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● *Original Contribution*

POTENTIAL INFECTION CONTROL RISKS ASSOCIATED WITH ULTRASOUND EQUIPMENT – A BACTERIAL PERSPECTIVE

Susan Campbell Westerway,*† Jocelyne M. Basseal,† Adam Brockway,‡ Jon A. Hyett,§¶ and Dee A. Carter‡

*Faculty of Dentistry & Health Sciences, Charles Sturt University NSW, Australia; †Australasian Society for Ultrasound in Medicine (ASUM), Sydney, NSW, Australia; ‡University of Sydney, Sydney, Australia; §Department of High Risk Obstetrics, Royal Prince Alfred Hospital, Sydney, Australia; and ¶Discipline of Obstetrics, Gynaecology and Neonatology, Central Clinical School, Faculty of Medicine, University of Sydney, Sydney, Australia

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Abstract—Ultrasound equipment used in trans-abdominal (TA) and trans-vaginal (TV) examination may carry bacterial contamination and pose risks to infection control during ultrasound examination. We aimed to describe the prevalence of bacterial contamination on ultrasound probes, gel, machine keyboard and cords and examined the effectiveness of low- and high-level disinfection techniques. This study was performed at a public hospital and a private practice. A total of 171 swabs were analyzed and bacterial species were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis and polymerase chain reaction (PCR). Sixty percent of TA probes and 14% of TV probes had evidence of bacterial contamination after an ultrasound examination. Low-level disinfection was partially effective, but 4% of probes were still contaminated by spore-forming species. Some heated gel samples were highly contaminated with the environmental bacterium *Brevundimonas aurantiaca,* suggesting the gel was conducive to bacterial growth. Ultrasound machines, probe cords and gels were identified as potential sources of bacterial contamination and need to be cleaned and changed regularly to minimize risks of infection. (E-mail: j.basseal@asum.com.au) **© 2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Bacterial contamination, Healthcare-associated infection, Patient safety, Disinfection.**

INTRODUCTION

Routine ultrasound examination may not be as safe as is assumed. This dynamic procedure provides a vehicle for cross-infection at several levels ranging from basic hand hygiene to exposure to ultrasound coupling gel and the ultrasound probe as well as transfer of infection from sources such as the probe cord and machine keyboard. In Australia, the National Health and Medical Research Council has reported that there are over 200,000 healthcare-associated infections in acute healthcare facilities each year. A number of organizations provide guidance that aims to reduce the risk of cross-infection. The National Health and Medical Research Council promotes a systems-based risk management framework, Standards Australia promotes best practice in disinfection and sterilization of reusable medical equipment and the Therapeutic Goods Administration regulates materials used for disinfection of medical equipment used in high-, medium- and low-grade infection environments. Ultrasound hygiene is promoted by professional organizations such as the Australasian Society for Ultrasound in Medicine, the American Institute of Ultrasound in Medicine and the World Federation of Ultrasound in Medicine and Biology.

Natural latex condoms are commonly used as probe covers but may not provide adequate protection against infection. A study examining 440 endocavity probes after covers were removed following (trans-vaginal) TV and trans-rectal scans found 68% had bacterial flora present, with pathogenic bacteria and viral nucleic acids, including human papilloma virus (HPV) on 3.4% and 1.5% of the probes, respectively (Kac et al. 2010). Another study (Casalegno et al. 2012) found that despite the use of probe covers, 24% of TV probes were contaminated by human DNA and 3.5% were positive for HPV.

Address correspondence to: Jocelyne M. Basseal, Australasian Society for Ultrasound in Medicine, Level 2, 511 Pacific Highway, Crows Nest, NSW 2065, Australia. E-mail: j.basseal@asum.com.au

Recommended techniques for low-level disinfection did not remove infective agents in all cases and 3% of probes remained contaminated after cleaning. The authors concluded that high-level disinfection should be used for all endocavity probes. Ultrasound gel has also been reported to be contaminated with a number of pathogenic organisms including *Pseudomonas aeruginosa* and *Klebsiella oxytoca,* but there are no regulations relating to post-purchase packaging or use of this product (FDA Safety Communications 2012).

We aimed to document the potential risk for contamination with pathogenic micro-organisms that women are exposed to in contemporary practice when they attend for a trans-abdominal (TA) or TV scan. The study examined the efficacy of low-level and high-level infection control regimens for cleaning TA and TV probes and also assessed the potential for contamination from the probe cord, coupling gel and machine keyboard. Our intention was to provide data that would inform the development of robust clinical guidelines.

METHODOLOGY

Study samples

This was a blinded study conducted in the ultrasound units of a public hospital (ultrasound unit A) and a private clinic (ultrasound unit B) in Sydney, Australia. Ethical approval was sought and deemed unnecessary as this study did not involve patients or patient samples. Using Sterilin transport swabs (Thermo Fisher Scientific, Waltham, MA, USA), duplicate culture swabs were taken from TV and TA probes, cords, ultrasound machine keyboard and ultrasound gel bottles. The following protocols were used:

1. TV probe swabs: were obtained before patient use (following high-level disinfection [HLD] after the previous patient) and following patient use. First, the condom or vinyl probe cover was removed, the gel was wiped with a paper towel and the probe was swabbed. The probe was then rinsed under cold running water, dried with paper towel and cleaned with an alcohol-based wipe (low-level disinfection [LLD]) and re-swabbed. It was then subjected to HLD by immersion in a 2.4% glutaraldehyde solution (Cidex, CIVCO Medical Solutions, Kalona, IA, USA) for the disinfection time recommended by the manufacturer and was swabbed again.
2. TA probe swabs: were also obtained following patient use. A paper towel was used to wipe the ultrasound gel from the probe before the first swab. An alcohol-based wipe was then used to clean the probe (LLD) and the probe was re-swabbed.
3. Ultrasound unit keyboard (keys and rolling trackball) and ultrasound probe cords: these were swabbed separately without undergoing any disinfection.
4. Ultrasound coupling gel: sampled from (i) unopened gel bottles; (ii) unheated refillable gel bottles; and (iii) refillable gel bottles reheated throughout the d in a dry heater.

Growth, enumeration and identification of micro-organisms

A total of 171 swabs were obtained and were plated onto sterile Nutrient Agar and incubated at 37°C overnight to allow bacterial growth. All plates were assessed qualitatively and bacterial growth was recorded on a scale from 0 to 3, where 0 = no growth, 1 = 1–3 colonies, 2 = 4–10 colonies and 3 = >10 colonies and up to confluent growth. Bacterial colonies were isolated and purified by streak plating, and were subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis using a Microflex LT/LRF MALDI-TOF (Bruker, Billerica, MA, USA) with a clinical database. In brief, each colony was spotted onto a MALDI-TOF target plate and overlaid with 1 μL trifluoroacetic acid followed by 1 μL matrix solution (1.5 mg α-cyano-4-hydroxycinnamic acid in 50% acetonite with 2.5% trifluoroacetic acid). Once dry, the plates were inserted into the Microflex to obtain spectral fingerprints that were compared to the database to obtain identifications. As recommended by the manufacturer, identification scores values > 1.7 were considered reliable identifications. All isolated colonies were tested in duplicate.

Colonies that could not be identified by MALDI-TOF were identified by 16 S polymerase chain reaction (PCR) sequencing. To extract DNA, bacterial colonies were emulsified in 500 μL of Milli-Q water in a beat-beating tube and vortexed for 30 s using a BenchMixer Vortexer (Benchmark Scientific, Sayreville, NJ, USA). Tubes were centrifuged for 10 min at 16,000 rpm and the supernatant containing the bacterial DNA was removed. PCR was performed in a reaction volume of 25 μL containing 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 1 μM dNTPs (Thermo Fisher Scientific), 0.5 μM each of 16 S full length universal primers 27 F (AGAGTTTGATCMTGGCTCAG) and 1492 R (CGGTTACCTTGTTACGACTT), 1 ng of genomic DNA prepared above and 1 unit of *Taq* polymerase (Qiagen, Hilden, Germany). PCR cycling included denaturation at 95°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 45 s repeated for 35 cycles, with a final extension at 72°C for 7 min.

PCR products were assessed for quality on a 1% agarose gel containing 1 x GelRed (Qiagen) with

Table 1. Bacterial growth on swab plates from samples obtained from ultrasound units

	Ultrasound unit A (96 samples)					Ultrasound unit B (75 samples)				
	Number of samples at each level of contamination* (% of total)					Number of samples at each level of contamination* (% of total)				
Sample type	0	1	2	3	Notable species present	0	1	2	3	Notable species present
TV probe (n = 63)										
Following removal of probe cover (n = 28)	13 (81)	2 (12)	0	1 (7)	*Brevundimonas spp.:* Environmental organism associated with human infection	11 (92)	1 (8)	0	0	*Micrococcus luteus:* Normal flora of the skin
LLD treatment† (n = 26)	14 (100)	0	0	0	-	11 (92)	1 (8)	0	0	*Terribacillus saccharophilus:* Environmental spore-former
HLD treatment (n = 9)	7 (100)	0	0	0	-	2 (100)	0	0	0	-
TA probe (n = 66)										
Following paper towel wipe (n = 32)	8 (44)	3 (17)	6 (34)	1 (5)	*Staphylococcus haemolyticus:* Hospital pathogen, emerging antibiotic resistance	5 (36)	7 (50)	2 (14)	0	*Staphylococcus warneri:* Emerging cause of nosocomial infections
LLD treatment† (n = 32)	17 (94)	1 (6)	0	0	*Bacillus subtilis:* Environmental spore former	14 (100)	0	0	0	-
HLD treatment‡ (n = 2)	2 (100)	0	0	0	-	-	-	-	-	-
Ultrasound gel (n = 20)										
Unopened (n = 2)	2 (100)	0	0	0	-	0	0	0	0	-
Unheated (n = 11)	6 (100)	0	0	0	-	5 (100)	0	0	0	-
Reheated (n = 7)	1 (25)	0	0	3 (75)§	*Brevundimonas aurantiaca:* Environmental organism, non-pathogenic, motile	3 (100)	0	0	0	-
Equipment (n = 22)										
TA probe cord (n = 7)	0	2 (67)	1 (33)	0	*Acinetobacter lwoffii:* Emerging cause of nosocomial infections	2 (50)	2 (50)	0	0	*Ureibacillus thermosphaericus:* Environmental organism
TV probe cord (n = 5)	0	2 (67)	1 (33)	0	*Pseudomonas spp.:* Environmental organism found in hospitals	1 (50)	1 (50)	0	0	*Micrococcus luteus:* Normal flora of the skin
Ultrasound computer keyboard (n = 10)	0	0	0	3 (100)§	*Enterococcus faecium:* Normal intestinal flora. Emerging antibiotic resistance in nosocomial infections	0	0	0	7 (100)§	*Pseudomonas stutzeri:* Environmental organism associated with human infection

HLD = high-level disinfection; LLD = low-level disinfection; TA = trans-abdominal; TV = trans-vaginal.
* 0 = no colonies; 1 = 1–3 colonies; 2 = 4–10 colonies; 3 = >10 colonies.
† Low-level disinfection.
‡ High-level disinfection; not used at ultrasound unit B for TA probes.
§ Indicates confluent growth.

electrophoresis at 120 V for 15 min. PCR products were then excised from the gel and purified using the QIAquick PCR Purification kit (Qiagen) according to the manufacturer's instructions and were sent for sequencing at the Australian Genome Research Facility (Sydney, Australia). Sequences were analyzed using Geneious software (version 6.1; Biomatters Ltd, Auckland, NZ) and bacterial identifications were obtained by BLAST, available through the National Center for Biotechnology Information.

Antibiotic resistance profiling of Enterococcus faecium

Enterococcus faecium, identified *via* MALDI-TOF, was streaked onto Nutrient Agar plates to obtain pure colonies. Vancomycin profiling was tested by PCR amplification of a pure colony using the Vancomycin Resistance Kit (Ausdiagnostics, Beaconsfield, Australia) according to manufacturer's instructions.

RESULTS

The results of the analysis of bacterial contamination in each ultrasound unit are summarized in Table 1. This includes the number of samples from each sample type (TA and TV probes, gel and equipment) at each level of contamination, along with the percent these comprise of the total samples for that sample type. Species that were considered notable due to high density or clinical importance are described, and a list of all species isolated from the various different sites is given in Supplementary Table S1.

Potential pathogens found on TA and TV probes pre-LLD/HLD

Sixty percent of the 32 TA probes sampled before any disinfection treatment had bacterial contamination. Notable contaminating species included *Staphylococcus haemolyticus* and *Staphylococcus warneri.* These are both emerging hospital pathogens, indicating that TA probes can harbor potential pathogens if they are not disinfected. Only one (3%) TA probe remained contaminated following LLD, where the environmental spore-former *Bacillus subtilis* was isolated.

Four (14%) of the 28 TV probes screened after removal of the probe cover had bacterial growth before disinfection. One (4%) remained positive after LLD, with the environmental spore-former *Terribacillus* spp. isolated. No contamination was detected on TV probes after HLD (pre- and post-patient).

The results from the two ultrasound units were highly congruent, suggesting these findings may be a reasonable representation of findings in ultrasound clinics that employ routine disinfection methods.

Ultrasound unit keyboards and probe cords yield high levels of bacterial contamination

Bacterial samples were cultured from 19 (86%) of 22 samples obtained from ultrasound keyboards and probe cords. Most of these were human commensals and environmental species that would be expected on non-sterilized surfaces (Table S1). A total of 9 (41%) of the 22 swabs showed evidence of contamination with potential pathogens, including *Acinetobacter lwoffii* and *Pseudomonas stutzeri*. Another notable organism cultured from a swab collected from a machine keyboard was *Enterococcus faecium*, which is an intestinal organism. Because vancomycin-resistant enterococci are a significant problem in the hospital setting, the *E. faecium* isolate was subjected to vancomycin resistance profiling and was found to be negative for vancomycin resistance.

Heated gel is conducive to bacterial growth

Three (42%) of 7 heated ultrasound gel samples yielded confluent growth of the environmental non-pathogen *Brevundimonas aurantiaca.* This was present in the heated gel only, which appears to provide an environment conducive to bacterial growth. All swabs taken from unopened and unheated gel were sterile.

DISCUSSION

We have shown that significant proportions of both TA and TV probes have bacterial contamination at the end of a procedure and that this can include potential pathogens. Although LLD measures were generally effective, a low (<5%) rate of bacterial contamination remained. HLD effectively removed all remaining contaminants from the probe. Infection control processes for ultrasound focus on the probe, but we have also shown that probe cords and machine keyboards present significant sources of infection and that this can include potential pathogens. This is consistent with other studies highlighting the importance of cleaning ultrasound equipment, which can be a potential vector in the transmission of infectious agents (Keys et al. 2015; M'Zali et al. 2014). While commercial gel is provided in sterile packaging, heating this product (for the comfort of the patient) appears to provide an environment conducive to bacterial growth.

Healthcare-associated infections are a major cause of morbidity and mortality, and various programs, such as the hand hygiene initiative, have been implemented in an attempt to decrease their prevalence. A recent Australasian survey found that significant numbers of operators do not comply with ultrasound disinfection guidelines (Basseal and Campbell Westerway 2015). This appears to be for a number of reasons, including

incomplete knowledge of the appropriate methods for LLD and HLD and poor understanding of infection control issues. While ultrasound operators undergo rigorous training to capture and interpret images, current curricula in Australia do not include details on infection prevention and control. Improving education on the risk of hospital-acquired infection and infection control would improve practitioner awareness of these issues, and thereby improve compliance with practice guidelines.

Added difficulties in managing infection control in ultrasound relate to the physical design of equipment and to the length and complexity of the examination. At present, disinfection policies focus on the "hot end" of equipment that comes in to direct contact with the patient. Probe handles and cords are not sterilized during this process, and systems that use fluid disinfectants can destroy electronic components on contact. Similarly, most ultrasound keyboards are not designed with infection control in mind and are difficult to clean thoroughly without affecting their function. The ultrasound scan is a dynamic real time investigation and the operator maintains contact with the probe, the patient and the machine to maximize image quality. The potential vectors for infection are therefore complex and multidirectional. Our data show that probe cords and machine keyboards present significant sources for infection and should be cleaned routinely. Equipment manufacturers should also be encouraged to design components that are easier to clean while using, for example, touch screens, waterproof silicone keyboards and track pads rather than traditional keyboards and trackballs.

While LLD was effective in removing most bacterial contamination, it was not completely effective in cleaning either TA or TV probes. Interestingly, the remaining contaminants were spore-forming species, which are recognized as presenting an added challenge in infection control. Some spore-formers are pathogenic, an example being *Clostridium difficile* that has been associated with hospital-acquired infections as its spores can survive treatment with a number of disinfectants (Barra-Carrasco and Paredes-Sabja 2014; Wilcox and Fawley, 2000). *C. difficile* spores have been found on the hands of a quarter of health-care workers after routine care of patients with *C. difficile* infection (Landelle et al. 2014), and the potential pathway for cross infection *via* the ultrasound machine is easily seen. If infection control policies continue to recommend LLD for TA probes, more studies evaluating the effectiveness of these methods of disinfection on different spore-formers are warranted.

In this study we found *Brevundimonas* (formerly *Pseudomonas*) *aurantiaca* in reheated gel bottles, suggesting that heating gel can promote colonization and growth by bacteria. Although the use of heated gel is more comfortable for the patient, this represents an infection hazard. *B. aurantiaca* is not pathogenic, but a closely related species, *B. vesicularis* has been implicated in infective endocarditis (Yang et al. 2006) and neonatal sepsis (Karadag et al. 2012). Ultrasound gel containers are also often refilled from bulk containers. We recommend that ultrasound operators are made aware of the issues with reheating and refilling coupling gel containers, and that new sterile gel bottles are used to prevent cross-infection.

The strength of this study lies in the prospective nature and breadth of sample collection and the sensitivity of the techniques used to define bacterial species. However, although organisms were identified, colony counting provides only a semi-quantitative means of defining load and it is therefore not possible to determine whether these species would be likely to cause nosocomial infection. There are very few documented episodes of bacterial infection acquired from ultrasound equipment and none of these have involved obstetric and gynecological imaging, however under-reporting may be possible. We did not directly compare LLD and HLD methods as the high-level process followed LLD and may have benefitted from the removal of most contaminants, however the presence of spore-forming bacteria following LLD but not HLD suggests HLD is more efficacious. Another limitation of the study was that it was confined to assessment of bacterial contamination and did not assess viral (*e.g.,* HPV) or fungal (*e.g., Candida*) infections that can also be significant in obstetric and gynecological practice.

CONCLUSIONS

In conclusion, we have demonstrated that both abdominal and vaginal probes have a significant chance of being contaminated by bacteria at the end of an examination and need to be properly cleaned. HLD is effective in sterilizing equipment. LLD removes most contaminants, but some spore-producing bacteria remained, and this may present a cross-infection risk that merits further evaluation. Ultrasound machines, probe cords and coupling gel are all potential sources of infection and need to be cleaned, changed regularly or upgraded to new formats to minimize risks of infection. Ultrasound operators and equipment manufacturers need to be aware of these issues so that they can improve the practice of infection control.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ultrasmedbio.2016.09.004.

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