ORIGINAL ARTICLE

Evaluation of Ultraviolet C for Disinfection of Endocavitary Ultrasound Transducers Persistently Contaminated despite Probe Covers

Guillaume Kac, MD; Isabelle Podglajen, PhD; Ali Si-Mohamed, MD; Aurelia Rodi; Christine Grataloup, MD; Guy Meyer, MD

OBJECTIVE. To determine the rate of bacterial and viral contamination of endocavitary ultrasound probes after endorectal or endovaginal examination with the use of probe covers and to evaluate the antimicrobial efficacy of a disinfection procedure consisting of cleaning with a disinfectant-impregnated towel followed by disinfection with ultraviolet C (UVC) light.

METHODS. Endovaginal or endorectal ultrasound examinations were performed for 440 patients in 3 institutions. All probes were covered by a condom or sheath during the examination. For bacterial analysis, 1 swab was applied lengthwise across one-half the surface of the probe just after removal of the probe cover. The second swab was similarly applied over the probe immediately after the end of a 2-step process consisting of cleaning with a towel impregnated with a disinfectant spray and a 5-minute UVC disinfection cycle. Swabs were applied onto plates and incubated for 48 hours. The number of colony-forming units was counted, and organisms were identified. A similar protocol was used for viral detection of Epstein-Barr virus, human cytomegalovirus, and human papillomavirus, except that an additional swab was applied along the entire external surface of the probe cover before its removal. Viruses were detected by means of a polymerase chain reaction–based protocol.

RESULTS. After removal of probe covers, contamination by pathogenic bacteria was found for 15 (3.4% [95% confidence interval, 2.0%–5.6%]) of 440 probes, and viral genome was detected on 5 (1.5% [95% confidence interval, 0.5%–3.5%]) of 336 probes. After cleaning with a towel impregnated with a disinfectant spray and disinfecting with UVC light, neither bacterial pathogenic flora nor viral genome was recovered from the probe.

CONCLUSIONS. Endocavitary ultrasound probes may carry pathogens after removal of covers under routine conditions. A disinfection procedure consisting of cleaning with a disinfectant-impregnated towel followed by disinfection with UVC may provide a useful method for disinfecting endocavitary ultrasound probes.

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The endocavitary ultrasound examination is a common diagnostic procedure performed for both inpatients and outpatients. In the United States, 624,000 transrectal ultrasound-guided prostate biopsies are estimated to be performed annually to evaluate patients for prostate cancer.[1] Transvaginal ultrasound examinations are routinely used for follow-up examinations of pregnant women or for diagnostic purposes. Endocavitary ultrasound scanning can cause cross-infection with organisms transmitted by blood and by genital or rectal secretions, such as the human immunodeficiency virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Trichomonas vaginalis.*[2] Outbreaks of *Pseudomonas aeruginosa* infection associated with the use of contaminated transrectal ultrasound equipment have occurred recently.[3,4]

International guidelines recommend strict hygiene procedures that include the use of a probe cover (condom or sheath) and chemical high-level disinfection immediately after each examination.[2,5-12] According to the guidelines, systematic use of a probe cover is considered insufficient to prevent endocavitary ultrasound probes from becoming contaminated with microbes because the probe cover frequently becomes perforated during transrectal and transvaginal ultrasound examinations. Perforation usually occurs in 1%–9% of cases and, in 1 study, occurred in 81% of cases.[13-19] However, little is known about the risk of microbial contamination

From Hygiène Hospitalière (G.K., A.R.), the Laboratoire de Bactériologie (I.P.), the Laboratoire de Virologie (A.S.-M.), the Service de Radiologie (C.G.), and the Service de Pneumologie (G.M.), Assistance Publique–Hôpitaux de Paris, Hôpital Européen Georges Pompidou, and Université Paris Descartes (I.P., G.M.), Paris, France.

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of ultrasound probes after transrectal or transvaginal examination. To our knowledge, the only study that has investigated this risk is one that reported a 2.2% rate of bacterial contamination of probes found after removal of condoms in 46 transvaginal examinations.[18] It is known that the routine probe disinfection recommended by health authorities is not always performed.[2,19] This may be due to the lack of data about bacterial and viral contamination of ultrasound probes despite the use of covers and to the multiple disadvantages of chemical high-level disinfection.[20] Thus, there is a need for a more precise estimate of probe contamination and alternative methods for ultrasound probe disinfection. Ultraviolet C (UVC) disinfection of ultrasound probes has recently been demonstrated to be useful in reducing the bacterial load on external ultrasound probes under routine conditions.[21] The aims of our study are thus (1) to determine the rate of bacterial and viral contamination of endocavitary (transrectal and transvaginal) ultrasound probes after use and removal of condoms or probe sheaths under routine conditions and (2) to evaluate the antimicrobial efficacy of a new disinfection procedure that consists of cleaning with a disinfectant-impregnated towel followed by disinfecting with UVC light.

METHODS

Setting

We performed a prospective study conducted during a 4-month period (May–October 2007) in 3 radiology wards in Paris, France, including 1 private radiology center and 2 radiology wards in institutions of Assistance Publique–Hôpitaux de Paris.

Sampling and Processing

Two types of disposable probe covers were used in the study: condoms ($n = 267$) and probe sheaths ($n = 173$) (Microtek Medical). The probes were covered by condoms or probe sheaths according to the routine use of each center. Ultrasound gel was applied to the probe prior to its placement in the condom or probe sheath. After each scan, the probe cover was carefully removed to ensure that the exterior surface of the cover did not come into contact with the interior.

A total of 440 patients were included for bacterial analysis, and a subset of 336 patients was included for viral analysis. For bacterial analysis, a pair of sterile cotton-tipped swab samples (Mast Diagnostic) were taken from the transducer heads after each transrectal or transvaginal ultrasound examination: (1) 1 swab was applied lengthwise across one-half the surface of the ultrasound probe just after removal of the probe cover, and (2) 1 swab was applied across the other one-half the surface of the probe immediately after the probe had undergone a 2-step process consisting of cleaning with a towel impregnated with a disinfectant spray that contained didecyldimethylammonium chloride, polyhexamethylene biguanide, glycolic acid, and 29% (vol/vol) ethanol (Aniospray 29; Anios) and a 5-minute disinfection cycle in the UVC chamber, as described elsewhere.[21] In vitro evaluation of the antimicrobial activity of the UVC chamber by use of coupons inoculated with suspensions of viable microorganisms has shown a mean reduction by a factor of more than 10^5 for bacteria (*P. aeruginosa, Staphylococcus aureus,* and *Escherichia coli*), by a factor of more than 10^5 for *Candida albicans* and *Aspergillus fumigatus,* by a factor of more than 10^5 for *Bacillus subtilis* and *Mycobacterium avium*, and by a factor of more than 10^4 for viruses (enterovirus, adenovirus, and orthopoxvirus), after 5 minutes (data not shown).

The UVC chamber is a closed chamber that contains a bracket from which the probe can be hung. The active parts of the probe are exposed to direct rays from 6 UVC low-pressure lamps (wavelength, 254 nm) fixed to the walls and the bottom of the chamber. One disinfection cycle lasts 5 minutes.

The swabs were then immediately streaked onto 2 plates that contained trypticase soy broth with agar (Oxoid), Sabouraud dextrose agar, and Generbag anaerobic culture medium (bioMérieux). The plates were incubated at 37°C for 48 hours under aerobic and anaerobic conditions. The numbers of colony-forming units (CFU) were counted, and microorganisms were identified and tested for antimicrobial susceptibility by means of standard procedures. Microorganisms other than coagulase-negative staphylococci, *Corynebacterium* species, *Micrococcus* species, and *Bacillus* species were considered to be potentially pathogenic organisms.

For viral analysis, a set of 3 sterile swab samples (Mast Diagnostic) was taken after each transrectal or transvaginal ultrasound examination: (1) 1 swab was applied across the entire external surface of the probe cover just before its removal, (2) 1 swab was applied lengthwise across one-half the surface of the ultrasound probe just after removal of the probe cover, and (3) 1 swab was applied across the other one-half the surface of the probe after the probe had undergone a 2-step process consisting of cleaning with a towel impregnated with a disinfectant spray (Aniospray 29; Anios) and a 5-minute disinfection cycle in the UVC chamber. After sampling, all probes were disinfected by means of chemical high-level disinfection using 2% glutaraldehyde for 20 minutes.

For viral analysis, sterile swabs were used for detection of 3 targeted viruses: Epstein-Barr virus (EBV), human cytomegalovirus, and human papillomavirus (HPV). For DNA extraction, 1 mL of universal transport medium was processed by use of a robotic workstation (Magna Pure LC Total Nucleic Acid Isolation Kit; Roche Diagnostics). Viral genome detection, for human cytomegalovirus and EBV, was assessed by means of the quantitative real-time polymerase chain reaction (PCR) commercial assay R-gene (Argene). Light Cycler (Roche Diagnostics), version 2.0, was used as the real-time PCR instrument. Finally, HPV DNA was assessed by use of the HPV Amplicor assay (Roche Diagnostics), which involved amplification of a target region within the genomic DNA, hybridization to a microwell plate, and subsequent colorimetric detection. The PCR used biotinylated primers specific

TABLE 1. Results of the Cultures Positive for Potentially Pathogenic Bacteria Obtained from Ultrasound Probes after Removal of the Probe Cover under Routine Conditions

Sample	Type of ultrasound examination	Type of probe cover	No. of colony-forming units	Microorganism isolated
1	Transrectal	Condom	1,000	*Escherichia coli*
2	Transrectal	Condom	21	*E. coli*
3	Transrectal	Condom	9	*E. coli*
4	Transrectal	Condom	3	*E. coli*
5	Transrectal	Condom	3	*E. coli*
6	Transrectal	Sheath	8	*E. coli*
7	Transrectal	Sheath	6	*E. coli*
8	Transvaginal	Sheath	57	*Klebsiella pneumoniae*
9	Transrectal	Condom	250	*Acinetobacter* sp
10	Transrectal	Condom	14	*Acinetobacter lwoffi*
11	Transrectal	Condom	6	*A. lwoffi*
12	Transvaginal	Condom	1,000	*Pseudomonas* sp
13	Transvaginal	Condom	15	*Pseudomonas stutzeri*
14	Transrectal	Condom	20	*Burkholderia fungorum*
15	Transrectal	Condom	4	*B. fungorum*

for a target sequence, approximately 165 base pairs in size, spanning a variable region within the polymorphic L1 gene of the HPV genome. These primers are specifically targeted to amplify HPV DNA from the same 13 high-risk anogenital types detectable (ie, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

For each ultrasound examination, a technician responsible for the sample collection filled out the following information on a standardized form: date, patient age and sex, date and type of ultrasound examination performed, type of probe cover used, and whether the probe cover ruptured during the examination. A visual inspection of the probe was performed to detect any blood or body fluids on the probe.

STATISTICAL ANALYSIS

Quantitative data were expressed as median values and ranges, and qualitative data were expressed as frequencies and 95% confidence intervals (CIs). The rates of bacterial and viral contamination were compared between groups with the use of the Fisher exact test or the χ^2 test, if conditions for a normal distribution were met. All analyses were performed on SAS software, version 9.1 (SAS Institute). A P value of less than .05 was considered to be statistically significant.

RESULTS

For bacterial analysis, a total of 440 sets of samples from ultrasound examinations were included, comprising 318 sets (72%) from male patients who underwent transrectal examinations and 122 sets (28%) from female patients who underwent transvaginal examinations. The median age was 63 years (range, 16–100 years).

After removal of the probe covers, bacterial flora was recovered from 301 (68.4%) of 440 samples of probe surfaces (median, 4 CFU/plate [range, 1–1,000 CFU/plate]). Pathogenic flora was recovered from 15 (3.4% [95% CI, 2.0%–5.6%]) of 440 samples of probe surfaces (median, 14 CFU/plate [range, 3–1,000 CFU/plate]), comprising 8 Enterobacteriaceae, 3 *Acinetobacter* species, 2 *Pseudomonas* species, and 2 *Burkholderia* species (Table 1). The rate of contamination with pathogenic flora was 3 of 122 (2.5% [95% CI, 0.5%–7.3%]) for transvaginal probes and 12 of 318 (3.8% [95% CI, 2.1%–6.6%]) for transrectal probes ($P = .8$); this rate was 3 of 173 (1.7% [95% CI, 0.4%–5.2%]) when the probe had been covered by a probe sheath during the examination and 12 of 267 (4.5% [95% CI, 2.5%–7.8%]) when the probe had been covered by a latex condom during the examination ($P = .2$). Neither rupture of the cover nor the presence of blood or body fluids on the probe was detected in any cases. After cleaning with a disinfectant-impregnated towel and disinfecting in the UVC chamber, microbial flora was recovered from 36 (8.2%) of 440 swab samples of probes (median, 0 CFU/plate [range, 0–17 CFU/plate]). The flora was composed only of coagulase-negative staphylococci, micrococci, and corynebacteria, which are recognized as skin colonizers and probably were a result of procedural contamination during sample processing. Pathogenic flora was not recovered in any case.

For viral analysis, a total of 336 ultrasound examinations (238 [71%] transrectal examinations of male patients and 98 [29%] transvaginal examinations of female patients) were included (with a ratio of examinations to patients of 1 : 1). The median age was 62 years (range, 16–99). The presence of at least 1 of the 3 targeted viruses studied was detected on the external surface of the probe cover for 56 patients (16.7% [95% CI, 13.1%–21.0%]); HPV was detected for 28 patients (8.3%), alone (for 25 patients) or associated with other viruses (for 3 patients). HPV was equally distributed among probes used in examinations of men (20 [8.4%] of 238 male patients

[95% CI, 5.5%–12.7%]) and probes used in examinations of women (8 [8.2%] of 98 female patients [95% CI, 4.0%–15.5%]) ($P = .9$). The median number of copies of viral DNA was 2.8×10^3 copies/mL (range, 12 to 1.6×10^5 copies/mL) for EBV and 7.02×10^5 copies/mL (range, 787 to 2.7×10^7 copies/mL) for human cytomegalovirus. The presence of viral genome on the external surface of the probe cover was not influenced by the type of ultrasound performed ($P = .2$). After removal of the probe covers, viral genome was detected on 5 (8.9% [95% CI, 3.5%–19.7%]) of the 56 probes with virally contaminated covers, or 1.5% (95% CI, 0.5%–3.5%) of the cohort studied. HPV was detected on 3 probes and EBV on 2 probes (Table 2). Viral genome was detected on 1 (1.0% [95% CI, 0.01%–6.1%]) of 98 transvaginal probes and 4 (1.7% [95% CI, 0.5%–4.4%]) of 238 transrectal probes ($P > .99$); viral genome was detected on 1 (1.5% [95% CI, 0.01%–8.6%]) of 68 probes that had been covered by a probe sheath and 4 (1.5% [95% CI, 0.4%–3.9%]) of 268 probes that had been covered by a condom ($P > .99$). The same type of virus was found both on the external surface of the probe cover and on the probe after its removal in 3 cases (2 cases of EBV and 1 of HPV). In contrast, in 1 case, EBV and HPV were found on the external surface of the probe cover, while only HPV was found on the probe after removal of the cover. In another case, EBV was detected on the outer surface of the probe cover, while HPV was detected on the probe after removal of the cover. Neither rupture of the cover nor presence of blood or body fluids was detected in any of these cases. After cleaning with a disinfectant-impregnated towel and disinfecting in the UVC chamber, viral genome was not recovered from any of the probes.

DISCUSSION

Our study suggests that probe covers are inefficient at preventing contamination of endocavitary ultrasound probes under routine conditions. Many studies have determined the rate of probe cover perforation,[13-19] but to our knowledge, only 1 study has evaluated the rate of microbial contamination during transvaginal sonography under routine conditions.[18] We observed a 3.4% global rate of bacterial contamination of probes with potentially pathogenic organisms (2.5% for transvaginal probes and 3.8% for transrectal probes). This rate is in accordance with the results observed in the study by Amis et al[18] in which *Acinetobacter* species were recovered from 1 (2.2%) of 46 probe swab samples after removal of condoms, but the precision of the estimate is greater in our study because of the large number of samples. The mechanism by which probe contamination occurs is unclear. It may involve microperforations in the covers that occur before or during the examination, or leakage of blood or other body fluids at the open rim of the sheaths. In our study, no break of the probe covers was detected in any case of contamination, and visual inspection did not reveal any presence of blood or body fluids on the probe. However, the possibility of contamination due to microscopic damage of the sheaths cannot be excluded. The poor sensitivity of visual inspection for detection of contamination has been noted elsewhere,[16,17] and our results underscore the necessity of routine probe disinfection after each examination in addition to the use of a protective cover for the prevention of bacterial or viral transmission among patients.

It is recognized that routine probe disinfection between examinations is not a universal practice.[2,19] This is partly due to the multiple disadvantages of chemical disinfection, such as toxic effects on healthcare workers (skin, eye, and especially respiratory tract irritation), time devoted to the disinfection procedure, and potential incompatibility with the materials used to construct the probes.[20] Thus, there is a need for alternative methods for ultrasound probe disinfection. UVC disinfection of ultrasound probes was recently demonstrated to be effective at reducing the bacterial load on external ultrasound probes under routine conditions.[21] Our results confirm these data regarding the absence of pathogenic microorganisms on the probes at the end of the UVC disinfection

TABLE 2. Results of the 5 Sets of Samples for which Viral Genome was Isolated from Ultrasound Probes after Removal of the Probe Cover under Routine Conditions

Set of samples	Type of ultrasound examination	Type of probe cover	Surface of probe cover[a]			Surface of probe after removal of cover					
						Before disinfection[b]			After disinfection[c]		
			EBV	HCV	HPV	EBV	HCV	HPV	EBV	HCV	HPV
1	Transrectal	Condom	…	…	+	…	…	+	…	…	…
2	Transrectal	Condom	+ (1,810)	…	+	…	…	+	…	…	…
3	Transrectal	Sheath	+ (120)	…	…	…	…	+	…	…	…
4	Transvaginal	Condom	+ (534)	…	…	+ (285)	…	…	…	…	…
5	Transrectal	Condom	+ (1,690)	…	…	+ (448)	…	…	…	…	…

NOTE. The number in parentheses indicates the number of copies of viral DNA per milliliter. EBV, Epstein-Barr virus; HCV, human cytomegalovirus; HPV, human papillomavirus; +, positive for virus.

[a] Samples obtained by applying a swab along the entire external surface of the probe cover just before removal of the probe cover.

[b] Samples obtained by applying a swab along one-half the length of the surface of the ultrasound probe just after removal of the probe cover.

[c] Samples obtained by applying a swab along one-half the length of the surface of the ultrasound probe after the probe had been cleaned with a towel impregnated with a disinfectant spray and then disinfected by means of a 5-minute disinfection cycle in the ultraviolet C chamber.

process. In addition to the high antimicrobial activity inside the UVC chamber, the disinfection process is traceable by means of data collected, including patient name, date of the procedure, and serial number of the probe, which is similar to the method used for endoscopes. Ultrasound endocavitary probes belong to the category of semicritical instruments because of their contact with mucous membranes, which justifies the need for a rigorous disinfection process and traceability. The disinfection process used for heat-sensitive semicritical patient-care instruments such as ultrasound probes must achieve a high level of microbial inactivation, including that of bacteria, mycobacteria, fungi, and viruses.[2] In our study, 3 viruses, including HPV, were targeted to evaluate the risk of probe contamination. Genital HPV is the most common sexually transmitted infection in the United States, with an estimated 6.2 million persons newly infected each year.[22] Although the majority of HPV infections are asymptomatic, persistent genital HPV infection can cause cervical cancer and other types of anogenital cancers and genital warts in both men and women.[23] HPV was detected in 8.3% of specimens collected on the external surface of probe covers in this study, reflecting HPV colonization in the genital and rectal secretions. This figure is in full agreement with the estimated 8% prevalence of HPV in the general population in Europe based on results of 27 studies including more than 70,000 persons.[24] This is of particular concern because the median HPV transmission rate has been estimated to be as high as 40%.[25] These data underline the need for physicians to better adhere to disinfection recommendations, including antiviral activity for reprocessing endocavitary probes in hospitals and also in outpatient clinics; otherwise, HPV cross-transmission through incorrectly disinfected ultrasound probes may occur.

Interestingly, in the present study, the comparison of condoms with probe sheaths specifically designed for ultrasound probes revealed no significant differences in the rates of bacterial and viral contamination. This is, to our knowledge, the first comparative study of the effectiveness of condoms and sheaths in preventing bacterial and viral contamination of endocavitary ultrasound probes under routine conditions. Our results are in agreement with those of a study that evaluated the rates of perforation of condoms and probe sheaths, in which it was determined that there was no superiority of probe sheaths over condoms.[15]

Potential limitations of this study may be inherent to the study protocol. First, we did not perform a randomized study comparing probe sheaths with condoms, because we searched primarily to evaluate the risk of bacterial and viral contamination of ultrasound probes under routine conditions without interfering with current hospital practice. Second, the sampling protocol included applying the swabs across the transducer heads after scanning and removal of the probe cover. The sampling protocol could artificially reduce the microbial load on the probe surface before cleaning.

In conclusion, we have shown that endocavitary ultrasound probes may carry potential pathogens, including HPV, unless properly disinfected between examination sessions. The use of a cover does not alter the requirement for disinfection after each examination. Cleaning of the probes with a disinfectant-impregnated towel followed by UVC disinfection may provide a useful, rapid, and easy-to-use method for disinfecting ultrasound endocavitary probes.

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Address reprint requests to Guillaume Kac, MD, Hygiène Hospitalière, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France (guillaume.kac@egp.aphp.fr).

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