Disinfection of Transvaginal Ultrasound Probes by Ultraviolet C – A clinical Evaluation of Automated and Manual Reprocessing Methods

Desinfektion transvaginaler Ultraschallsonden – klinischer Vergleich einer automatischen Technik mittels ultravioletter Strahlung und einer manuellen Wischtuchmethode

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Key words

hygiene, disinfection, transvaginal ultrasound, antigermix, UV-C

received 12.10.2018
accepted 06.03.2019

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DOI https://doi.org/10.1055/a-0874-1971
Published online: 2019
Ultraschall in Med

ISSN 0172-4614

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ABSTRACT

Purpose Since pathogens can be transmitted to patients via transvaginal ultrasound probes, it is of particular importance that cleaning and disinfection are performed adequately. This study was designed to do a qualitative comparison of a low-level disinfection technique with disinfectant-impregnated wipes and an automated disinfection technique using ultraviolet C radiation in a clinical setting.

Materials and Methods The transvaginal ultrasound probes used in two groups of 160 patients were compared in a prospective controlled study regarding the effectiveness of manual low-level disinfection (Mikrozid® sensitive wipes) and automated disinfection using ultraviolet C radiation (Antigermix® AS1). Microbiological samples were taken from the whole surface of the probe before and after the disinfection process.

Results Before disinfection, 98.75 % (316/320) of the samples showed bacterial contamination. After automated and manual disinfection, the contamination rates were 34.2 % (54/158, automated) and 40.5 % (64/158, disinfectant wipes) ($p > 0.05$). Pathogens with the potential to cause healthcare-associated infections, such as Enterococcus faecalis and Klebsiella pneumoniae, were removed completely by both techniques. Manual disinfection showed a lower contamination rate after disinfection of bacteria that usually belong to the vaginal, pharyngeal and skin flora (disinfectant wipes 10.6 %, 11/104, automated 32.5 %, 38/117) ($p < 0.001$).

Conclusion For the clinical routine, automated disinfection with ultraviolet C is a promising technique for transvaginal ultrasound probes because of the simple handling and time efficiency. In our study, this method was completely effective against nosocomial pathogens. However, the study didn't show any significant difference in terms of effectiveness compared to low-level wipe disinfection.

ZUSAMMENFASSUNG

Ziel Da Krankheitserreger über transvaginale Ultraschallsonden auf Patientinnen übertragen werden können, ist eine adäquate Reinigungs- und Desinfektionstechnik von besonderer Wichtigkeit. Ziel dieser Studie war der qualitative und klinische Vergleich einer herkömmlichen Wischtuchdesinfektion mit einer automatischen Desinfektion durch ultraviolette Strahlung.

Material und Methode 2 Gruppen à 160 Patientinnen wurden in einer prospektiven, kontrollierten Studie hinsichtlich der Effektivität der manuellen low-level-Wischtuchdesinfektion (Mikrozid® sensitive wipes) im Gegensatz zur automatischen Desinfektion mit ultravioletter Strahlung (Antigermix® AS1) für transvaginale Ultraschallsonden untersucht. Dafür wurden mikrobiologische Abstriche von der gesamten Sonden-Oberfläche vor und nach Desinfektion genommen.

Ergebnisse Vor Desinfektion wurde auf 98,75 % (316/320) der Abstriche bakterielles Wachstum nachgewiesen. Nach der Desinfektion ließ sich bei 34,2 % (automatische Desinfektion, 54/158) und 40,5 % (Wischtuchdesinfektion, 64/158) noch bakterielles Wachstum nachweisen ($p > 0,05$). Erreger,

die zu nosokomialen Infektionen führen können, wie beispielsweise Enterococcus faecalis und Klebsiella pneumoniae, wurden durch beide Techniken komplett entfernt. Gegen Keime der Vaginal-, Rachen- und Hautflora zeigte die Wischtuchdesinfektion eine niedrigere Kontaminationsrate von 10,6 % (11/104) (automatische Desinfektion 32,5 %, 38/117) ($p < 0{,}001$).

Schlussfolgerung Die automatische Desinfektion von vaginalen Ultraschallsonden mit UV-C-Strahlung ist aufgrund einfacher Handhabung und Zeiteffizienz eine erfolgversprechende Methode für den klinischen Alltag. Nosokomiale Pathogene wurden in unserer Studie durch die Desinfektion vollständig entfernt, allerdings konnte kein signifikanter Unterschied bezüglich der Wirksamkeit im Vergleich zur Wischtuchdesinfektion festgestellt werden.

Introduction

Since transvaginal ultrasound probes may carry pathogens, such as *Human papillomavirus* [10] or *Staphylococcus aureus* [23], which can be transmitted between patients and cause serious infections, there is an ongoing discussion about an appropriate reprocessing technique. According to the German recommendations for disinfection by the German Federal Institute for Drugs and Medical Devices (BfArM) and the Robert Koch Institute (RKI) in 2001 (last revised in 2012) [8, 18], transvaginal ultrasound probes are considered semi-critical group A medical products that require cleaning and bactericidal, virucidal and fungicidal disinfection prior to use in addition to sheathing in a protective cover. Wipe disinfection, as usually used in Germany, is described as sufficient if used adequately.

However, a recently published recommendation by the German Society of Ultrasound in Medicine (DEGUM) declares wipe disinfection to be insufficient for rigid endoscopic ultrasound probes, since not every spot is reliably disinfected [20]. In addition, current studies show that standard LLD is not totally effective against specific pathogens such as HPV [21, 30] and that probe covers have perforation rates of 0.9 – 81 %, depending on the material [3, 15, 19, 26, 29]. Additionally, probe handles might also carry pathogens and should therefore also be disinfected [22]. Contamination of the ultrasound gel cannot be ruled out [11, 24].

Altogether, a thorough disinfection technique such as high-level disinfection (HLD), which is already standard in other countries like the United States [2], Canada [9] and Australia [14], should be preferred according to ultrasound organizations such as the World Federation of Ultrasound (WFUMB) [1] and the German Society for Ultrasound in Medicine (DEGUM) [20]. Automated disinfection techniques have the advantage of good validation, standardization and user independence. While producers of transvaginal ultrasound probes still publish ambiguous recommendations [25] leading to uncertainty among some users, the clinical gynecological routine is in need of a thorough, fast and affordable technique.

One automated system is the Trophon® EPR (Nanosonics Ltd., Sydney, Australia) using hydrogen peroxide for disinfection, which showed a significantly higher success rate compared to LLD not only against bacterial pathogens but also against viruses such as norovirus and HPV in two studies [4, 7].

This study compared an automated system working with ultraviolet C (UV-C) radiation: Antigermix® AS1 (Germitec, Ivry-sur-Seine, France) to a wipe disinfection method. This UV-C system showed efficiency against HPV in vitro and in clinical conditions [17].

Methods

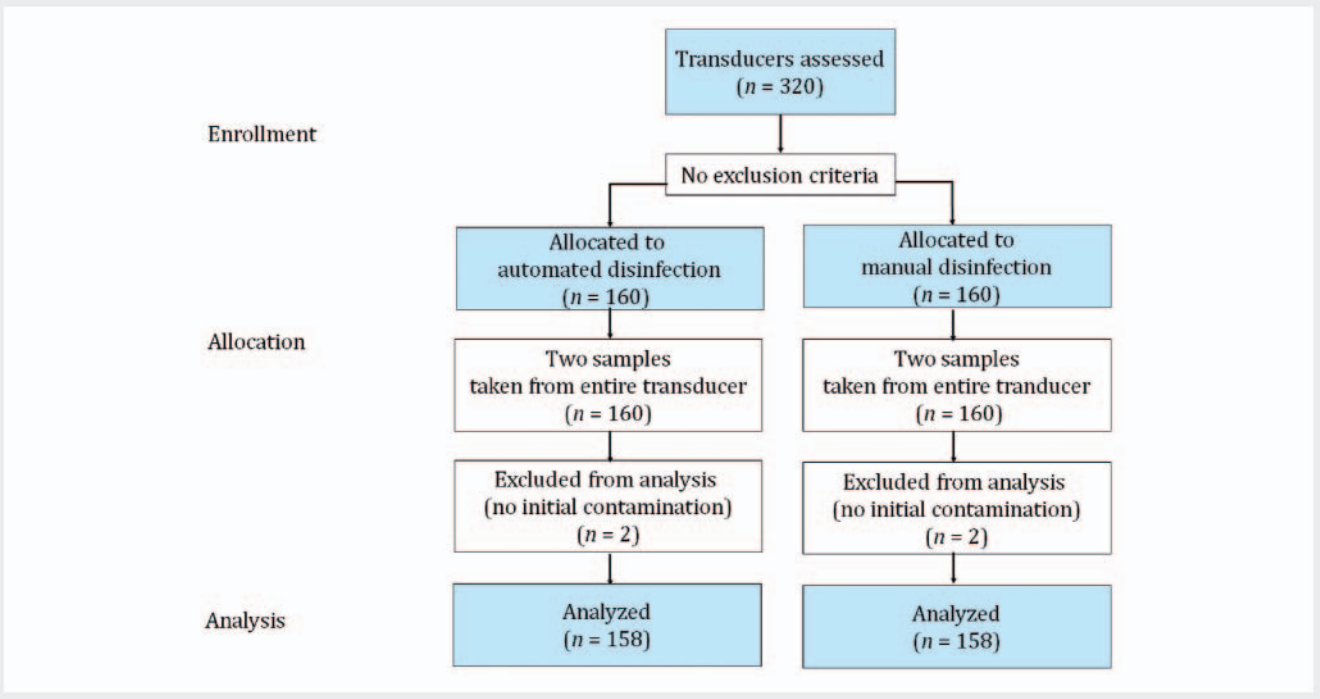
The study was designed according to the Declaration of Helsinki and was approved by the institutional ethics board. Patient consent was not required, as patient details or samples were not obtained.

During a three-month period, a total of 320 transvaginal ultrasound examinations were performed by 6 physicians for prenatal checkup or annual gynecological screening. Patients were randomly allocated to two examination rooms, each with one type of disinfection method. They were generally healthy.

Ultrasound examinations were performed with two identical probes (C10 – 3v, Philips Medical Systems, Andover, MA, USA) of the same age and condition, protected by a non-sterile transducer cover. After concluding each transvaginal ultrasound examination, the physician removed the ultrasound gel thoroughly with a paper cloth. Subsequently, when both patient and physician had left the examination room, the first swab was taken of the entire transducer including the handle, but not the cable. All swabs were taken by two specially trained medical staff members. ▶ **Fig. 1** shows the order of the sample series.

In the first room, a second cleaning of the whole transducer with wipes provided by Germitec followed. These cleaning wipes have no disinfection claim as they are impregnated by a mixture of non-ionic and amphoteric surfactants and use DIDAC (Didecyldimethyl ammonium chlorine) as a preservative. 90 seconds of automated disinfection using ultraviolet radiation with Antigermix® AS1 (Germitec) completed the process. Antigermix® AS1 disinfects the whole transducer in a disinfection chamber without the need of disconnecting it [13]. Afterwards, the second swab was taken.

In the second room, the whole probe was disinfected by Mikrozid® sensitive wipes (Schülke & Mayr GmbH, Norderstedt, Germany). These are ready-to-use impregnated wipes using quaternary ammonium compounds [27]. After an application time of at least one minute according to the manufacturer's instructions, the second swab was taken.



▶ **Fig. 1** Conducting of the sample series from transvaginal ultrasound transducers before and after disinfection using a manual and an automated method.

Sample collection was carried out with sterile gloves and Polywipe™ (Medical Wire & Equipment, Corsham, UK) pre-moistened sponge swabs. After incubation at 36 °C for 48 hours in enrichment broth (Tryptic Soy Broth with neutralizer, Merck Life Science, Eppelheim, Germany), samples were applied to blood and MacConkey agar and incubated for another 24 hours. In the case of unsuccessful phenotypical identification, species identification was performed by means of MALDI-TOF (matrix-assisted laser desorption/ionization – time of flight). The broth was validated with different numbers of CFU before using the product.

Statistical analysis

We created a two-by-two contingency table with binary variables (contaminated or not contaminated) in order to carry out a univariable analysis. An "explorative statistical analysis" was performed using IBM SPSS Statistics 24 for Windows (IBM Corporation, Somers, NY, USA). P-values ≤ 0.05 will be described as "significant". Hence, P-values will not be adjusted for multiplicity.

Results

After vaginal ultrasound examination, 316 out of 320 samples (98.75 %) showed bacterial contamination, the remaining two samples per group without bacterial contamination were excluded from the study. Thus, 158 transducers went through the different disinfecting processes in each group. All examined probes were visually clean and did not show any obvious cover damage.

Efficacy of disinfection

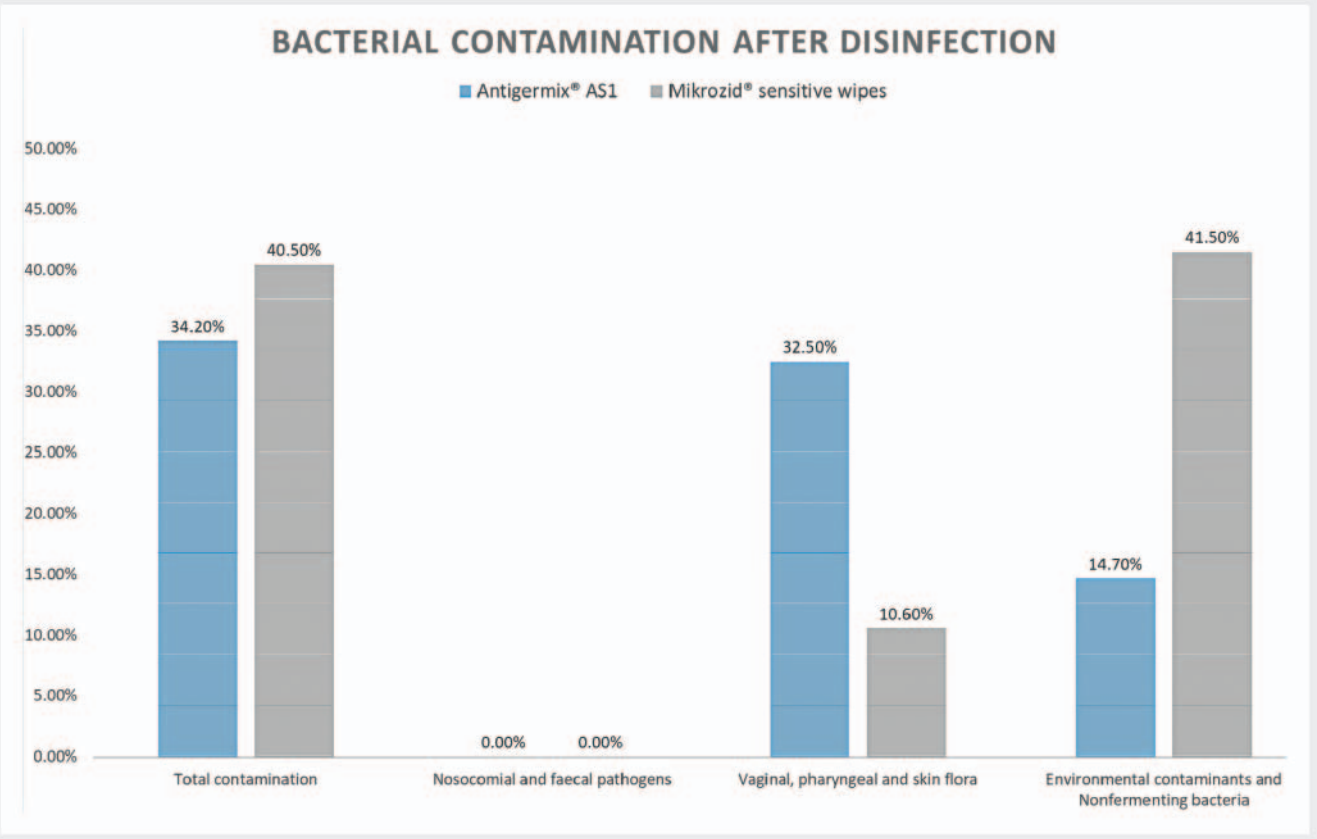
In both groups, up to four different bacterial species were found on the same transducer before disinfection. After the disinfection process with Antigermix® AS1, 34.2 % (54 positive results) were contaminated in contrast to 40.5 % (64 positive results) after LLD with Mikrozid® sensitive wipes. This difference with a 6.3 % higher success rate of the Antigermix® AS1 was not statistically significant (odds ratio (OR), 1.3; 95 % CI, 0.8 – 2.0; P = 0.25). ▶ **Fig. 2** shows the proportion of contaminated transvaginal ultrasound transducers after disinfection according to bacterial species

Microbial testing

Altogether, 38 different species of bacteria and no fungi were revealed after microbiological analysis. In order to specify the results, they were separated into three groups of "environmental contaminants and nonfermenting bacteria", "vaginal, pharyngeal and skin flora" and "nosocomial and fecal pathogens". ▶ **Table 1** names each detected bacterial species.

Regarding the decisive group of "nosocomial and fecal pathogens", which includes pathogens causing nosocomial infections such as *Staphylococcus aureus* [23], *Enterococcus faecalis* [28] and *Klebsiella pneumoniae* [5, 11, 28], both methods of disinfection showed a reduction of 100 %. Before disinfection, 25.0 % (Antigermix® AS1) and 16.9 % (Mikrozid® sensitive wipes) of the samples showed potential pathogen contamination. This effect was not statistically significant (p = 0.07).

In the group of "vaginal, pharyngeal and skin flora", 32.5 % (38/117) of the samples were contaminated after disinfection with Antigermix® AS1, while after disinfection with Mikrozid® sen-



▶ **Fig. 2** Proportion of contaminated transvaginal ultrasound transducers after disinfection by an automated or manual method in total and according to the bacterial species.

▶ **Table 1** Type of contaminant/pathogen identified.

nosocomial and fecal pathogens	Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Acinetobacter baumannii, Klebsiella pneumoniae, Streptococcus agalactiae, Pantoea agglomerans, Citrobacter freundii, Streptococcus pneumoniae
vaginal, pharyngeal and skin flora	Coagulase negative Streptococcus, Aerococcus viridans, Micrococcus spp., Staphylococcus epidermidis, Staphylococcus hominis, Corynebacterium spp., viridans streptococci, Micrococcus luteus, Corynebacterium tuberculostearicum, Corynebacterium striatum, Lactobacillus, Staph. warneri, Aerococcus sanguinicola, Globicatella sulfidifaciens
environmental contaminants and nonfermenting bacteria	Bacillus spp., Stenotrophomnas rhizophilia, Moraxella osloensis, Achromobacter xyloxidans, molds, Pseudomonas stutzeri, Bacillus circulans, Bacillus simplex, Acinetobacter lwoffi, Acinetobacter pitii, Acinetobacter johnsonii, Acinetobacter radioresistens, Acinetobacter spp., Stenotrophomonas maltophilia

sitive wipes the rate of contamination was 10.6 % (11/104). This shows a 21.9 % higher success rate of manual disinfection. The effect was statistically significant ($P < 0.001$). After disinfection with Antigermix® AS1, Micrococcus spp. showed an increase from 2 to 15 colonized samples.

"Environmental contaminants and nonfermenting bacteria" was the main group colonizing the samples before disinfection (129 positive results Antigermix® AS1, 130 positive results Mikrozid® sensitive wipes). The contamination rate was 14.7 % after automated (19/129) and 41.5 % (54/130) after manual disinfection. This effect was statistically significant ($P < 0.001$).

Discussion

In our study, the comparison of automated disinfection by ultraviolet C to LLD with disinfecting wipes leads to similar results in both groups, as 34.2 % (Antigermix® AS1) and 40.5 % (Mikrozid® sensitive wipes) of the probes were contaminated after the completed disinfection process. There is a tendency toward better results in the automated group, but it is not statistically significant.

In order to explain the high rates of bacterial contamination found in our study, especially the sampling technique might have

an important impact. In general there are three ways of taking a swab for bacterial analysis: Firstly a cotton wool wad, which is made for taking swabs in wounds, from mucous membranes or other moist surfaces. Secondly a RODAC plate, which would allow a quantitative analysis, but is not as sensitive as the sponges and doesn't allow wiping of the whole surface of the ultrasound probe because of its geometry. Thirdly the sponges we used: They have a high sensitivity and can detect single bacteria, and it is easy to wipe the whole probe.

Of course it also would have been interesting to do a quantitative analysis to see whether samples meet the requirements for disinfection (a 5 log decline in CFU (colony forming unit)). However, for clinical hygiene, the most important aim of disinfection is an elimination of pathogens that can cause relevant infections in the patient. The presence of normal skin or environmental bacteria is not that relevant in this case. In this study pathogens with the potential to cause healthcare-associated infections, such as *Enterococcus faecalis* and *Klebsiella pneumoniae*, were removed completely by both techniques.

In some cases in our study, bacteria was found in the second swab taken after disinfection, which we had not found in the first swab taken before disinfection including 19 in the Antigermix group and 7 in the wipes group (12 % and 4.4 %). These are possible reasons for this finding: First, we did not take the swabs under a sterile workbench. Therefore, even with perfect adherence to the requirements for sterile working conditions, it is possible for contamination to occur while taking the swab. In the study by Buescher et al. [7], sham samples from sterile petri dishes were taken in our department under very similar conditions and showed that there was a contamination of 10.8 %. Second, the method is very sensitive, but as always there is also a certain detection limit. So it is possible that there is a certain variation and divergent results might occur.

Another explanation is that Mikrozid® sensitive wipes might have a higher resistance to recontamination compared to Antigermix® AS1, because active residues are left on the transducer after the disinfection process. These residues might prevent recontamination, e. g. by manipulation with hands or environmental factors for a short time after disinfection. They may inhibit the growth of microorganisms and overestimate the efficacy of the chemical-based reprocessing procedure. To avoid this, we used enrichment broth with neutralizer. The neutralizer blocks any effect of disinfectant residue that might still be on the sponge. The sponges were put into the broth within a short time. So this makes the contamination rate we found in the wipes group comparable to that of the Antigermix group, even if Antigermix doesn't use a liquid disinfectant.

Other studies have already proven the suitability of Antigermix® AS1 for disinfection. One study was carried out by Kac et al. [17], who reported complete bactericidal and virucidal effectiveness after disinfecting endovaginal or endorectal ultrasound transducers with a disinfectant-impregnated towel and a 5-minute UV-C disinfection cycle. Before disinfection, only 3.4 % of the probes showed contamination. However, Kac et al. used cotton tip swabs instead of sponges which do not cover uneven surfaces. Moreover, samples were only taken from one half of the probe which might lead to false-negative results. Bloc et al. [6] also detected sterile (< 10 colony-forming units) results after disinfection of ultrasound probes used for regional anesthesia with disinfectant-impregnated paper and a 90 s UV-C disinfection cycle by Antigermix® AS1. In comparison to the results of Kac et al. [16], examining the effectiveness of Antigermix® AS1 for the disinfection of transthoracic ultrasound transducers, the germ reduction of 100 % was significant. This study used a 10-minute UV-C cycle by Antigermix® AS1 without disinfectant-impregnated wipes. Compared to our study, these favorable results might be attributed to differences in the duration of the disinfection cycle, disinfection and detection method and probe shape. Both studies did not use any enrichment broth. However, a prolongation of the UV-C cycle and a combination of disinfecting wipes and UV-C-radiation should be discussed for transvaginal ultrasound probes.

With Trophon EPR®, Buescher et al. [7] already examined an automated disinfection procedure in our department under similar circumstances and with the same transducers used in our study. Trophon EPR® is an automated disinfection system working with hydrogen peroxide that takes 7 minutes for disinfection of the whole transducer. The study protocols were not the same, so it is not possible to directly compare the results. One difference between the studies is a shorter incubation time of 24 hours in the Trophon study, which was now extended to 48 hours due to changes in our lab's standard operating procedures. Due to further investigations (conducted in our department with 50 additional samples), this prolongation was not responsible for higher numbers of contaminated probes in our study. Another important difference between the two studies is that in the Trophon study swabs were taken separately from the body and the handle of the probe, whereas in this study we took one swab of the whole probe including the handle. ▶ **Table 2** shows a large variety of bacteria before the disinfection process, many of them vaginal and skin flora. Since we found so many different bacterial species despite probe covers, additional bacteria on the handle could be an explanation for different results.

There are more factors in the clinical use of transvaginal ultrasound probes that have an influence on their contamination. They are worth a discussion although they did not influence the results of this study. Additional pathogen contamination can occur as a result of the ultrasound transducer holder. Directly after examination, the transducer is often positioned in the holder and the disinfection process only starts after the patient has left the room. Pathogens transmitted to the holder might be transmitted back to the transducer after disinfection when the probe is placed back in the holder. Using an automated disinfection system e. g. Antigermix® AS1 opens up the possibility of leaving the probe in the disinfecting machine until it is used for the next patient, which would lead to a reduction of bacterial contamination.

Another factor is the strict time limits of the clinical routine leading to shortcomings of manual disinfection attributed to inconsistent human performance. The residence time in our protocol of at least one minute met the manufacturer's standards, but might be reduced in busy departments. Furthermore, disinfection of the whole transducer is important, as previous studies have already confirmed a high contamination rate of the handle [7, 22]. Thorough disinfection might be disregarded because it is considered laborious. Moreover, to prevent bacterial transmis-

▶ **Table 2** Contamination before disinfection.

Antigermix	wipes
nosocomial and fecal pathogens: Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Acinetobacter baumannii, Klebsiella pneumoniae, Streptococcus agalactiae, Pantoea agglomerans, Steptococcus pneumoniae	nosocomial and fecal pathogens: Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus agalactiae, Enterococcus faecium, Citrobacter freundii
vaginal, pharyngeal and skin flora: Coagulase Negative Streptococcus, Aerococcus viridans, Micrococcus spp., Staphylococcus epidermidis, S taphylococcus hominis, Corynebacterium spp., Viridans streptococci, Corynebacterium striatum, Staph. warneri, Aerococcus sanguinicola, Globicatella sulfidifaciens	vaginal, pharyngeal and skin flora: Coagulase Negative Streptococcus, Aerococcus viridans, Micrococcus spp., Staphylococcus epidermidis, Corynebacterium spp., Viridans streptococci, Corynebacterium striatum, Staph. warneri
environmental contaminants and nonfermenting bacteria: Bacillus spp., Stenotrophomonas rhizophilia, Moraxella osloensis, Achromobacter xyloxidans	environmental contaminants and nonfermenting bacteria: Bacillus spp., Stenotrophomonas rhizophilia, Moraxella osloensis, Acinetobacter Iwoffi, Acinetobacter radioresistens, Stenotrophomonas maltophilia

sion, abiding by hand and surface disinfection standards is of high importance as the hands of the examiner could transmit potential pathogens. Additional ways of germ transmission are via ultrasound gel [11, 24] and nonsterile covers with a certain permeability [3, 15, 19, 26, 29].

In conclusion, our results emphasize the importance of probe disinfection after transvaginal ultrasound examinations, as 98.75 % of the samples showed bacterial contamination before the disinfection process, including pathogenic species causing nosocomial infections.

Antigermix® AS1 has the potential to become the thorough and automated disinfection technique the clinical gynecological routine needs. In terms of clinical applicability, it has the advantage of being non-toxic to the physician and patient and non-corrosive to the ultrasound probes [12]. Additionally, the expenditure of time for the disinfection process is just 90 seconds and, therefore, the process can be easily integrated into the clinical routine. Thus, from a gynecologists' view, automated disinfection with ultraviolet C is a promising disinfection technique. Nevertheless, the microbiological results of this study are not fully satisfactory as both techniques showed high contamination rates even after disinfection.

Nosocomial pathogens were removed by both techniques, so that the ultrasound probes did not carry the risk of relevant infections for the patient. In order to prove if all criteria for disinfection are fulfilled, a future study should do a quantitative analysis to compare the exact contamination rates.

Conflict of Interest

Germitec contributed to the project by funding the sampling costs and loaning an Antigermix® AS1 system. Investigators received no additional benefit. A contract guaranteed scientific independence for the investigating institution. Collection and analysis of data remained solely with the investigating institution. The authors have no relation to the funding cooperation beyond the conduct of this study. The terms of this arrangement have been reviewed and approved by the University of Muenster in accordance with its policy on objectivity in research.

Acknowledgements

We are grateful to everyone involved in the study who voluntarily dedicated his or her time and effort. A special thanks goes to Katrin Sunkovsky and Christine Thies for taking samples and helping out whenever possible, to Margret Junge and Sven Rottmann for technical support in the laboratory and Prof. Dr. med. Alexander Mellmann for his scientific advice.

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