Title

Identification and Analysis of Bacterial Contamination of Ultrasound Transducers and Multiuse Ultrasound Transmission Gel Bottle Tips Before and After the Aseptic Cleansing Technique

Journal
Journal of Ultrasound in Medicine, 39(10)

ISSN
0278-4297

Authors
Mullins, Kevin
Burnham, Kevin
Henricson, Erik K
et al.

Publication Date
2020-10-01

DOI
10.1002/jum.15300

Title:

Identification and Analysis of Bacterial Contamination of Ultrasound Transducers and Multi-Use Ultrasound Transmission Gel Bottle Tips Before and After the Aseptic Cleansing Technique

Authors:

Mullins Kevin M, MD (University of California, Davis - kevinmullinsmd@gmail.com)

Burnham Kevin J, MD (University of California, Davis - kjburnham@ucdavis.edu)

Cohen Stuart J, MD (University of California, Davis – stcohen@ucdavis.edu)

Fair James, MD (University of Utah – james.fair@hsc.utah.edu)

Ray Jeremiah W, MD (University of California, Davis – jwray@ucdavis.edu)

Corresponding Author:

Jeremiah W. Ray, MD, FACEP, CAQSM

University of California, Davis Health

One Shields Avenue

264 Hickey Gymnasium, Sports Medicine

Davis, CA 95616

Cell Number: 209-256-0084

Work Number: 530-752-7515

Fax Number: 530-754-4371

Email: jwray@ucdavis.edu

Word Counts

Abstract: 250
Manuscript: 2949

Acknowledgments:

None

Structured Abstract:

Objective: Provide a descriptive analysis for species identification of culture and gram-stain results from ultrasound transducers and multi-use ultrasound transmission gel bottle tips in active clinical use, as well as compare bacterial cultures from ultrasound transducers before and after aseptic cleansing.

Methods: A prospective, blinded descriptive analysis study. 18 distinct clinical care sites within 1 primary clinical institution. 194 samples from ultrasound transducers and multi-use gel bottle tips. Transducers were cleansed utilizing disinfectant-impregnated disposable towels. Before and after the cleanse, transducers were pressed against tryptic soy agar contact plates. Plates were de-identified, submitted for blind incubation, gram stain, and species identification with microsequencing. Plates cultured for 5 days. Any formed bacterial colonies underwent DNA microsequencing for organism identification. Results were classified as clinically relevant (CR) bacteria or non-clinically relevant (NCR) bacteria.

Results: 60 pre-cleanse samples (74.1%) grew cultures with CR bacteria, and 21 samples (25.9%) did not. *Staphylococcus simulans*, represented 31.7% of all positive culture samples. 13

post-cleanse samples (16.1%) grew cultures with CR bacteria, equating to a 58% reduction of CR bacterial growth (LR 58.92, $p < 0.001$).

Conclusion: Ultrasound transducers have significant CR bacterial burden and may serve as potential vectors for infection. The aseptic cleansing protocol effectively eliminates most of the bacterial load from ultrasound transducers, but leaves persistent bacteria that present risk for nosocomial infection with ultrasound-guided interventions. These findings support AIUM 2018 guidelines intended to ensure an appropriate level of transducer preparation based on examination type, while emphasizing rational infection control measures to minimize risk for potential patient harm.

Full Text

Introduction:

Ultrasonography use in clinical medicine has become increasingly common, and is now considered the standard of care for many diagnostic and therapeutic interventions.[1] However, while the popularity of ultrasound-guided procedures continues to rise, the methods utilized for cleaning remain variable among medical practitioners. [2] Despite available international guidelines for ultrasound cleaning[3,4], it has been reported that 87% of academic medical centers do not have a mandated protocol or standard contact time for transducer disinfection.[5] At present, the aseptic technique is widely utilized for many ultrasound-guided procedures, in which the ultrasound transducer is cleansed with antimicrobial wipes rather than using a sterile ultrasound transducer cover.[6] It is known that ultrasound transducers commonly demonstrate a bacterial burden after contacting patient skin.[7,8,9] Visual inspection alone cannot exclude contamination, as one study found only 51% of blood-contaminated ultrasound units were visibly stained.[10] A second study demonstrated that, of clinical ultrasound equipment that practitioners deemed ready for patient use, 26% had bacterial contamination.[11] Several significant ultrasound-associated bacterial infections resulting in patient harm have been reported in the literature.[12, 13, 14, 15, 16, 17, 18] Review of these case series reveal that endocavity ultrasound interventions are the most common cause of significant ultrasound-associated bacterial infections. The other notable etiology of iatrogenic infection in ultrasound-guided procedures is the use of contaminated ultrasound transmission gel from multi-use bottles. A recent case-control study evaluated 40 patients who developed post-procedure soft tissue or bloodstream infections during a 3-year period and found a positive association with contaminated ultrasound gel. After replacement of the contaminated gel, there were no new cases detected during 18 months of follow-up.[19] In another review, including all cases of septic arthritis in Iceland over a 12-year period, the iatrogenic etiology of septic arthritis tripled, with the leading cause being arthrocentesis and joint injections.[20]

Sterile ultrasound transducer covers and sterile ultrasound gel are widely available, but with drawbacks such as increased cost, increased length of procedure, as well as possible diminished image quality.[21] While some advocate for complete sterile technique with every interventional ultrasound procedure,[22] others have proposed that non-sterile gel has no relevant bacterial burden.[23] Adding to the uncertainty of bacterial seeding from ultrasound-guided interventions is the inability for surgical preparation solutions to adequately remove bacterial burden.[24] Previous articles have evaluated bacterial growth on ultrasound devices; however, it remains unclear if full sterile technique should be recommended for all ultrasound guided procedures, particularly in orthopedic and musculoskeletal settings (Table 1).

To further understanding of the appropriate technique for ultrasound-guided procedures, this study aims to: 1) provide descriptive analysis of culture and gram-stain results from ultrasound transducers and multi-use ultrasound transmission gel bottle tips in active clinical use; 2) compare bacterial cultures from ultrasound transducers before and after aseptic cleaning.

Methods:

The study was reviewed, approved and funded by the University of Utah Medical Group Quality Assurance Committee. Informed consent was not necessary for this study, as there were no patients involved. Ultrasound transducers and multi-use gel bottle tips from active clinical use were evaluated in 18 distinct clinical care sites. The transducers and multi-use gel bottle tips were pressed against tryptic soy agar contact plates (Carolina Biological Supply Company, Burlington, NC). These plates were then de-identified and submitted to Nelson Laboratories (Salt Lake City, UT) for blinded incubation, gram stain, and species identification with microsequencing. All transducers were then cleansed utilizing manufacturer recommended disinfectant-impregnated disposable towels containing dimethyl benzyl ammonium chloride (Professional Disposables International, Inc., Orangeburg, NY). The cleansed transducers were then pressed to a second agar media plate. All agar media plates were cultured for 5 days. Nelson Laboratories technicians, who were blinded to the agar plate source, analyzed all agar media plates. Any formed bacterial colonies then underwent DNA microsequencing for organism identification.

Prior studies demonstrated approximately 60% of ultrasound transducers have bacterial isolates after coming in to contact with patients[25] and about 4% of transducers have bacterial isolates after antimicrobial cleansing.[26] Utilizing free software from DSS Research (Fort Worth, TX) for power calculation, assuming an alpha error level of 5%, one-tailed, which corresponds to a 95% confidence interval, a sample size of 50 ultrasound transducers yields a statistical power of 100%. Data results were then verified utilizing Stata Data Analysis and Statistical Software (StataCorp) at the University of California, Davis. Fisher Exact Test was used to analyze the positive culture rates before and after disinfectant wipe cleaning. A simple prevalence of positive cultures was relayed with respect to multi-use ultrasound transmission gel bottle tips, with breakdown by organism.

Results:

A total of 194 samples were obtained across 18 distinct clinical care locations. 162 of these samples were obtained directly from ultrasound transducers, while 26 were from multi-use ultrasound transmission gel tips, and 2 were from the data collector's pen and badge. The remaining 4 collected samples did not have a label to accurately identify the source from which they were obtained; thus, these samples were excluded from the study.

Table 2 outlines the sites where samples were obtained. The largest number of samples was collected in radiology (31). Within each clinical setting, samples were obtained from varying transducer types and gel tip bottles. Table 3 illustrates the distribution of transducer type from which the samples were gathered. Initial samples from the ultrasound transducers were categorized into clinically-relevant (CR) microorganisms, not clinically-relevant (NCR) microorganisms, or no microorganisms. A positive sample was classified as one containing cultures with either CR growth, NCR growth, or both CR and NCR growth. In total, there were 14 different microorganisms identified in this study, 7 of which were classified as CR, and the other 7 as NCR. The delineation between CR and NCR microorganisms was based on careful literature review pertaining to the potential for human harm of each respective organism.

Of the total pre-cleanse samples obtained from ultrasound transducers in this study, there were 60 samples (74.1%) that grew cultures with CR bacteria, and 21 samples (25.9%) that did not. In comparison, after cleaning the transducers, only 13 samples (16.1%) of the post-cleanse cultures contained CR bacteria, equating to a 58.0% reduction of CR bacterial growth on samples (LR 58.92, $p = <0.001$). There was one ultrasound transducer from which the post-cleansing sample was not obtained; the pre-cleansing results were imputed forward. There was a statistically significant relationship between cleaning and reduction in CR bacteria.

The most frequently cultured microorganism was *Staphylococcus simulans,* representing 31.7% of all positive culture samples as demonstrated in Table 4. In total, the CR microorganisms collectively occurred at a much higher frequency than the NCR microorganisms, by an approximate ratio of 10-to-1. Growth of four of the seven CR microorganisms (*Staphylococcus simulan*s, *Micrococcus luteus*, *Paenibacillus provencensis*, and *Brevibacterium pityocampae*) was significantly reduced after cleaning (Table 5). The three CR microorganisms that did not demonstrate statistically significant reduction were noted to have small sample sizes. Two of the seven NCR microorganisms were found to have statistically significant reduction growth, while the remaining five had small sample sizes, for which p-values remained above threshold.

Discussion:

We performed a descriptive analysis of culture and gram-stain results from ultrasound transducers and multi-use ultrasound transmission gel bottle tips in active clinical use throughout a single healthcare system. All ultrasound transducer surfaces tested in our study were considered ready for patient use. Pre-cleanse samples grew CR microorganisms at a high rate (74.1%), which supports conclusions drawn from prior literature studies that cleanliness standards based on visual inspection alone are insufficient, and there remains a need for further education as well as implementation of cleaning guidelines. Aseptic cleaning with disinfectant-impregnated disposable towels containing dimethyl benzyl ammonium chloride reduced the prevalence of CR microorganisms, from 74.1% to 16.1%; a statistically significant relationship between cleaning and CR microorganisms, (LR 58.92, $p = <0.001$) was observed. These findings indicate that the aseptic technique reduces ultrasound transducer bacterial burden.

Of the remaining bacterial contaminants post-cleanse, *Staphylococcus simulans* was the most prevalent microorganism, which is a common animal pathogen that may occasionally colonize the human skin. Human infections with *S. simulans* have rarely been reported, but do occur in patients who have repeated contact with animals such as butchers and veterinarians. The majority of cases associated with *S. simulans* include cardiac or osteoarticular infections.[27] [28] [29]

Ultrasonography use in clinical practice has become progressively more common in the United States, a trend that will likely continue as portable ultrasound machines become more accessible [30], and Sports Medicine Fellowship Programs continue to implement ultrasound curriculums across the nation.[31] As stated by the American Institute of Ultrasound in Medicine (AIUM), “Infection control is an integral part of the safe and effective use of ultrasound in medicine.”[32] However, despite increased ultrasound utilization,[33] institutions have adopted widely varied approaches to ultrasound cleaning. While some hospitals have yet to implement a cleaning protocol of any sort[34], others have mandated full sterilization autoclaves prior to all ultrasound procedures. AIUM recently introduced new guidelines intended to ensure appropriate level or transducer preparation based on examination type, recommendations that our data supports. Review of the current literature and the data from our current study emphasize the importance of adherence to AIUM guidelines. Based upon our findings, which sampled the largest number of health care settings of any study to date, we recommend low level disinfection (LLD) in conjunction with the use of single-use, sterile ultrasound transducer covers and sterile ultrasound gel for all interventional ultrasound guided applications. Given microbial persistence after LLD, we do not recommend aseptic techniques alone prior to percutaneous procedures. All medical-grade protective barriers are regulated by an acceptable quality level (AQL); thus, we do not believe high-level disinfection (autoclave) is required prior to percutaneous office-based procedures. We do recommend high level disinfection prior to endocavity procedures, based on the increased risk of bacterial transmission in this setting.

Strengths of the study include the prospective, blinded study design and high volume of samples collected across a wide array of clinical environments. To our knowledge, no other study in the literature has assessed ultrasound machines among multiple departments within a health care system. Despite meticulous care for the large number of samples, there were unfortunately 4 post-cleanse samples that were lost during transit. However, the pre-cleansing results were imputed forward, thus decreasing the chance of a type 1 error.

There were some limitations to this study. For instance, although a large number of cultures were collected from ultrasound transducers, no samples from additional surfaces of the ultrasound machine were obtained. Recent literature has suggested that potential vectors for infection are complex and multidirectional. Ultrasound transducer handles, cords and keyboards can all present as significant sources for infection and should be cleaned routinely.[35] Unfortunately, these surfaces are sometimes difficult to clean due to physical design and some electrical equipment such as keyboards may be damaged by fluid disinfectants. Additional studies may be warranted to assess these factors. Another limitation is that gel tips were cultured at room temperature: recent literature has demonstrated that warmed ultrasound gel can promote colonization and growth by bacteria. Consequently, the prevalence of bacterial growth in our study may be falsely underrepresented when compared to a clinical practice that routinely heats ultrasound gel for patient comfort.[37]

Conclusion:

We demonstrated ultrasound transducers in clinical use have a significant CR bacterial burden and may serve as a potential vector for infection. The aseptic cleansing protocol effectively eliminates most of the bacterial load from ultrasound transducers, but leaves persistent bacteria that present risk for nosocomial infection with ultrasound-guided interventions. Our data support the use of single-use, sterile ultrasound transducer covers and sterile ultrasound gel for all percutaneous ultrasound guided procedures. Given that medical barriers are regulated for quality, high level disinfection between patients adds no additional benefit outside operative and endocavity applications. Overall, our findings support AIUM 2018 guidelines intended to ensure an appropriate level of transducer preparation based on examination type. We strongly agree with emphasizing rational infection control measures to minimize risk for potential patient harm.

Acknowledgments:

None

References:

[1] Finnoff JT, Hall MM, Adams E, et al. American Medical Society for Sports Medicine Position Statement: Interventional Musculoskeletal Ultrasound in Sports Medicine. Clin J Sport Med. 2015; 25:6-22.

[2] Westerway SC, Basseal JM. The ultrasound unit and infection control – Are we on the right track? Ultrasound. 2017. Vol. 25(1) 53-57.

[3] Miyague AH, Mauad FM, Martins WP, Benedetti AG, Ferreira AT, Mauad-Filho F. Ultrasound scan as potential source of nosocomial and cross-infection: a literature review. 2015; 48(5):319

[4] Nyhsen CM, Humphreys H, Koerner RJ, Grenier N, Brady A, Sidhu P, Nicolau C, Mostbeck G, D'Onofrio M, Gangi A, Claudon M. Infection prevention and control in ultrasound – best practice recommendations from the European Society of Radiology Ultrasound Working Group. Insights Imaging. 2017; 8:523-535.

[5] Hoyer R, Adhikari S, Amini R. Ultrasound transducer disinfection in emergency medicine practice. Antimicrobial Resistance and Infection Control. 2016; 5:12 DOI 10:1186/s13756-016-0110-y

[6] Baima J, Isaac Z. Clean versus sterile technique for common joint injections: a review from the physiatry perspective. Curr Rev Musculoskelet Med. 2008; 1:88-91.

[7] Ejtehadi F, Ejtehadi F, Teb JC, et al. A safe and practical decontamination method to reduce the risk of bacterial colonization of ultrasound transducers. J Clin Ultrasound. 2014; 42:395-398.

[8] Karadeniz YM, Kilic D, Altan SK, et al. Evaluation of the role of ultrasound machines as a source of nosocomial and cross-infection. Invest Radiol. 2001; 36:554-558.

[9] Sanz GE, Theoret J, Liao MM, et al. Bacterial contamination and cleanliness of emergency department ultrasound probes. Canadian Journal of Emergency Medicine. 2011; 13:384-389.

[10] Keys M. Sim B, Thom O, Tunbridge M, Barnett A, Fraser J. Efforts to attenuate the spread of infection (EASI): a prospective observational multicenter survey of ultrasound equipment in Australian

emergency departments and intensive care units. Crit Care Resusc 2015; 17:43-6

[11] Whiteley GS, Glasbey TO, Westerway SC, Fahey PP, Basseal J. A new samples algorithm demonstrates that ultrasound equipment cleanliness can be improved. American Journal of Infection Control. 2018; 0196-6553

[12] Olshtain-Pops, K, Block C, Tempter V, et al. An outbreak of *Achromobacter xylosixidans* associated with ultrasound gel used during transrectal ultrasound guided prostate biopsy. J Urol. 2011; 185:144-147.

[13] Keizur J, Lavin B, Leidich R. Iatrogenic urinary tract infection with *Pseudomonas cepacia* after transrectal ultrasound guided needle biopsy of the prostate. J Urol. 1993; 149:523-526.

[14] Hutchinson J, Runge W, Mulvey M, et al. *Burkholderia cepacia* infections associated with intrinsically contaminated ultrasound gel: the role of microbial degradation of parabens. Infect Control Hosp Epidemiol. 2004; 25:291-296.

[15] Weist K, Wendt C, Petersen L, Versmold H, et al. An outbreak of pyodermas among neonates caused by ultrasound gel contaminated with methicillin-susceptible *Staphylococcus aureus*. Infect Control Hosp Epidemiol. 2000; 21:761-764.

[16] Gaillot O, Maruejouls C, Abachin E, et al. Nosocomial outbreak of *Klebsiella pneumonia* producing SHV-5 extended-spectrum beta-lactamase, originating from a contaminated ultrasonography coupling gel. J Clin Microbiol. 1998; 36:1357-1360.

[17] Jacobson M, Wray R, Kovach D, Henry D, et al. Sustained endemicity of *Burkholderia cepacia* complex in a pediatric institution, associated with contaminated ultrasound gel. Infect Control Hosp Epidemiol. 2006; 27:362-366.

[18] Chittick P, Russo V, Sims M, et al. Outbreak of *Pseudomonas aeruginosa* respiratory tract infection sin cardiovascular surgery associated with contaminated ultrasound gel used for tranesophageal

echocardiography – Michigan, December 2011-January2012. MMWR Morb Mortal Wkly Rep. 2012; 61:262-264.

[19] Cheng A, Sheng WH, Huang YC, Sun HY, Tsai YT, Chen ML, Lui YC, Chuang YC, Huang SC, Chang CI, Chang, LY, Huang WC, Hsueh PR, Hung CC, Chen YC, Chang SC. Prolonged postprocedural outbreak of *Mycobacterium massiliense* infections associated with ultrasound transmission gel. Clinical Microbiology and Infection. 2016; 22: 382.el-382.e

[20] Geirsson AJ, Statkevicius S, Vikingsson A. Septic arthritis in Iceland 1990-2002: increasing incidence due to iatrogenic infections. Ann Rheum Dis. 2008; 67:638-643.

[21] Baima J, Isaac Z. Clean versus sterile technique for common joint injections: a review from the physiatry perspective. Curr Rev Musculoskelet Med. 2008; 1:88-91.

[22] Olexzkowicz SC, Chittick P, Russo Victoria, et al. Infections associated with use of ultrasound transmission gel: proposed guidelines to minimize risk. Infect Control Hosp Epidemiol. 2012; 33:1235-1237.

[23] Sherman T, Fergsuon J, Davis W, et al. Does the use of ultrasound affect contamination of musculoskeletal injections sites?. Clin Orthop Relat Res. 2015; 473:351-357.

[24] Saltzman MD, Nuber GW, Gryzleo SM, et al. Efficacy of surgical preparation solutions in shoulder surgery. J Bone Joint Surg Am. 2009; 91:1949-1953.

[25] Sanz GE, Theoret J, Liao MM, et al. Bacterial contamination and cleanliness of emergency department ultrasound probes. Canadian Journal of Emergency Medicine. 2011; 13:384-389.

[26] Karadeniz YM, Kilic D, Altan SK, et al. Evaluation of the role of ultrasound machines as a source of nosocomial and cross-infection. Invest Radiol. 2001; 36:554-558.

[27] Razonable RR, Lewallen DG, Patel R, Osmon DR. Vertebral osteomyelitis and prosthetic joint infection due to *Staphylococcus simulans*. *Mayo Clin Proc*. 2001;76(10):1067-1070.

[28] Vallianou N, Evangelopoulos A, Makri P, et al. Vertebral osteomyelitis and native valve endocarditis due to Staphylococcus simulans: a case report. *J Med Case Rep*. 2008;2:183.

[29] Désidéri-Vaillant C, Nédelec Y, Guichon JM, et al. *Staphylococcus simulans*osteitis in a diabetic patient. *Diabetes Metab*. 2011;37(6):560-562.

[30] Comstock, Jonah. "Butterfly IQ Gets FDA Clearance for Chip-Based, Smartphone-Connected Ultrasound." *MobiHealthNews*, 31 Oct. 2017, www.mobihealthnews.com/content/butterfly-iq-gets-fda-clearance-chip-based-smartphone-connected-ultrasound.

[31] Finnoff JT, Berkoff D, Brennan F, DiFiori J, Hall MM, Harmon K, Lavallee M, Martin S, Smith K, Stovak M. American Medical Society for Sports Medicine recommended sports ultrasound curriculum for sports medicine fellowships. Clin J Sport Med. 2015 Jan;25(1):23-9.

[32] American Institute of Ultrasound in Medicine. Guidelines for Cleaning and Preparing External- and Internal-Use Ultrasound Probes Between Patients, Safe Handling, and Use of Ultrasound Coupling Gel, 2018. http://www.aium.org/officialStatements/57

[33] Mehta P, Rand EB, Visco CJ, Wyss J. Resident Accuracy of Musculoskeletal Palpation With Ultrasound Verification. J Ultrasound Med. 2018 Jul;37(7):1719-1724

[34] Poonja Z, Uppal J, Netherton S, Bryce R, Lyon A, Cload B. Evaluation of emergency department ultrasound machines for the presence of occult blood. Canadian Association of Emergency Physicians. 2018 Oct;0(0):1-4

[35] Westerway SC. Basseal JM, Brockway A, Hyett JA, Carter DA. Potential Infection Control Risks Associated With Ultrasound Equipment – A Bacterial Perspective. Ultrasound Med Biol. 2017 Feb;43(2):421-426

Keywords:

ultrasound; transducers; gel bottle tips; bacterial contamination; aseptic cleansing; bacteria

Table 1: Literature Comparison for Ultrasound Cleansing

Study Name	Number of Departments	Number of Machines	Number of Transducers	Number of Bottles	Number of Cultures	Pre-Clean Growth Rate	Post Clean Growth Rate
Ray (2019)	18	41	82	26	194	11.92%	3%
Whiteley (2018)	5	NR	NR	NR	750	26%	6%
Westerway (2017)	2	NR	60	7	171	38.3%	3.3%
Laurence et al (2014)	9	43	82	NR	320	5.60%	NR
Chu (2014)	1	31	31	0	31	22.60%	NR
Ejtehadi	1	1	3	NR	50	98%	21%

Table 2: Number of samples by location (N=188).

Sample Location	n (%)
Radiology Department (4)	31 (16.4)
Main Operating Room (1)	18 (9.6)
Emergency Room Main (6)	15 (8.0)
Huntsman Operating Room (3)	14 (7.4)
Trauma Bay (7)	14 (7.4)
Orthopedic Center (15)	12 (7.4)
Burn ICU (14)	9 (4.8)
SJ Emergency Room (17)	9 (4.8)
PACU Orthopedic Center (16)	8 (4.3)
Medical ICU (13)	8 (4.3)
Echocardiogram Lab (5)	8 (4.3)
Neonatal ICU (12)	7 (3.7)
Cardiovascular ICU (11)	7 (3.7)
SJ Sports Clinic (18)	7 (3.7)
Pre-Operative Clinic (2)	6 (3.2)
Surgical ICU (10)	6 (3.2)
Labor & Delivery (8)	6 (3.2)
OBEM (9)	3 (1.6)
Additional Samples	
ID Badge	1
Marking Pen	1
Unlabeled	4

Table 3: Number of samples by surface type (N=188).

Sample Type	n (%)
Phased Transducer (2)	72 (38.3)
Linear Transducer (1)	48 (25.5)
Curved Transducer (3)	32 (17.0)
Hockey Transducer (5)	8 (4.3)
Endo Transducer (4)	2 (1.1)
Gel Bottle Tip (6)	26 (13.8)

Table 4: Frequency on ultrasound transducers and bottle tips (number indicates a positive culture, N=177; CR: clinically-relevant; NCR: non clinically-relevant).

CR Microorganism	n (%)	NCR Microorganism	n (%)
Staphylococcus simulans	55 (31.7)	*Bacillus pumilus/sefensis*	6 (3.4)
Micrococcus luteus	45 (25.4)	*Exiguobactlerium artemiae*	3 (1.7)
Paenibacillus provencensis	24 (13.6)	*Brevundimonas species*	2 (1.1)
Brevibacterium pityocampae	20 (11.3)	*Bacillus altitudinis*	2 (1.1)
Bacillus simplex	8 (4.5)	*Microbacterium aaccharophilum*	1 (0.6)
Bacillus thuringiensis	6 (3.4)	*Alternaria alternata*	1 (0.6)
Staphylococcus warnei	3 (1.7)	*Pseudomonas mucidolens/sacch*	1 (0.6)

Table 5: Frequency of all microorganisms pre and post clean on ultrasound transducers (number indicates a sample with at least one microorganism culture growth; CR: clinically-relevant; NCR: non clinically-relevant).

CR Microorganism	Pre-Clean	Post-Clean	P Value
Staphylococcus simulans	43	5	<0.001
Micrococcus luteus	39	4	<0.001
Paenibacillus provencensis	16	6	0.020
Brevibacterium pityocampae	19	0	<0.001
Bacillus thuringiensis	5	1	0.083
Bacillus simplex	5	1	0.083
Staphylococcus warner	2	0	0.094
NCR Microorganism	**Pre-Clean**	**Post-Clean**	**P Value**
Bacillus pumilus/sefensis	6	0	0.003
Exiguobactlerium artemiae	3	0	0.040
Brevundimonas species	2	0	0.094
Bacillus altitudinis	2	0	0.094
Microbacterium saccharophilum	1	0	0.238
Alternaria alternata	1	0	0.238
Pseudomonas mucidolens/sacch	1	0	0.238