bacteria should be considered for future research when it is a viable recovery option.

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