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Brief Report

Survival of *Staphylococcus aureus* on therapeutic ultrasound heads

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Therapeutic ultrasound (US) is commonly used in the rehabilitation of soft tissue injuries including wounds. US heads and coupling gel come into direct contact with patient skin, increasing the risk for health care-associated infections owing to cross contamination. In this study, nearly 80% of *Staphylococcus aureus* placed on US heads in gel survived for 1 hour, with survival of 3 days possible in other types of organic matter.

Therapeutic ultrasound (US) is used by many health care providers including physical and occupational therapists. Use of US heads on patient skin requires coupling gel. Improper cleaning of gel from US heads between patients increases the risk for cross contamination,[1] possibly contributing to health care–associated infections (HAIs).[2]

The purpose of this study was to determine survival of *Staphylococcus aureus* on US heads. *S aureus* can be antibiotic resistant (eg, methicillin-resistant *S aureus*),[3] and has been found on US gel bottles within clinics.[4] Understanding the survival of this pathogen on US heads, particularly when mixed into organic-rich residues, will provide a better understanding of potential links between this treatment and HAIs.

METHODS

Handheld US units with aluminum heads from 3 manufacturers (Appendix Table A1) were used. The units were cleaned prior to use (dilute Sparkleen detergent, Fisher Scientific, Suwanee, GA) and rinsed (Type 1 ultrapure water, Direct-Q, Millipore Sigma, Burlington, MA). The US heads were then dipped into disinfectant (Roccal-D Plus, Pharmacia & Upjohn Co, Div Pfizer, Inc, New York, NY), for 30 seconds, rinsed in ultrapure H_2O, and dried using sterile Kimwipes (Kimberly-Clark Global Sales, LLC, Roswell, GA).

Overnight cultures of *S aureus* (ATCC #700699) were diluted in sterile saline solution or tryptic soy broth (TSB), and counted using viable plate counts on tryptic soy agar for each experiment (range of colony forming units [cfu]/mL was 7.5 e^8 to 3 e^9). Using sterile pipet tips, 25 μL of the diluted cultures (containing from 1.9-7.5 e^4 cfu *S aureus* [mean = 3.74 e^4 cfu]), approximating bacterial counts on healthy skin,[5] were placed in an area of 1 cm^2 on each of 9 disinfected US heads for each experiment. The cell mixtures were dried using low-pressure filtered air. Matrices into which *S aureus* was mixed included 0.85% saline solution, TSB at 3 concentrations (full, 2/3, and 1/3 strength), US coupling gel, and massage lotion. When using US coupling gel (Aquasonic 100, sterile single-use packets, Parker Laboratories, Fairfield, NJ) the 25 μL of *S aureus* was added to 1 mL of the sterile gel. For the massage lotion (Free Up, Meyer PT, Hudson, OH), using a freshly opened bottle from which aliquots added to TSB yielded no growth, the 25 μL of *S aureus* was added to 1 mL of the lotion. Using the same 9 US heads for the 6 different matrices, all trials were performed in triplicate, incubated at room temperature, and sampled at 1, 24, and 72 hours after drying.

Surviving *S aureus* was removed from US heads after incubation using sterile transport swabs (with liquid Stuart's medium, Fisherbrand, Fisher Scientific, Suwanee, GA). These swabs were placed in tubes of sterile saline solution, mixed, and diluted. Surviving *S aureus* was quantified on tryptic soy agar plates after incubation at 37°C for 24 hours.

Calculations of percent survival of *S aureus* were made for the different matrices tested using the following relationship:

$$\%\ Survival = (cfu\ from\ US\ head\ @\ incubation\ time\ and\ matrix) / (cfu\ in\ initial\ aliquot\ added\ to\ US\ head)$$

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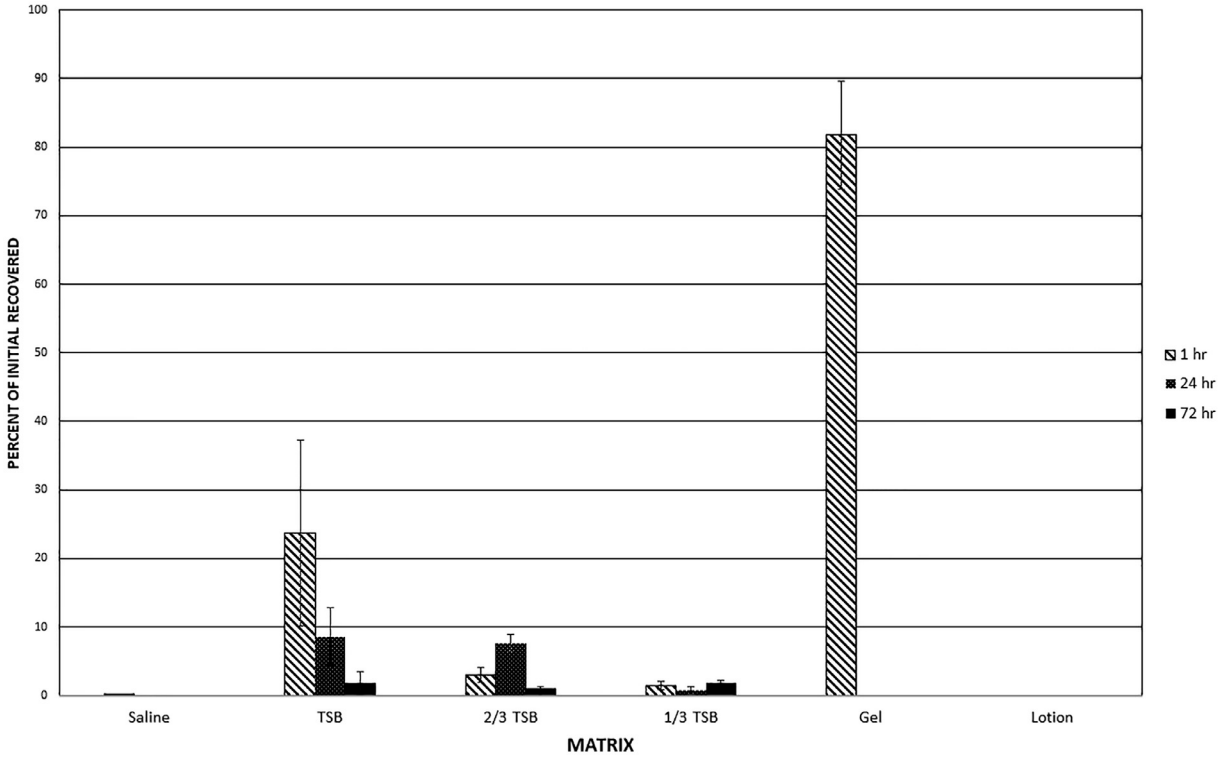

Fig 1. Survival of *Staphylococcus aureus* on ultrasound heads as percent of cells initially added, eventually recovered at different incubation times when mixed into saline solution, various concentrations of tryptic soy broth, coupling gel, or massage lotion. Mean values presented, +/- 1 SE, n = 3.

Estimates of potential *S aureus* loss owing to swabbing and drying were made at 5 minutes post drying (mean survival = 4.4%). Because this estimation was not made for each matrix (only saline solution), and because the same procedure was used for all experiments, any *S aureus* loss should be constant. Therefore, our survival numbers represent an underestimation of actual *S aureus* survival.

Statistical comparison of the data were made using a factorial analysis of variance (time by matrix) with post hoc analysis; IBM SPSS statistics, version 24 (IBM Corp, Armonk, NY).

RESULTS

Survival of *S aureus* on the US heads varied by incubation time and matrix. At 1 hour, *S aureus* survival in gel and full-strength TSB was significantly greater than all other matrices ($P < .01$), and survival in gel was also significantly higher than in full-strength TSB ($P < .01$) (Fig 1). Suspending the bacteria in saline solution resulted in little survival after 1 hour (0.04% survival +/- 0.03%, SE), and no survival thereafter (Fig 1). No bacteria survived in massage lotion at any incubation time.

When *S aureus* was mixed into different concentrations of TSB, survival was variable. At 24 hours, survival of *S aureus* in full-strength and 2/3 TSB were significantly higher than in all other matrices ($P < .01$), and were not significantly different from each other (Table 1). At 72 hours, about 1% of the *S aureus* in any TSB concentration survived, however the difference between TSB matrices did not reach statistical significance.

DISCUSSION

The results of this study suggest that *S aureus* can survive on US heads for 24 hours, and possibly up to 3 days, especially if dried in organic matter. Survival of this pathogen in high numbers for 1 hour in gel on a US head suggests that cross contamination between patients could contribute to HAIs if gel is not removed after treatment. This potential link between HAIs and US treatment is even

Table 1
Survival of *Staphylococcus aureus* on ultrasound head surfaces when embedded in different organic matrices or in saline solution

	Average percent survival of *Staphylococcus aureus* on ultrasound heads after incubation of:		
Matrix	1 hr (+/- 1 SE)	24 hr (+/- 1 SE)	72 hr (+/- 1 SE)[§]
Saline solution	0.04 (0.03)	0 (0)	0 (0)
TSB	23.69 (13.50)[†]	8.59 (4.21)[‡]	1.88 (1.54)
2/3 TSB	3.00 (1.04)	7.64 (1.25)[‡]	1.09 (0.19)
1/3 TSB	1.52 (0.62)	0.73 (0.60)	1.77 (0.48)
Coupling gel	81.78 (7.89)[*]	0 (0)	0 (0)
Lotion	0 (0)	0 (0)	0 (0)

TSB, tryptic soy broth.
[*]Coupling gel and TSB at 1 hour versus all other matrices ($P < .01$).
[†]Coupling gel versus TSB at 1 hour ($P < .01$).
[‡]TSB and 2/3 TSB versus all other matrices at 24 hours ($P < .01$).
[§]No significant difference between any groups observed at 72 hours ($P > .05$).

more problematic when considering that an earlier study found bacterial contamination (including methicillin-resistant *S aureus*) on 52.7% of gel bottle tips sampled in clinics.[4]

Survival of *S aureus* was greatest when mixed into organic matrices. Embedding bacteria in Aquasonic 100 gel resulted in the highest survival of any matrix tested. Preservatives in this gel have previously been found to be ineffective against *S aureus.*[1] Ohara et al[6] also found 70%-97% survival of *S aureus* after 1 hour when added directly to different coupling gels. They suggested that water content may have resulted in the variation in survival they observed. We did not measure water content of our dried matrices but noted that dried gel formed a thick membrane-like residue on the US heads, possibly retaining water.

Survival of *S aureus* in dried TSB matrices for up to 72 hours suggests that something in this medium protects *S aureus*. Ingredients found in TSB include a large amount of digested protein, glucose, NaCl, and a buffer (https://www.bd.com/resource.aspx?IDX=30505). Studies of the survival of bacteria on metal surfaces in the food industry have indicated similar survival for *S aureus*,[7] and for other gram-positive bacteria survival is possibly linked to protein or carbohydrate-rich residues.[8] Lipids may not play a role in the survival of bacteria on metal surfaces, as TSB lacks lipids, and because *S aureus* did not survive in lipid-rich massage lotion here. Cleaning/disinfecting US heads after each use is recommended to prevent cross contamination by *S aureus*.

CONCLUSIONS

These findings have direct clinical implications related to bacterial cross contamination of patients undergoing US therapy. Although based on limited numbers of samples, the significant survival of *S aureus* on US heads demonstrated here, and its potential link to HAIs,[2] suggests a need for improved cleaning/disinfecting of US heads in clinics. Further research is warranted to investigate best options to reduce the threat of HAIs due to *S aureus* from therapeutic US.

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APPENDIX

Table A1

Appendix Table A1
Ultrasound heads used in this study. Note that the heads varied in diameter. To account for differences in the sizes of the ultrasound heads, bacterial mixtures were spread into an area of 1 cm^2, which fit completely on the surface of all ultrasound heads used.

Manufacturer	Model	Diameter (cm)
Richmar*	410-176	5
Richmar	410-160	5
Richmar	410-115	5
Chattanooga Group†	78047	5
Chattanooga Group	27336	2
Chattanooga Group	27334	2
Chattanooga Group	27335	2
METRON‡	L-4E6S	5
METRON	L-005	5

*Richmar, Chattanooga, TN.
†DJO, LLC, Vista, CA.
‡Sportstek Physical Therapy Supplies Pty Ltd, Oakleigh VIC 3166, Australia.

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