



Incidence of residual bacterial contamination of transvaginal ultrasound probes

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Received: 21 January 2019 / Accepted: 8 March 2019 / Published online: 2 April 2019
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Abstract

Purpose The aim of the study was to investigate if ultrasound probes are reusable medical devices that risk becoming contaminated after a patient examination in Japan.

Methods The level of bacterial contamination on transvaginal ultrasound (TVU) probes following current routine probe cleaning at a university hospital (site A) and a clinic (site B) in Japan was investigated.

Results A total of 98.1% of probes were found to be contaminated at site A (median CFU 40, IQR 10, 132.5) and 94.1% were found to be contaminated at site B (median CFU 50, IQR 20, 85). Of the contaminated probes, 52.9% at site A and 64.6% at site B harbored potentially pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA).

Conclusion These findings suggest that there is a high rate of ultrasound probe residual bacterial contamination in Japan.

Keywords Cleaning · Contamination · Decontamination · Pathogen · Transvaginal ultrasound

Introduction

Ultrasound probes are used throughout healthcare in a wide variety of procedures and may come into contact with intact skin, mucous membranes, broken skin, sterile tissue, and blood in the course of use. Like all reusable medical devices, ultrasound probes can become contaminated with pathogens after a patient examination. A meta-analysis of 32 studies from several countries including Japan found a pooled prevalence of 12.9% for pathogenic bacteria and 1% for pathogenic viruses after wiping or spraying transvaginal and transrectal probes and using a cover between patients [1]. A number of studies and outbreaks have demonstrated that ultrasound probes can become contaminated, which may lead to infection risk [1–17].

Therefore, high-level disinfection (HLD) is required to control cross infection via transvaginal ultrasound (TVU) probes by official regulations in the United States, Canada, Europe, Australia, and New Zealand. However, no data and

regulations exist for residual bacterial contamination of TVU probes in Japan, where gynecologists use probe covers and simple dry paper towel cleaning with or without low-level disinfection (e.g., wipes moistened with alcohol or quaternary ammonium compounds) as routine probe cleaning. We investigated the level of bacterial contamination on TVU probes following current routine probe cleaning/disinfection practice in Japanese clinical settings.

Materials and methods

We collected samples at an outpatient office of a university hospital (site A) and a private maternity clinic (site B) on randomly chosen dates in Saitama and Tochigi prefectures in Japan between July 30 and October 2, 2018. We performed the study at sites with different patient characteristics in different environments. However, the cleaning/decontamination method itself was the same at both facilities. In total, 103 microbial samples were collected by swabbing the surface of whole ultrasound transducers including both the head and the handle with moistened sterile swabs after routine cleaning by wiping off with paper towels. No gynecologists changed their practice during this research. Patients were not selected and no patient information was collected. The study

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protocol was presented to the respective institutional review boards and deemed exempt.

Sample collection from examination rooms occurred after patient examination and cleaning the probe with a paper towel. Using a moistened sterile swab, the surface of the probe was swabbed aseptically by a trained sample collector. The swab was then placed into the sample collection tube containing sterile transport medium (PBS), labeled, and stored on ice. A negative control was collected at a random point in the day by holding the moistened swab in the air for 20 s and sealing it in the collection tube.

Determination of bacterial counts (CFU)

The samples (including negative control swab) were processed immediately upon arrival at the analysis laboratory, or refrigerated and processed within 24 h. The swabs were sonicated for 5 min and vortexed for 30 s before being serially diluted in tryptone soya broth (TSB; Becton–Dickinson) to allow detection in the range of 0–10⁶ colony forming units (CFU). One milliliter of the diluted sample was transferred to a sterile Petri dish and covered with 18–20 ml of tryptone soya agar (TSA; Becton–Dickinson). Plates were incubated at 37 °C for 3 days and the remaining sample incubated in TSB at 37 °C for 3 days. The number of colonies was counted and tubes checked for growth. A positive control was set on each day of testing and involved plating a known amount of *Bacillus subtilis* with an expected 100 CFU per plate. We defined samples as “contaminated” when bacterial growth was observed on either or both cultures.

Species identification

The predominant colony on each cultured plate was isolated by streak plating on soybean casein digest (SCD) and subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis using a Microflex LT MALDI-Biotyper (Bruker) with a clinical database. Each colony was spotted onto a MALDI-TOF target plate, overlaid with alpha-cyano-4-hydroxycinnamic acid (HCCA) solution, and dried to insert into the Microflex. Spectral fingerprints were searched against the database to obtain identifications. As recommended by the manufacturer, identification score values greater than 1.7 were considered reliable identifications.

Bacterial colonies that could not be identified by MALDI-TOF were identified by 16S ribosomal DNA microsequencing. Total DNA was extracted using GenCheck Extraction Reagent (Fasmac) and used to amplify 5' terminus 500 bp fragment of 16S rDNA gene using AMpliTaq Gold (Applied Biosystems). The amplified DNA fragment was determined by its nucleotide sequence and identified on the MicroSeq[®] Identification Systems (Thermo Fisher).

Methicillin resistance analysis

Isolated *Staphylococcus aureus* clones were streaked on TSB and grown overnight at 35 °C. Total DNA was extracted using InstaGene Extraction Reagent (Bio-Rad Laboratories) and subjected to multiplex PCR for *mecA* and *femB* genes to identify genotypes of methicillin resistance, if present, in the isolated *Staphylococcus aureus* clones [18]. *mecA*+/*fXemB*+ clone was identified as methicillin resistant (MRSA), and *mecA*-/*femB*+ was identified as methicillin sensitive (MRSS).

Results

Transvaginal ultrasound probes were contaminated at both sites (Table 1). At site A, 52 probes were sampled and 98.1% were found to be contaminated (median CFU 40, IQR 10, 132.5). At site B, 51 probes were sampled and 94.1% were found to be contaminated (median CFU 50, IQR 20, 85). Of the contaminated probes, 52.9% at site A and 64.6% at site B harbored potentially pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Pseudomonas oryzihabitans*, and *Micrococcus luteus* (Tables 1, 2). In total, 25 unique species of bacteria were identified (Table 2).

Discussion

The data show that almost all transvaginal probes remain contaminated following patient examination, sheath removal, and routine probe cleaning. We selected two clinical settings with different characteristics. The findings from both sites had similar characteristics. Both Site A and

Table 1 Incidence of bacterial contamination on transvaginal probes after patient examination and sheath and gel removal

| Descriptive statistics | Site A | Site B |
|--|------------|------------|
| Total probes sampled (<i>n</i>) | 52 | 51 |
| Contaminated probes <i>n</i> (%) ^{*1} | 51 (98.1%) | 48 (94.1%) |
| Proportion with pathogenic bacteria <i>n</i> (%) ^{*2} | 27 (52.9%) | 31 (64.6%) |
| Median (CFU) | 40 | 50 |
| Interquartile range (CFU) | 10, 132.5 | 20, 85 |
| Maximum (CFU) | 2020 | 590 |
| Minimum (CFU) | 0 | 0 |

CFU bacterial counts

^{*1}No significant difference between site A and B ($p=0.217$)

^{*2}No significant difference between site A and B ($p=0.065$)

Table 2 Frequency of bacterial species identified on transvaginal ultrasound probes

| Bacterial Species* | Site A (n) | Site B (n) | Total |
|---|------------|------------|-----------------------|
| Methicillin resistant <i>Staphylococcus aureus</i> (MRSA) | 1 | 4 | 5 |
| Methicillin sensitive <i>Staphylococcus aureus</i> (MSSA) | 0 | 1 | 1 |
| <i>Staphylococcus capitis</i> | 3 | 4 | 7 |
| <i>Staphylococcus condimenti</i> | 0 | 1 | 1 |
| <i>Staphylococcus epidermis</i> | 8 | 7 | 15 |
| <i>Staphylococcus hominis</i> | 4 | 3 | 7 |
| <i>Staphylococcus haemolyticus</i> | 1 | 1 | 2 |
| <i>Staphylococcus saprophyticus</i> | 1 | 1 | 2 |
| <i>Staphylococcus warneri</i> | 1 | 3 | 4 |
| <i>Bacillus megaterium</i> | 0 | 1 | 1 |
| <i>Corynebacterium mycetoides</i> | 1 | 0 | 1 |
| <i>Kocuria rhizophila</i> | 1 | 0 | 1 |
| <i>Kytococcus schroeteri</i> | 1 | 0 | 1 |
| <i>Micrococcus luteus</i> | 5 | 4 | 9 |
| <i>Pseudomonas oryzihabitans</i> | 0 | 1 | 1 |
| <i>Bacillus arsenicus</i> | 0 | 1 | 1 |
| <i>Bacillus firmus</i> | 1 | 0 | 1 |
| <i>Bacillus megaterium</i> | 0 | 1 | 1 |
| <i>Bacillus mojavenis</i> | 0 | 1 | 1 |
| <i>Bacillus subtilis</i> | 11 | 5 | 16 |
| <i>Brachybacterium faecium</i> | 0 | 1 | 1 |
| <i>Rothia amarae</i> | 2 | 0 | 2 |
| <i>Sphingomonas aerolata</i> | 1 | 0 | 1 |
| <i>Sphingomonas hankookensis</i> | 0 | 1 | 1 |
| <i>Sphingomonas hominis</i> | 1 | 0 | 1 |
| No ID | 8 | 7 | 15 |
| Total | 51 | 48 | 99[§] |

*Shading indicates potentially pathogenic organism

[§] 1 probe at Site A and 3 probes at Site B returned 0 CFUCFU bacterial counts, MRSA methicillin-resistant *Staphylococcus aureus*, MSSA methicillin-sensitive *Staphylococcus aureus*

Site B had a high probe contamination rate (98.1% and 94.1%, $p = 0.217$) and a similar prevalence of potentially pathogenic bacteria (52.9% and 64.6%, $p = 0.065$). More than half of the potentially pathogenic isolates identified were common to both sites (8/15 common). This suggests that similar contamination issues exist in different types of medical settings. Overall, 25 unique species of bacteria were identified across both sites. Five of the six *Staphylococcus aureus* isolates were methicillin resistant. MRSA is one of the most significant causes of healthcare-acquired infections, with a recent Japanese study estimating that each MRSA infection costs \$33 548 USD and is associated with a 22.9% mortality rate [19]. Other staphylococci included *Staphylococcus saprophyticus*, a leading cause of urinary tract infection [20, 21], and several other coagulase-negative species [22–27].

Further opportunistic pathogens that have been implicated in infection were isolated. *Pseudomonas oryzihabitans* has been associated with a small number of reported infections, mainly in immunocompromised patients with indwelling catheters [28]. *Bacillus megaterium* has been associated with a case of brain abscesses, lamellar keratitis following an eye surgery, and primary cutaneous infection [29]. *Kocuria rhizophila*, *Kytococcus schroeteri*, *Micrococcus luteus*, and *Corynebacterium* spp. have also been reported to cause infections in immunocompromised patients [30–33].

Bacterial contamination of probes could arise from multiple sources including the previous patient, the environment, and clinical staff via handling during cleaning or during the procedure itself, particularly if they are not wearing gloves. It is important to remember that standard precautions including hand hygiene, glove use, and aseptic technique are critical for preventing patient exposure to infection risks.

We found that almost all TVU probes were contaminated with bacteria even after usual cleaning practices in Japan. Most of the probes were contaminated with pathogenic bacteria including MRSA. However, these results are based on samples collected from handles and transducers together. As an initial study, we surveyed the gross situation of bacterial contamination that can be the potential risk of cross contamination or infection. Previous studies mainly in western countries suggest that contamination on either heads or handles can be a risk for cross contamination/infection. We are considering conducting a study in the future that samples bacterial contamination on head and handle separately. There are several possible routes of contamination. Internationally, an increasing number of guidelines require a minimum of high-level disinfection of transvaginal ultrasound probes even when a sheath will be used, as the probe sheath can fail [34, 35]. Conversely, our study of residual human papilloma virus (HPV) on TVU probes showed no residual HPV on probes after appropriate glove changes at cover change for each patient [36]. Also, the clinically significant

level of contamination that causes cross infection via TVU probe is still unknown.

Conclusion

This study demonstrated that almost all transvaginal probes remained contaminated with bacteria following current routine ultrasound probe cleaning practices in Japan. The majority of the probes were contaminated with potentially pathogenic bacteria including MRSA. While some studies suggest that ultrasound probes can become contaminated and lead to infection risk, further research is required to determine the precise route of contamination and implement practical solutions for preventing cross infection in Japanese clinical settings.

Funding Nanosonics Ltd. (NSW, Australia) provided funding for the microbiological testing as a part of this study.

Compliance with ethical standards

Conflict of interest All authors declare no conflicts of interest.

Ethical approval This study was approved by our Institutional Review Board.

Informed consent Informed consent was not needed because the samples in this study were taken after routine probe cleaning, not directly from each patient.

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