



Hosting the Unwanted: Stethoscope Contamination Threat

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Authors' contributions

This work was carried out in collaboration between all authors. Author GM conceived and designed the study, acquired data, carried out the data analysis, drafted the article, did critical revision of the manuscript for important intellectual content. Author EC carried out the data analysis, collaborated in drafting the article. Author SB conceived and designed the study, collaborated in drafting acquired the article. Author CR acquired data, collaborated in drafting the article. Author NN conceived and designed the study, did critical revision of the manuscript for important intellectual content. Author LM conceived and designed the study, collaborated in drafting the article. Author LT conceived and designed the study, collaborated in drafting the article. Author DL conceived and designed the study, did critical revision of the manuscript for important intellectual content. Author PM conceived and designed the study, did critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Stethoscopes represent a vehicle of bacteria and other microorganisms and may play a role in the spread of health-care associated infections (HAIs). We aimed to evaluate the contamination levels of stethoscopes before and after use of a disinfecting technique (DT).

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Study Design: Matched cross-over study.

Place and Duration of Study: The study was conducted in July 2012 and involved three hospitals in Siena Province (Italy). Two were public hospitals with about 750 and 140 beds, and the other was private with 40 beds.

Methodology: We evaluated: i) contamination on 74 shared and non shared stethoscopes; ii) bacterial load before and after use of a DT. Total bacterial count (TBC) at 36°C and 22°C, *Staphylococcus* spp., molds, *Enterococcus* spp., *Pseudomonas* spp., *Escherichia coli* and total coliforms bacteria were evaluated. Mann Whitney and Wilcoxon tests were used for comparisons ($p < 0.05$).

Results: Before DT, 49 stethoscopes were positive for TBC at 36°C, 48 for TBC at 22°C, 40 for *Staphylococcus* spp., 18 for methicillin-resistant *Staphylococcus aureus*, 33 for coliforms (9 for *Escherichia coli*), 5 for *Enterococcus* spp. and 2 for molds. After cleaning, the percentage reduction in CFUs was close at 100% in most comparisons. Shared stethoscopes proved to be less contaminated than non shared ones ($p < 0.05$).

Conclusion: Our results suggest that stethoscopes may be potential vehicles of HAIs. The DT was effective in reducing bacterial contamination.

Keywords: Stethoscope; health care-associated infections; hospital, medical devices; hygiene.

ABBREVIATIONS

HAI: Health-care associated infections; DT: Disinfecting technique; TBC: Total bacterial count; CFU: Colony forming unit; MRSA: Methicillin-resistant Staphylococcus Aureus.

1. INTRODUCTION

Health care-associated infections (HAIs), also referred to as "nosocomial" or "hospital" infections, are contracted in hospitals or other health care facilities without being present or incubating at the time of admission. They can affect patients in any type of care setting and can also appear after discharge. HAIs are the most frequent adverse event of health care [1]. Hospital infections may be caused by any agent, including bacteria, fungi and viruses, as well as other less common types of pathogens. They represent a significant cause of morbidity and mortality and may increase health care costs [2,3]. Most involve the urinary tract, bloodstream, surgical sites and respiratory tract. They are also a considerable problem for certain categories of patients, such as those with immune deficiency or suppression, intensive care patients, chemotherapy patients, recipients of organ transplants, diabetics and so forth [4,5].

For primary prevention it is essential to identify reservoirs of microorganisms that cause nosocomial infections. Hands are the main sources, followed by medical devices, such as: catheters, ventilators, endoscopes, sphygmomanometers, otoscopes, thermometers, stethoscopes, computer keyboards etc [6-9]. Practices such as hand-washing and barrier protection remain the simplest and most important infection-control measures [4,10,11]. Stethoscopes are probably the most common medical device used by physicians/health professionals and they are used in close contact with patients' skin. Several studies have shown that stethoscopes are important vectors of infection [3-5,12-19], while other studies have investigated microbial contamination on stethoscopes [9,20,21]. Other researches have examined the ability of certain products to decrease microbial contamination [3,22].

Considering all these aspects, the education and sensitization of young health care providers to use of disinfecting techniques remain important. The aim of this study was to evaluate contamination levels of stethoscopes before and after use of a disinfecting technique (DT).

2. MATERIALS AND METHODS

2.1 Settings

A matched cross-over study involving three hospitals in Siena Province (Italy) was conducted in July 2012. Two were public hospitals with about 750 and 140 beds, and the other was private with 40 beds. To represent the heterogeneity of hospital departments and staff, the following hospital units were selected: intensive care, operating theatres, emergency units and medical units such as cardiology. These units provided different scenarios. Intensive care units have doctors/nurses who follow strict protocols and hygiene is a high priority. Patients may be unconscious and are generally critical, some with immunodeficiency or infections. Operating theatres are designed and operated to have a low contamination load. Emergency units have a very high volume of patients and many doctors/nurses participate in daily activity, making hygiene heterogeneous. Medical units are places where patients have contact with doctors and visitors.

Before the study began, meetings were held between the hospital management and the principal researcher. This was necessary to explain the project, establish the necessary contacts, and avoid any bias in conducting the study. It was considered important to avoid bias caused by doctors/nurses knowing when the investigation would be run, as this might prompt changes in hygiene. It was also decided that stethoscope sampling would be on the same day in each hospital, to prevent news of the study circulating and modifying hygienic behaviour.

2.2 Study Population

74 stethoscopes were analyzed, including shared (47) and personal (27) ones.

2.3 Disinfecting Technique

A putty compound, having a malleable elastic consistency was used, it adheres, removing dirt, and disinfects at the same time. These two characteristics distinguish this DT from traditional methods of cleaning and disinfection. The main sanitizing principle was ethanol (29%), in addition the compound contained purified water (51%), guar (6%), glycerine (7%), and minor quantity of other substances such as boric acid, colorants and odorants. This technique has a disinfectant efficacy, evidenced by studies conducted according to the indications in the U.S. Pharmacopeial Convention (USP), chapter <1072> "*Disinfectants and antiseptic*" and according to CONFARMA protocol number 229100911 A-B which is based on the: i) guideline of the Germany Society for Hygiene and Microbiology from 1991, ii) norms EN 1040 "*Chemical disinfectants and antiseptics Basic bactericidal activity Test method and requirements*" and iii) the norm EN 13697 "*Chemical disinfectants and antiseptics-Quantitative non-porous surface test for evaluation of bacteria and/or fungicidal activity of chemical disinfectants used in food, industrial. Domestic and institutional areas-Test method and requirements without mechanical action*" [23].

2.4 Data Collection

The experimental protocol required a first sample (swab) H(0) from one half of each stethoscope membrane before cleaning it with the product, and a second sample H(1) from the other half of the stethoscope membrane after cleaning. Samples were obtained by swabbing the stethoscope surface with sterile cotton pads for approximately 5 seconds per sample. Cleaning the stethoscope diaphragm with the product took approximately 20-25 seconds. All samples were obtained by the principal investigator who was escorted by a doctor of the hospital management. All doctors/nurses encountered during the visit to the units were informed by the principal researcher/hospital management doctor of the study and were asked if there was any problem about taking stethoscope samples. There were no objections. A new pack of product was used for every stethoscope. The following information was also recorded at the time of sampling: hospital ID, department ID, doctor/nurse ID. Records were indexed with a unique ID. The same ID was assigned to the pack of sanitizing product. All the information was recorded and stored in a database for future analysis.

2.5 Laboratory Analysis

Analysis was carried out in the Hygiene and Environmental Laboratory of the University of Siena, where the swabs were placed in 1ml of phosphate buffered saline, shaken in a vortex mixer and the liquid sown (0.1ml/plate) in Petri dishes containing: plate count agar (PCA) for total microbial load of mesophilic and psychrophilic microorganisms incubating at 36°C and 22°C, respectively; mannitol salt agar for *Staphylococcus* spp., *Pseudomonas* cetrimide for *Pseudomonas* spp., Slanetz & Bartley medium for *Enterococcus* spp., Brilliance *E. coli*/coliform spp. chromogenic medium for *Escherichia coli* and coliform bacteria, *Acinetobacter* base for *Acinetobacter* spp, and Brilliance methicillin-resistant *Staphylococcus aureus* (MRSA) MRSA2 medium for methicillin-resistant *Staphylococcus aureus* incubating at 36°C. *Clostridium difficile* agar base was supplemented with *Clostridium difficile* selective supplement and 7% defibrinated horse blood for *Clostridium difficile* spp, with incubation for 48 hours at 36°C in an anaerobiosis jar. Anaerobiosis was obtained using a gas generating kit.

All the sowings were made by the same technician of the Department of Physiopathology, Experimental Medicine and Public Health involved in the study. The Petri dishes were read by the principal researcher and the technician. The results were expressed as colony-forming units per swab (CFU/ 0.1ml). The plates were read 24 and 48 hours after sowing. All bacteria/mould counts were added to the previous database for further use.

2.6 Statistical Analysis

Data cleaning of the database was performed. Descriptive analysis (mean, standard deviation, median, interquartile range, minimum, maximum) of the data for all types of microbes/molds was performed at H(0) and H(1). To reveal differences in bacterial contamination before and after use of the product the Wilcoxon signed-rank test was used, while the Mann-Whitney test was used to detect difference between personal and shared stethoscopes and differences among three hospitals.

For each microorganism, the number of positive samples at H(0) and H(1) was counted and their percentages were calculated. The total quantitative CFU count of the 74 stethoscopes at H(0) and H(1) and the percentage reduction after use of the product (Table 1) were also calculated. Significance level was set $P < 0.05$. Stata ® SE, version 12.1, Stata Corp, College Station, Texas, USA software was used for the analysis.

Table 1. Descriptive statistics of stethoscope variables at H(0) and H(1): Number and percentage of positive samples, overall CFU count, percentage reduction in CFUs between H(0) and H(1), mean, standard deviation, median, interquartile range, minima and maxima

Culture medium	Time	Positive sample (%)	CFU total count *	% CFU reduction	Mean ^a	Standard deviation ^a	Median ^a	Interquartile RANGE (25%-75%) ^a	min ^a	max
PCA 36	H(0)	49(66,2)	5329	-99,8	108,8	228,1	15	5 to 41	1	1110
	H(1)	6(8,1)	10		1,7	0,5	2	1 to 2	1	2
PCA 22	H(0)	48(64,9)	5509	-99,9	114,8	274,7	10	3.5 to 46	1	1508
	H(1)	3(4,1)	5		1,7	0,6	2	1 to 2	1	2
<i>E.coli</i>	H(0)	9(12,2)	140	-100	15,6	23,7	5	2 to 11	1	71
	H(1)	0(0)	0		-	-	-	-	-	-
<i>Coliforms</i>	H(0)	33(44,6)	1739	-99,7	52,7	99,5	12	3 to 28	1	361
	H(1)	3(4,1)	5		1,7	1,2	1	1 to 3	1	3
<i>Enterococci</i>	H(0)	5(6,8)	15	-100	3	3,9	1	1 to 2	1	10
	H(1)	0(0)	0		-	-	-	-	-	-
<i>Staphylococci</i>	H(0)	40(54,1)	3188	-99,8	79,7	201,9	10	4 to 46	1	1003
	H(1)	4(5,4)	5		1,3	0,5	1	1 to 1.5	1	2
MRSA	H(0)	18(24,3)	233	-100	12,9	18,7	5	2 to 16	1	68
	H(1)	0(0)	0		-	-	-	-	-	-
Molds	H(0)	2(2,7)	2	-50	1	-	1	1	1	1
	H(1)	1(1,4)	1		1	-	1	1	1	1

Pseudomonas spp., *Acinetobacter spp.* and *Clostridium difficile* were not detected on the stethoscopes.

* summing all CFUs on the stethoscopes,
^aonly positive samples

3. RESULTS AND DISCUSSION

The Table 1 shows the variables at H(0) and H(1) with mean, standard deviation, median, interquartile range, minima, maxima, overall CFU count, percentage reduction in CFUs between H(0) and H(1), number of positive samples and their percentages for all stethoscopes. No samples contained *Pseudomonas*, *Acinetobacter* spp. or *Clostridium difficile* at H(0) or H(1). The CFUs' reduction is close at 100% in most comparisons. In cases the number of CFUs in H(1) did not correspond to 0, statistical tests were carried to highlight differences between pre- and post- cleaning. Significant differences were detected in the comparison for TBC at 36°C and 22°C, *Coliforms*, *Staphylococcus* spp. ($p < 0.001$). No differences emerged in the molds comparison ($p < 0.563$). Only 2/74 stethoscopes were contaminated with molds before the DT, and one after cleaning. Two stethoscope diaphragms also carried a visible film of undefined material (solidified gel or other dirt) which was only partly removed by the disinfecting technique (Fig. 1). These stethoscopes, at H(1), did not show microbial contamination.

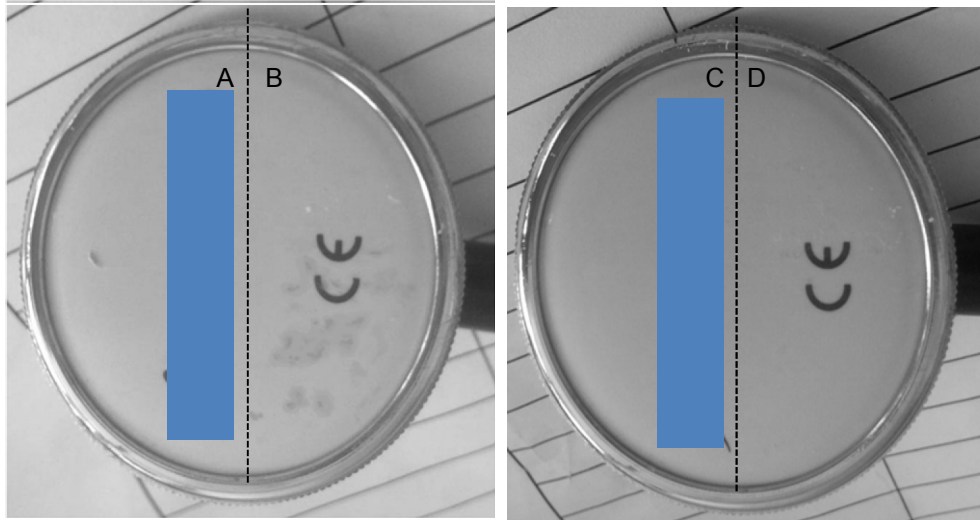
The comparison between physicians'/nurses' stethoscopes and shared ones showed significant differences in some cases. We recorded increased contamination of private stethoscopes by *E. coli* ($P = 0.0360$), *Enterococcus* spp. ($P = 0.0024$), *Staphylococcus* spp. ($P = 0.0164$) and MRSA ($P = 0.0060$). No significant statistical difference was found between shared and private stethoscopes for TBC at 36°C ($P = 0.2496$), TBC at 22°C ($P = 0.2235$) and coli form ($P = 0.3583$) contamination. The (Fig. 2) shows an example of cultures for MRSA from swabs taken before and after cleaning. No statistical differences were also found in contamination among the three hospitals ($P > 0.05$).

Stethoscopes are a universal tool of the medical profession and a potential source of nosocomial infections. They are used in direct contact with numerous patients every day and are often not routinely cleaned [24]. Several studies, as well as our own, have investigated microbial contamination of stethoscopes and, consequently, their role in the transmission of health-care-associated infections. Some of these infections are very dangerous, for example in the European Union, methicillin-resistant *Staphylococcus aureus* (MRSA) is frequently isolated in hospitals of Italy, Spain, Greece, Portugal and Great Britain [25].

In line with our results, the bacteria most commonly isolated are gram-positive cocci, especially *Staphylococcus* spp. [5,19,20,24,26]. Other bacteria frequently isolated are Enterobacteriaceae: *Enterobacter* spp., coliforms spp, *Citrobacter* spp., *Klebsiella* spp. and *Serratia* spp. being microorganisms considered by several studies [5,18,19,26-28]. We too found coli forms and *E. coli* in our samples; indeed, before cleaning, 40 out of 74 were positive for *Staphylococcus* spp., including 18 for MRSA, 33 for coliforms (with 3 *E. coli*), 5 for *Enterococcus* spp. and 2 for molds.

This and other studies have been conducted with the additional aim of determining the effectiveness of certain sanitizing techniques in reducing microbial contamination of stethoscopes. The compounds most commonly used for cleaning and disinfection of stethoscopes are ethyl and isopropyl alcohol or disinfectants based on them, non-ionic detergents and antiseptic soaps. The results showed that ethyl and isopropyl alcohol have similar effectiveness in reducing CFUs. Ethyl alcohol and ethanol-based cleansers reduce bacterial count by 92.8% and 96%, isopropyl and isopropyl-based compounds reduce it by 92.5% and 99%. However, repeated use of alcohol can dry out stethoscope rubber seals and damage the tubing.[29] Antiseptic soaps are less effective, reducing CFUs by about 74-75% [4,10,18,26,29,30]. After use of the disinfecting technique, the swab sometimes

removed some of the print on membranes of cheaper stethoscopes. This was probably due to the moist and adhesive nature of the product and the wiping action of the swab.



Left:

- A) first sample H(0): use of cotton swab, no use of DT
- B) second sample H(1): use of DT, before of cotton swab

Right:

- C) first sample H(0): use of cotton swab, no use of DT
- D) second sample H(1): use of DT, use of cotton swab

The disinfecting technique eliminated bacterial load, but did not always remove dirt from stethoscopes (see part B)

Fig. 1. Stethoscopes pre and post use of disinfecting technique

The cleaning product contains about 30% ethyl alcohol and has elastic consistency that attaches to and removes dirt. Both features disinfect. Our study demonstrates that after cleaning the percentage reduction in CFUs in all samples was 99.8% for TBC at 36°C and 99.9% for TBC at 22°C. These values are larger than the percentage reductions obtained in the surveys mentioned above. This disinfecting technique also determined a 99.7% reduction in *coliforms*, 99.8% in *Staphylococcus* spp., and 100% in *E. coli*, *Enterococcus* spp. and MRSA at H(1). Molds showed a different pattern, decreasing from two to one positive sample. This apparent ineffectiveness of the technique for molds could be due to a relative initial absence of molds on the stethoscopes. To test the disinfecting technique for molds, greater initial mould contamination is necessary. The tested product does not wet the article to be cleaned, dispensing with a drying step.

The Healthcare Infection Control Practices Advisory Committee recommends that stethoscopes be disinfected when visibly soiled and on a regular basis but does not specify what constitutes a regular schedule. There is no consensus about the appropriate frequency of cleaning [31]. Thus the cleaning of stethoscopes by healthcare professionals varies in frequency. A study conducted in 2001, evaluated stethoscope cleaning by 150 personnel and showed that: 49% cleaned them daily and 7% admitted to never cleaning them [29]. A survey of 1382 health care workers in 2012 showed that only 24% disinfected their

stethoscopes after every use, 32% cleaned them many times per day but not after every use, 11% cleaned them weekly and 3.8% never cleaned them [17].

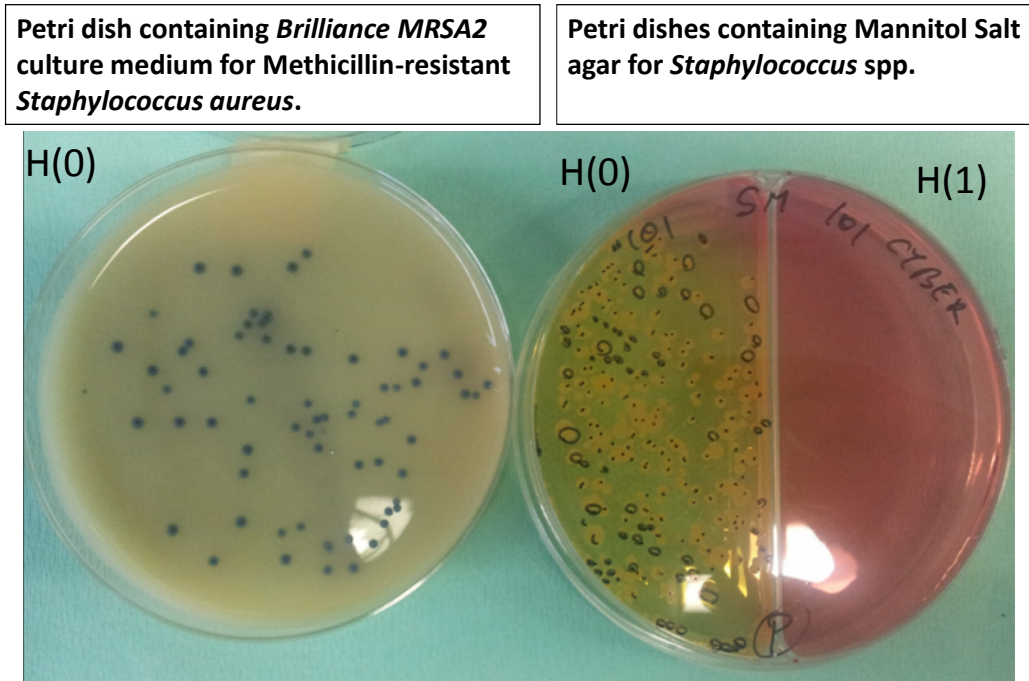


Fig. 2. Example of cultures of swabs from stethoscopes taken pre and post cleaning

We also compared microbial contamination of stethoscopes of physicians/nurses and shared stethoscopes and found some significant statistical differences. We recorded greater contamination on non-shared stethoscopes for: *E. coli*, *Enterococcus* spp, *Staphylococcus* spp. and MRSA. Since health care professionals presumably use their own stethoscopes more often than shared ones, the former are more likely to be contaminated. Other reasons for greater contamination of personal stethoscopes could be that shared stethoscopes are subject to established hygiene practices. In fact, we found that in intensive care units, standard protocols require the disinfection of stethoscopes which are placed at every bedside. Other departments may also follow this procedure. On the contrary, a study by Whittington et al. conducted in an intensive care unit showed that the diaphragms of bedside stethoscopes had greater bacterial contamination than personal ones, though the latter are more frequently contaminated by pathogenic bacteria [22]. This could sustain our hypothesis that personal stethoscopes are used with greater frequency than shared ones and are therefore more often colonized by pathogenic bacteria.

Although our study involved different departments, the overall number of stethoscopes was small compared to other studies [18,21]. It would be useful to follow this population for a longer period of time in order to highlight the effects of continued use of the disinfecting technique on bacterial contamination. It would also be interesting to know the capacity of the

technique to keep microbe concentrations low with repeated use and to test how long the effect lasts.

Our results indicate that educational programmes on disinfection procedures for doctors are important, especially for young staff. They help make health personnel more aware of these aspects, often overlooked or considered marginal. Habits acquired early in professional life are more likely to last.

It would be also useful to calculate the attributable risk of nosocomial infection caused by stethoscopes. This information, linked to hospital expenses, would infer savings in health care costs.

4. CONCLUSION

The results of the present study suggest that the disinfecting technique was effective in reducing stethoscope microbiological load, however its efficacy should not be a reason to neglect standard hygiene and cleanliness practices. Stethoscopes may also be disinfected by simple traditional methods, such as swabbing with sodium hypochlorite which normally eliminates bacteria.

CONSENT

The study did not involve patients, so the consent has not been applied. However we were authorized by the medical administrations of Siena teaching hospital "Le Scotte", the Rugani Clinic and Alta Val D'Elsa Hospital in Siena Province Italy.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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