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## Major article

## A new UV-LED device for automatic disinfection of stethoscope membranes

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**Key Words:**  
Biotechnology  
Disinfection  
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Stethoscope  
UV-LED

**Background:** Stethoscopes are widely used by doctors and nurses. Poor stethoscope hygiene is a potential source of nosocomial infection. This study aimed to propose an innovative solution, based on the latest advances in ultraviolet (UV) light-emitting diodes (LEDs), for disinfecting stethoscope membranes automatically and efficiently.

**Methods:** *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were sown on 28 stethoscope membranes and then transferred to Petri dishes. Treatment involved illuminating exposed Petri dishes with a UVC LED for 1 minute. For each microbe, the number of colony-forming units (cfu) at 36°C was compared in control and treated dishes using the Wilcoxon signed-rank test. The Kruskal-Wallis test was used to assess percent reductions in bacteria. Statistical significance was set at 99%.

**Results:** A significant reduction in cfu counts after UV treatment ( $P < .01$ ) was found for all bacteria: 85.5% for *E faecalis*, 87.5% for *S aureus*, 94.3% for *E coli*, and 94.9% for *P aeruginosa*. No significant differences in percent reduction in cfu were found between bacteria ( $P > .01$ ).

**Conclusion:** The stethoscope, symbol of medicine and health care professionals, has been demonstrated to be a carrier of microorganisms. The treatment technique was effective and efficient in disinfecting the membranes. These promising results represent a step forward toward eliminating stethoscope membrane contamination with an innovative approach.

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The medical literature has demonstrated the importance of nosocomial infections and their negative consequences, including damage to patient health, clinical complications, mortality, and longer admissions, with the need for extended care and corresponding increased costs.<sup>1</sup> A 2012 meta-analysis of studies conducted in various settings and countries showed that the cost per case of infections acquired in hospital is typically between \$2,000 and \$15,000 and increasing.<sup>2</sup> Patients who contract nosocomial infections during admission to intensive care units cost an average

of \$10,000, compared with an average of \$4,000 for those who remain infection-free.<sup>3</sup>

Another recent meta-analysis on the financial impact of nosocomial infections on the US health care system found a total annual cost of \$9.8 billion for the 5 major health care–associated infections: surgical site infection, central line–associated bloodstream infection, catheter-associated urinary tract infection, ventilator-associated pneumonia, and *Clostridium difficile* infection.<sup>4</sup> These high figures actually may be underestimates, however, considering the opinion that an accredited academic journal noted that published outbreaks of infection are only the “tip of the iceberg” of all nosocomial epidemics.<sup>5</sup>

Nosocomial infections often result from inadequate or superficial management of cleaning, disinfection, and sterilization.<sup>6,7</sup> The hands of health care personnel are the main vehicle of transmission of microbes and viruses.<sup>8</sup> All objects that come into contact with

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Conflicts of interest: The authors are co-founders of a Start up company, which is currently trying to put into application and practice the innovative approach developed in years of research about the issues described in the article.

and are shared between personnel and patients are possible carriers of microorganisms.

Stethoscopes are widely used by health professionals, and it has been conclusively demonstrated that their membranes can transmit microbes and viruses from 1 patient to another and from health care worker to patient.<sup>9–15</sup> Crespo et al<sup>16</sup> recovered the same strain of *P. aeruginosa* (serotype O12) from skin and stethoscopes in affected units, although not from the hands of staff. Gastmeier et al<sup>17</sup> reported the same strain of *Klebsiella pneumoniae* on the stethoscopes of incubators of a neonatal intensive care unit as in clinical isolates from 2 patients with bloodstream infections caused by this bacterium.

The simplest solution to the problem is to disinfect the stethoscope membrane before each use, to avoid contamination and its buildup with repeated uses (Fig 1). Unfortunately, this is rarely done in clinical practice, for various reasons, including poor hygiene practices by medical staff, forgetfulness in managing the various stages of medical care, lack of awareness/consideration of the importance of the procedure, and the cumbersome process of disinfecting with swabs moistened with chemical disinfectant.<sup>10</sup> The stethoscope has become so important in spreading bacteria that a recent article highlighting major strategies for preventing hospital infections dedicated a special recommendation to cleaning of stethoscopes between patients to avoid increasing contamination.<sup>18</sup> Another study found that the substantial bacterial contamination level on stethoscopes is similar to that on physicians' hands, a known major source of nosocomial infections.<sup>19</sup> These findings are a first step toward rewriting guidelines and regulations for proper use and cleaning medical devices, especially stethoscopes.

A plausible approach could involve ultraviolet (UV) light, which has already been used for such therapeutic purposes as stimulating vitamin D production and treating psoriasis, as well as for sanitizing air, water, and the environment. At its typical wavelength of 200–280 nm, UVC radiation induces pyrimidine dimers in thymine and cytosine, breaking DNA molecules, inactivating germs and preventing them from growing or reproducing.<sup>20,21</sup>

Several previous studies have demonstrated that the use of UVC on a wheeled device to disinfect hospital rooms and environments is plausible, fast, and practicable.<sup>20,22,23</sup> In another study, Nerandzic et al<sup>24</sup> tested portable units emitting UVC for disinfection of the environment. In contrast, there has been little study of the use of UVC with innovative techniques and equipment, such as light-emitting diodes (LEDs), which allows the creation of small, portable, low-energy, easy-to-use devices for disinfection. In this article, we propose an innovative technological approach, based on the latest advances in ultraviolet LEDs, that enables automatic and efficient disinfection and sterilization of stethoscope membranes.

## MATERIALS AND METHODS

### Ultraviolet LED

We chose UVC light because of its good biocidal effect. UVC inactivates microbes with a few seconds and prevents their replication (spores included) on exposed surfaces under the following conditions: (1) reduced distance between surface to be disinfected and the UVC source; (2) wavelength preferably in the 255–280 nm range; (3) appropriate exposure time; and (4) homogeneous surface illumination. Miniaturization of UV-LED technology, as well as its low power consumption, long life, and decreasing cost, allow innovative applications for disinfection/sterilization in the biomedical field.<sup>25</sup> To satisfy the foregoing conditions, we used a UVTOP UVC LED (UVTOP255TO39FW;

Sensor Electronic Technology, Columbia, SC), with a peak wavelength of 260 nm, a lighting power of 300  $\mu$ W, a forward voltage of 6.5 V, and an irradiation angle of 120°.

### Device prototyping

A prototype, intended for use in an experimental environment, was created with a view toward developing a portable device that is safe for health professionals and patients alike. It is configured as a simple circular cover for application to the head of the stethoscope. The size of the cover was obtained by analyzing the classical dimensions of stethoscope membranes. The design was realized with Sketchup 3D modeling software (Sketchup, Boulder, CO) and a 3D printer.

The microelectronic component of the device was designed using the latest embedded system technology with UVC LEDs as the principal components. It included a battery power supply and a microcontroller that supplies a constant direct current of 20 mA. Figure 2 shows the prototype hardware. Note the circular shape with the UV LED in the center.

When a flat circular surface, such as a stethoscope membrane, is placed in front of the LED, the irradiation angle of 120° makes it possible to fully illuminate the surface when the LED is at a distance of  $d = r/\sqrt{3}$ , where  $r$  is the radius of the membrane.

### Laboratory analysis and experimental design

Our analysis was performed in the hygiene and environmental laboratory of the University of Siena. All precautions were taken to ensure that the experiment was conducted under safe conditions. UVC light can be hazardous for the skin and eyes of the operator. Although the possibility of exposure to the radiation was low and the emission of UVC light was very limited in space, all precautions were taken to avoid any possible risk. The experimental protocol was conducted using the UVC LED prototype, illuminating several stethoscope membranes of 40 mm diameter ( $260 \pm 12$  nm).

We tested the efficacy of the device with 4 strains of bacteria that are common stethoscope contaminants<sup>9–12,16</sup>: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. The membranes were cultured on plate count agar (PCA) in 90-mm Petri dishes, as in previous studies.<sup>24</sup> Several colonies were withdrawn from each culture to prepare bacterial suspensions in phosphate-buffered saline up to a 0.5 McFarland turbidity standard. From these initial suspensions, which were shaken in a vortex mixer, the following dilutions were made:  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ . The latter dilution was used for testing.

A 50- $\mu$ L suspension of each species of bacteria was uniformly sown on the stethoscope membranes using sterile swabs. Eight membranes, 2 per bacterial species, served as controls, and were placed in contact with fresh PCA in other 60-mm Petri dishes for at least 20 seconds. The other 20 stethoscope membranes, 5 per bacterial species, were illuminated with UVC for 1 minute at a distance of 11.5 mm and then placed in contact with fresh PCA in other Petri dishes for at least 20 seconds. The distance between the LED and the stethoscope membrane was such that the light cone illuminated the entire membrane surface. UVC exposure time was set to 60 seconds to achieve an exposure dose (fluence) sufficient to kill the microorganisms at a maximum distance of 23 mm, that is, the distance from the LED to the membrane perimeter.<sup>26</sup>

All sowings were done by the same technician from the university's Department of Molecular and Developmental Medicine, and the Petri dishes were read by the principal researcher and the technician. The results are expressed as colony-forming units per stethoscope membrane, each sown with 50  $\mu$ L of  $10^{-3}$  diluted

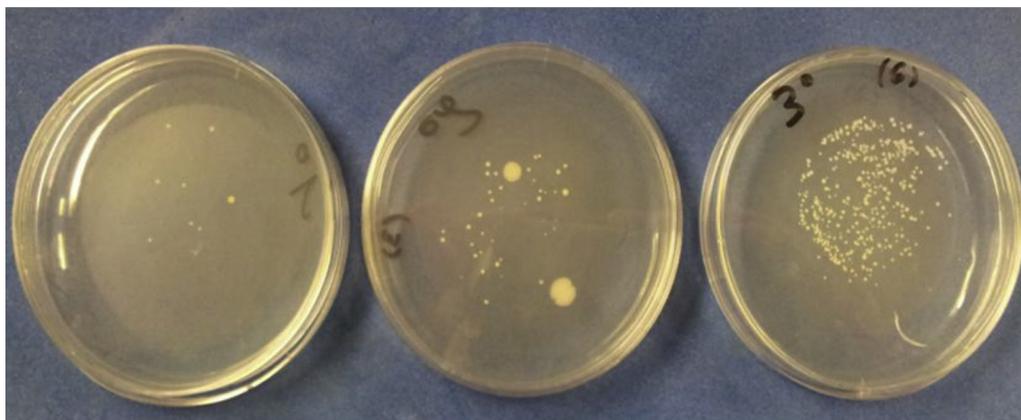


Fig 1. Petri dishes showing stethoscope contamination after use on 1, 2, and 3 patients respectively.

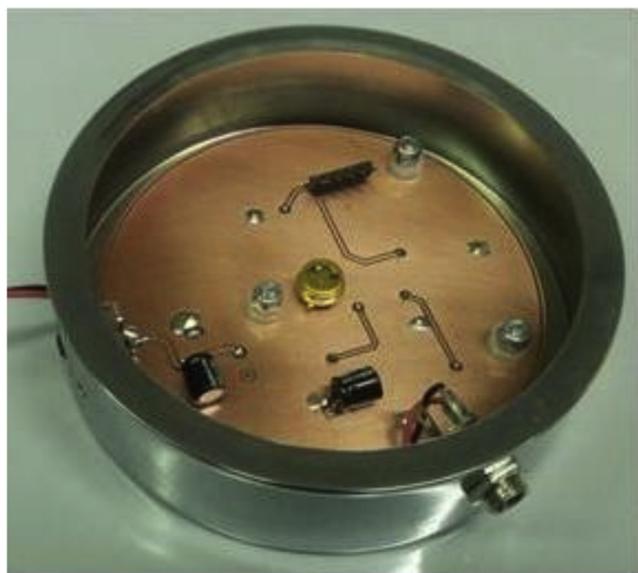


Fig 2. Device prototype (circular shape with the UV LED in the center).

suspension. The plates were read at 24 and 48 hours after sowing. We opted for a double count, at 24 hours to prevent vigorous bacterial growth from rendering some colonies uncountable at 48 hours, and at 48 hours to avoid missing bacterial species/colonies with slower growth.

#### Statistical analysis

We double-checked the database for input errors before statistical analysis. Descriptive statistics included median, interquartile range, and range of variation of all 4 species of bacteria. For each species, we calculated the total quantitative cfu count and the percent reduction between control cultures and treated stethoscope membrane cultures. This matched approach was taken to provide better control of confounders, minimizing their effects and increasing the reliability of the results. When cfu counts from treated membranes were above zero, statistical tests were conducted to verify differences with respect to controls. The Wilcoxon signed-rank test was used to reveal differences in

Table 1

Median, IQR, range of variation, percent reduction, and Wilcoxon *P* value, in cfu, between control cultures and cultures from UVC-treated stethoscope membranes for the 4 bacteria studied

Bacteria	Median CFU	IQR	Range of variation in CFU	Percent reduction in CFU	<i>P</i> value
<i>Staphylococcus</i> spp					
Control	56	51-64	49-67	—	
Exposed to UVC	7	5-8	5-12	87.5	<.01
<i>E coli</i>					
Control	35	27-43	24-46	—	
Exposed to UVC	2	1-3	1-4	94.3	<.01
<i>Pseudomonas</i> spp					
Control	39	38-41	38-42	—	
Exposed to UVC	2	2-3	1-5	94.9	<.01
<i>Enterococcus</i> spp					
Control	228	189-261	176-272	—	
Exposed to UVC	33	25-36	18-38	85.5	<.01

bacterial contamination before and after UV disinfection, and the Kruskal-Wallis test was used to detect differences among the 4 bacteria studied.

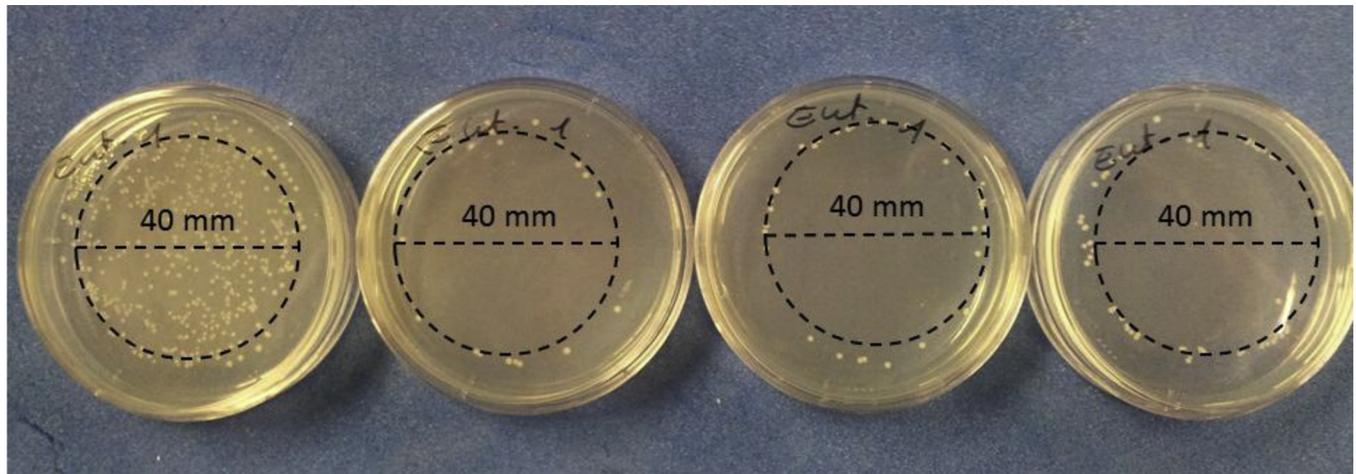
A statistical significance level of 99% ( $P < .01$ ) was applied for the inferential analyses. All statistical analyses were performed using Stata version 12.1 (StataCorp, College Station, TX).

## RESULTS

Median, interquartile range, and range variation of controls and UV-irradiated samples are reported together with the percent reduction in cfu for all 4 species of bacteria in Table 1. In all cases, the Wilcoxon signed-rank test showed significant differences in cfu count after UV disinfection ( $P < .01$  for all comparisons). The percent reductions were very high, all above 85%. As shown in Figure 3, residual bacteria were found around the periphery of the stethoscope membrane. No significant differences in percent reduction of cfu count were found among the 4 species of bacteria ( $P > .01$ , Kruskal-Wallis test).

## DISCUSSION

Almost 35% of epidemic nosocomial infections are attributed to direct contact between doctor and patient, 12% are associated with



**Fig 3.** Petri dishes in which stethoscope membranes had been placed in contact with the culture medium for at least 20 seconds. The first Petri dish on the left is the control. Residual bacteria remained only around the periphery of the stethoscope membranes after UVC treatment.

the environment, 11% are linked to biomedical/care instruments, and as many as 40% are related to unknown causes.<sup>27</sup> The large latter proportion could hide sources that are rarely disinfected owing to a perception of low contamination and a minor role in nosocomial infections.

Many routine hygienic practices, such as sterilization of surgical instruments, use of gloves and masks during medical procedures, and disinfection of the skin before injections, became established only after many years of hard work. In the nineteenth century, Ignác Fülöp Semmelweis introduced handwashing, a significant advance in hygiene practice. This procedure was initially disputed by many colleagues, despite its positive results in term of avoidable mortality. The same may be true for the cleaning of stethoscopes, an extension of health professionals' hands, which is not yet standard practice. Figure 1 shows stethoscope contamination after use on 1, 2, and 3 patients. At the third use without any cleaning procedure, contamination was high and similar to that found on control plates in the present laboratory experiment.

Also noteworthy is the fact that today doctors deal with informed patients, capable of assessing not only the outcome of hospital treatment, but also the medical and nursing care that they receive. If the quality of service falls short of expectations, patients may take legal action for damages caused by bad practice or omissions, such as poor standards of cleanliness or sterilization.<sup>28,29</sup> Elimination of hospital infections is in the best interest not only of doctors and patients, but also of hospitals themselves, to reduce the cost of extended hospital stays for additional treatments.<sup>3</sup>

Our present results show that it is possible to disinfect stethoscope membranes with UVC emitted by a LED. The percent reduction in cfu was between 85.5% and 94.9% (0.84-1.29 log<sub>10</sub> reduction) for all 4 bacteria tested, and considering the magnitude of the differences detected, the sample size ensured sufficient statistical power (>80%). We also calculated 99% confidence intervals, to reduce the uncertainty of detecting statistically significant differences in sample comparisons.

Previous studies also have demonstrated that UVC light can be an effective and economical technique for disinfecting surfaces and environments in general. Depending on the bacteria, exposure dose and time, distance between the light source and its target, and surface characteristics, reductions in cfu count ranging from <1 log<sub>10</sub> to ~7 log<sub>10</sub> have been reported. For example, Nerandzic et al<sup>24</sup> reported that a hand-held far-UV radiation device delivering

a radiant dose of 100 mJ/cm<sup>2</sup> for ~5 seconds reduced the recovery of methicillin-resistant *S aureus* (MRSA) by 5.4 log<sub>10</sub> and of vancomycin-resistant *Enterococcus* (VRE) by 6.9 log<sub>10</sub>. Similarly, wheeled devices for environmental disinfection, based on pulsed xenon ultraviolet or pressure mercury bulbs, achieved a 1.85 log<sub>10</sub> reduction in MRSA and a 0.6-1.68 log<sub>10</sub> reduction in VRE, with killing efficacy dropping dramatically with increasing distance.<sup>20,23</sup> Those studies were conducted with devices that irradiate with high energy using conventional types of bulbs. Our research, based on the same physical approach to disinfection, is different by virtue of the LED source of UVC light, which provides good disinfection if properly used and is more eco-friendly than mercury bulb lamps. It has a low-energy starter, and its lifespan is not decreased by frequent switching on and off. In addition, LEDs can be inserted into narrow inaccessible spaces and can be controlled more accurately and safely.

Our results could be further improved by exposure times longer than 60 seconds and the use of a more powerful LED and a wider angle of illumination, such as 140°. In fact, these parameters could speed up and increase the disinfection/sterilization effect.

It also should be noted that our cfu reductions may be somewhat of an underestimate, owing to the large distance from the LED and the fact that in the laboratory experiment, the membrane was placed with its outer edge on a cardboard support that shielded it from irradiation (Fig 3). Another approach would be to use a 280-nm LED, which, although less biocidal, can emit higher radiation power. One advantage of the present method is that it is a physical rather than chemical method of disinfecting or sterilization. Microorganisms and spores may resist disinfectants in different ways and even develop resistance, whereas when used properly, UVC radiation produces sterilization of all exposed surfaces.<sup>25,30</sup> It is also a "green" or ecologically sound method, producing no residues or special waste products requiring disposal.

Our positive results suggest that further investigations should be conducted in a real environment. They also indicate the need for testing over a longer period to assess the impact on overall infection rates. The UVC LED should be promoted as a personal device to facilitate its use and promote a disinfection routine. All health professionals could carry one, attached to a coat pocket, for example, so that it can be quickly coupled to a stethoscope worn around the neck. The device could be equipped with a means to prevent hazardous or accidental emission of UVC light; for example, when the head of the stethoscope is not properly

coupled to the device, sensors could detect it and interrupt emission of UVC light.

It was observed that microbes under the rim of the stethoscope membrane survived the sterilization treatment, because UVC light did not reach the part of the stethoscope membrane in contact with the device. This is a critical point that cannot be addressed by any device or technique. The only solution is to take the stethoscope apart for sterilization and then reassemble it. Although the risk of bacterial contamination of the rim is much lower than that of the main surface of the stethoscope membrane, it does exist; however, regular disinfection of the stethoscope after every use could also reduce the presence of bacteria under the rim.

Another potential limit of this physical method, negligible in the present context, is that prolonged and repeated exposure to UVC radiation can alter substrates.<sup>26</sup> This is also true of alcohol-based disinfectants, however.<sup>31</sup> Clearly, disinfection of stethoscope membranes cannot be avoided to promote a longer working life. The problem is solved by the use of more recently developed materials, such as membranes coated in polymers resistant to UV radiation.<sup>26</sup> Periodically replacing the membrane is an easy and economical solution. In addition, the UVC light tested had a lighting power of 300  $\mu$ W; substrate color changes occur after long, continuous exposures and at highlighting power, which were not the case in the present study.

Faced with the high costs of nosocomial infections, Western countries, especially the United States, are working to contain costs.<sup>4</sup> The US Patient Protection and Affordable Care Act introduced payment for performance, which regulates payment with expected outcome, penalizing/incentivizing on the basis of the quality and efficiency of service.<sup>32,33</sup> Health care centers, via their health management offices, can use risk management as cover, providing greater security to patients and cost savings by reducing the enormous costs of hospital infections, which can be prevented in a significant percentage of patients. Managing clinical risk in the context of hospital infections requires hygiene and antiseptic measures, barrier measures under standard conditions and for specific isolation, procedures to contain the spread of infectious agents, and implementation of an antibiotic policy.

The aim of the present study was to demonstrate the application of a novel technique for disinfecting stethoscope membranes. This technique could directly reduce (albeit perhaps to a limited degree) hospital-acquired infections. Displaying the proposed device on the coats of health care personnel also could serve as a reminder for good hygiene practice, indirectly reducing infections.

## CONCLUSION

UVC LED technology will become more widely used to sterilize medical devices and will revolutionize approaches to disinfection and sterilization. The prototype that we tested is an innovative, practical, and effective device able to disinfect the stethoscope membrane. Further research in a real context is needed to confirm these encouraging results. The device was engineered to disinfect stethoscope membranes, but the technology could be used for other small instruments requiring disinfection/sterilization, such as needles, barbers' equipment, and beauticians' tools.

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