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ORIGINAL ARTICLE

Aseptic Barriers Allow a Clean Contact for Contaminated Stethoscope Diaphragms

Rajiv Vasudevan; Ji H. Shin; Jessica Chopyk, PhD; William F. Peacock, MD; Francesca J. Torriani, MD; Alan S. Maisel, MD; and David T. Pride, MD

Abstract

Objective: To determine whether a single-use stethoscope diaphragm barrier surface remains aseptic when placed on pathogen-contaminated stethoscopes.

Methods: From May 31 to August 5, 2019, we tested 2 separate barriers using 3 different strains of 7 human pathogens, including extended-spectrum β-lactamase–producing *Escherichia coli,* methicillin-resistant *Staphylococcus aureus,* and vancomycin resistant *Enterococcus faecium.*

Results: For all diaphragms with either of the 2 barriers tested, no growth was recorded for any of the pathogens. Stethoscopes with aseptic barriers remained sterile for up to 24 hours. These single-use barriers also provided aseptic surfaces when stethoscope diaphragms were inoculated with human specimens, including saliva, stool, urine, and sputum.

Conclusion: Disposable aseptic diaphragm barriers may provide robust and efficient solutions to reduce transmission of pathogens via stethoscopes.

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Health care–associated infections (HAIs) pose a significant health risk to acute-care patients,[1] especially when involving susceptible or immunocompromised hosts.[2,3] According to the Centers for Disease Control and Prevention, there were an estimated 687,000 documented HAIs within the United States in 2015, responsible for approximately 72,000 deaths.[4] These HAIs lead to significant increases in lengths of stay and hospital costs.[5] Annual direct health care costs attributed to HAIs in the United States are estimated to be between $28 billion and $45 billion.[6] Hand hygiene interventions have been used extensively to reduce the transmission of pathogens responsible for HAIs[7-10] because physical contact represents the primary means by which providers examine their patients and thereby potentially introduce cross-contamination and transmit pathogens. However, the stethoscope is used in the cardiopulmonary assessment of nearly every patient but has never been effectively targeted for infection prevention interventions.

Stethoscopes harbor similar levels of microbial colonization as one's hand, warranting being called the "third hand" of the physician.[11,12] Several pathogens have been discovered on stethoscope diaphragms, including methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli,* vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa,* and *Clostridiodes difficile.*[11,13-16] When these bacteria colonize stethoscope diaphragms, they may be transmitted to the patient's skin after as few as 3 seconds of contact.[17] Typical auscultation procedures involve several minutes of contact with the skin and therefore present sufficient opportunities for pathogen transfers.

Studies have been performed to characterize stethoscope hygiene in clinical settings to identify obstacles that reduce proper hygiene practices. Hygiene rates are estimated to vary from 10% to 80%[18-22] when assessed by surveys, but the wide variation in these results likely reflects reporting biases.[23] Direct observational studies report much lower stethoscope hygiene, in the range of 11% to 16%.[24,25] Cited barriers to hygiene performance include lack of time and poor access to disinfecting materials. These barriers persist despite most providers being aware that

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stethoscopes can be colonized with microbes and potentially serve as a vector for transmission.[26]

The Centers for Disease Control and Prevention guidelines classify the stethoscope as a noncritical medical device (ie, in contact with intact skin, no bodily fluids), for which they recommend cleaning for at least 1 minute after each patient interaction using an alcohol- or bleach-based disinfectant.[27] However, recent literature suggests that common disinfectants might not completely eliminate contaminating bacteria.[28] For example, *Enterococcus faecium* demonstrates increasing tolerance to 70% isopropyl alcohol solutions[29] and may continue to colonize stethoscopes despite cleaning. This emerging literature highlights a need for alternatives to alcohol- and bleach-based stethoscope disinfectants.

In this study, we evaluated 2 different single-use aseptic barriers to determine their efficacy in preventing transmission of microbes that colonize stethoscope diaphragms. Our goals were to: (1) determine whether these diaphragms could prevent the transmission of different types of microbes, including bacteria and yeasts; (2) examine the period over which these barriers might prevent transmission; and (3) determine whether the barriers prevent transmission of microbes from human specimens.

Human subject involvement in this study was approved by the University of California, San Diego Administrative Panel on Human Subjects in Medical Research and was certified as category 4 exempt, which does not require informed consent on behalf of the study participants.

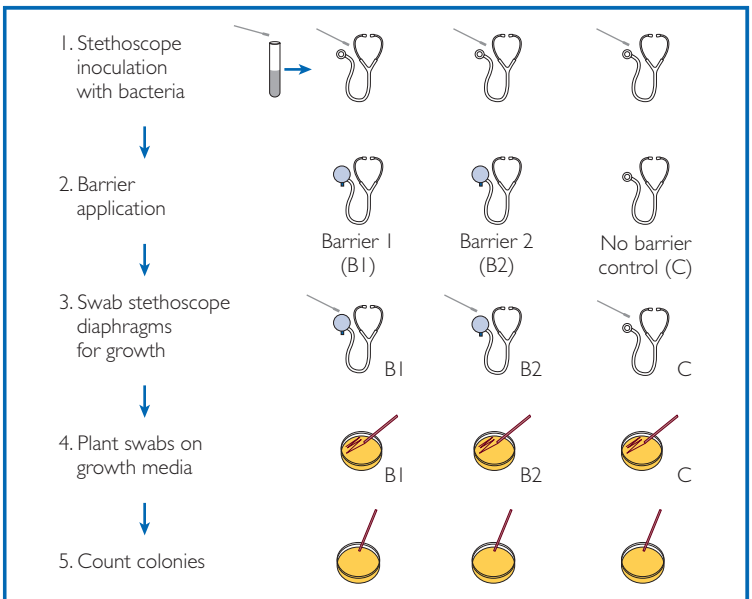

FIGURE 1. Work flow for determining barrier efficacy in preventing transmission of microbes via stethoscope diaphragms.

METHODS

Cultivation of Microbes

The bacteria used in this study were recovered from the University of California San Diego Health Clinical Microbiology Laboratory using specimens with documented culture results using standard-of-care clinical microbiology procedures. Saliva specimens were previously collected from healthy individuals. Stool was obtained from individuals being evaluated for the presence of *Helicobacter pylori* stool antigen. Urine was collected from individuals with documented urinary tract infections, and sputum was collected from individuals with pathogens, including *S aureus*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *P aeruginosa* in their sputum samples.

The identity of each microbe was confirmed using the Bruker matrix-assisted laser desorption time-of-flight assay using the Research Use Only Database (Brucker Scientific). Antibiotic susceptibilities for bacteria were performed on the Becton Dickenson Phoenix M50 machine (Becton, Dickinson and Co), which uses the microbroth dilution technique.[30] Cultures of each pathogen were grown in brain-heart infusion broth to approximately 10^8 colony-forming unit (CFU)/mL and diluted to 10^6 CFU/mL before use in this study. The *Candida albicans* was diluted to 10^7 CFU/mL because in preliminary studies, few colonies could be obtained at 10^6 CFU/mL. Each of these organisms was an aerobe or facultative anaerobe and was capable of growing under aerobic conditions; the exceptions were the anaerobic *Bacteroides* species, which were chosen instead of *C difficile* to test the barriers' effectiveness against anaerobes. For the *Bacteroides* species, we inoculated directly from swabs of the plate cultures rather than using the broth dilutions.

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Evaluation of Aseptic Barriers With Human Pathogens

From May 31, 2019, until August 5, 2019, three replicate clinical strains of 6 bacteria species and 1 yeast were prepared (n=21 samples). Four of these species were common multidrug-resistant (MDR) pathogens: MRSA, extended-spectrum β-lactamase–producing *E coli* (ESBL), VRE, and MDR *P aeruginosa.* Three other microbes were also tested: *Staphylococcus epidermidis, C albicans,* and *Bacteroides* species. Only fresh cultures (growth <16 hours) were used in this study. We grew the cultures as detailed and used the dilutions to inoculate the microbes onto 3 stethoscope diaphragms (Medline Industries, Inc) per strain (n=54), testing 2 medical-grade aseptic barrier tapes (Aseptiscope Inc) and 1 no-barrier control.

We used Copan FLOQswabs (Copan Diagnostics Inc), dipped them into the vortexed diluted cultures, and used them to inoculate the stethoscope diaphragms (Figure 1). The diaphragms were allowed to dry for 10 minutes, then the barriers were subsequently applied. The diaphragms were then swabbed with a clean swab, placed into ESwab media, and planted on blood agar, chocolate agar, and MacConkey agar (gram-negative selective) plates using the Copan WASP automated planting system (Copan Diagnostics Inc). Cultures were incubated at 37°C for 24 to 48 hours, and colonies were counted manually.

For *Bacteroides* species, the plates were swabbed and the stethoscope diaphragms were directly inoculated from these swabs and allowed to dry. The diaphragms were then swabbed using a clean swab and placed into ESwab media and planted on the media types described previously. These plates were incubated for 48 hours anaerobically using an Anoxomat Mark II System (Advanced Instruments Inc), and colonies were counted manually.

Barrier Efficacy With Human Samples

We tested the potential for pathogenic transmission through the barrier from human patient specimens. Four specimens were used: saliva previously collected from healthy individuals, stool from patients being evaluated for *H pylori* stool antigen, urine from patients with documented urinary tract infections, and sputum from patients with *S aureus, M catarrhalis, H influenzae*, and *P aeruginosa* pneumonia or bronchitis. Microbe identification and antibiotic susceptibilities were performed using the mentioned techniques. Copan FLOQSwabs were dipped directly into each specimen and used to inoculate the stethoscope diaphragms according to the same protocol as bacterial isolates.

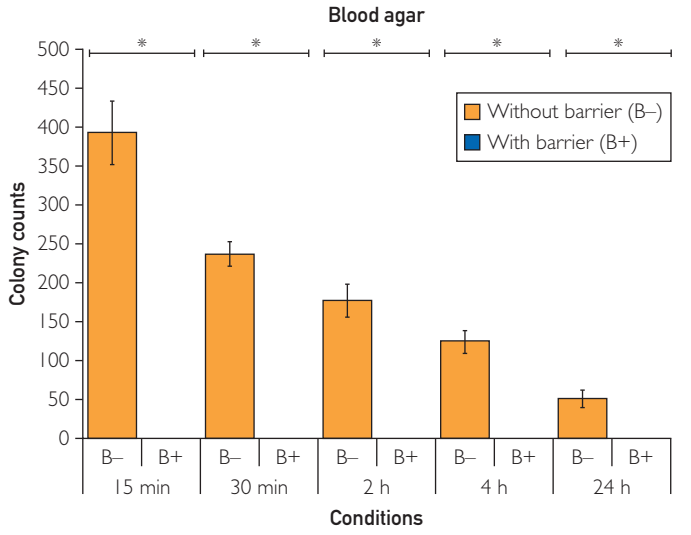
Longitudinal Effectiveness of Aseptic Barriers

In the event that a barrier is applied for an extended period, we sought to evaluate its ability to provide aseptic surfaces over time. We diluted fresh cultures of *S epidermidis* (a common bacterium found on the skin) to 10^7 CFU/mL and inoculated the stethoscope diaphragms with a swab of the culture. After the diaphragms were dried, stethoscopes were either kept without barriers (n=3) or had a barrier placed, with both barriers being tested (n=6). After 5 separate trials with periods of 15 minutes, 30 minutes, 2 hours, 4 hours, and 24 hours, we used FLOQswabs to swab the diaphragms and planted the cultures on blood agar and chocolate agar plates using the WASP automated planting system. Cultures were incubated aerobically at 37°C for 24 hours and colonies were counted manually.

Controls in these experiments involved direct inoculation of the bacterial preparations onto media using the WASP automated planting system. For the individual bacteria and yeast experiments, we tested 3 replicates of 2 aseptic barriers for a total of 6 total replicates. We combined the replicates because there were no microorganisms recovered from any of the barriers. For experiments using specimens of stool, saliva, sputum, and urine, we tested 5 replicates on 2 different barriers for a total of 10 replicates. An additional control was performed by swabbing barriers with microbes to discern whether barriers provide a physical hindrance to microbe transmission or they had direct antimicrobial activity.

Statistical Analyses

Statistical analyses were performed in RStudio, version 1.0.153. Differences in colony counts between stethoscope diaphragms with and



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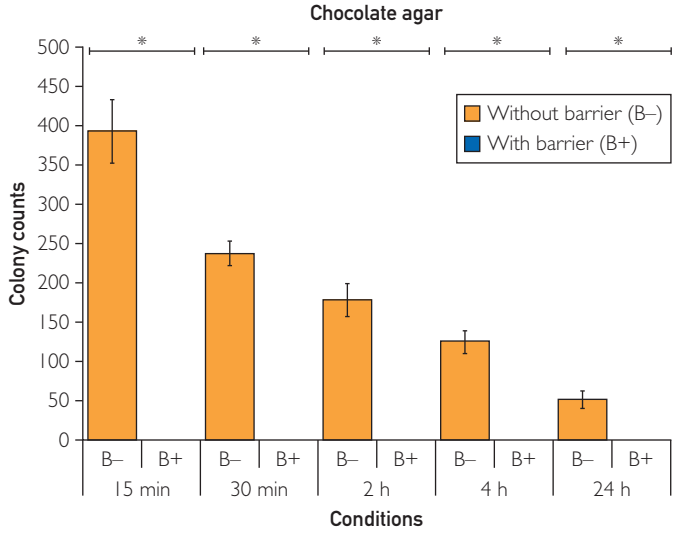

B

FIGURE 2. Bar charts represent mean colony counts ± standard error for *Staphylococcus epidermidis* on stethoscope diaphragms across time. Colonies were counted for *S epidermidis*—colonized diaphragms without disc barriers (B—) and stethoscope diaphragms with disc barriers (B+). The y-axis represents mean colony counts, and the x-axis represents the conditions and times that *S epidermidis* was placed on diaphragms before plating. Colony counts on (A) sheep blood agar plates and (B) chocolate agar plates. For B+ stethoscope diaphragms, no colonies were recovered on any plate types regardless of time period. *Values that are statistically significant ($P \leq .05$) comparing colony counts with (B+) and without (B—) disc barriers using a 1-tail *t*-test.

without barriers were determined using 1-tail *t* tests. In addition, analysis of variance with post hoc Tukey honestly significant difference tests were used to compare colony counts from stethoscope diaphragms with barriers, without barriers, and with barrier controls.

RESULTS

Longitudinal Assessment of Aseptic Barriers

We evaluated the ability of the barriers to remain aseptic at several time points up to 24 hours. We were able to cultivate *S epidermidis* from the diaphragms without barriers reliably at 15 minutes, 30 minutes, 1 hour, 4 hours, and 24 hours (Figure 2) and tested the difference in colony counts using a 1-tailed *t* test. At each time point, there was significant ($P \leq .05$) growth of *S epidermidis* on the stethoscope diaphragms without the barriers, but no growth on the diaphragms with the barriers.

Barriers Protect Against Aerobic/Facultative Anaerobic Pathogens

We tested the efficacy of the aseptic barriers against 5 bacteria and 1 yeast. We cultivated each of these pathogens on blood and chocolate agar plates, but also grew them on MacConkey agar, which is selective for gram-negative pathogens such as *P aeruginosa* and *E coli*. We observed identical trends on blood agar and chocolate agar plates (Figure 3A and B), on which there was significant growth of each of the 6 pathogens without the barriers, but no growth when the barriers were applied. Using analysis of variance with a post hoc Tukey honestly significant difference test, we found that these results were significant ($P \leq .05$) for 5 of 6 pathogens on blood agar and 4 of 6 pathogens on chocolate agar. On the MacConkey agar, only the *E coli* and *P aeruginosa* grew, but they demonstrated the same significant results observed on the other media types, on which there was pathogen growth without barriers, but none when barriers were present (Figure 3C). When microbes were placed on top of the barriers, they were capable of growing on the barrier surface in all circumstances, suggesting that the barriers provided physical but not directly antimicrobial activity against contaminating pathogens (Figure 3). These data demonstrate that aseptic barriers

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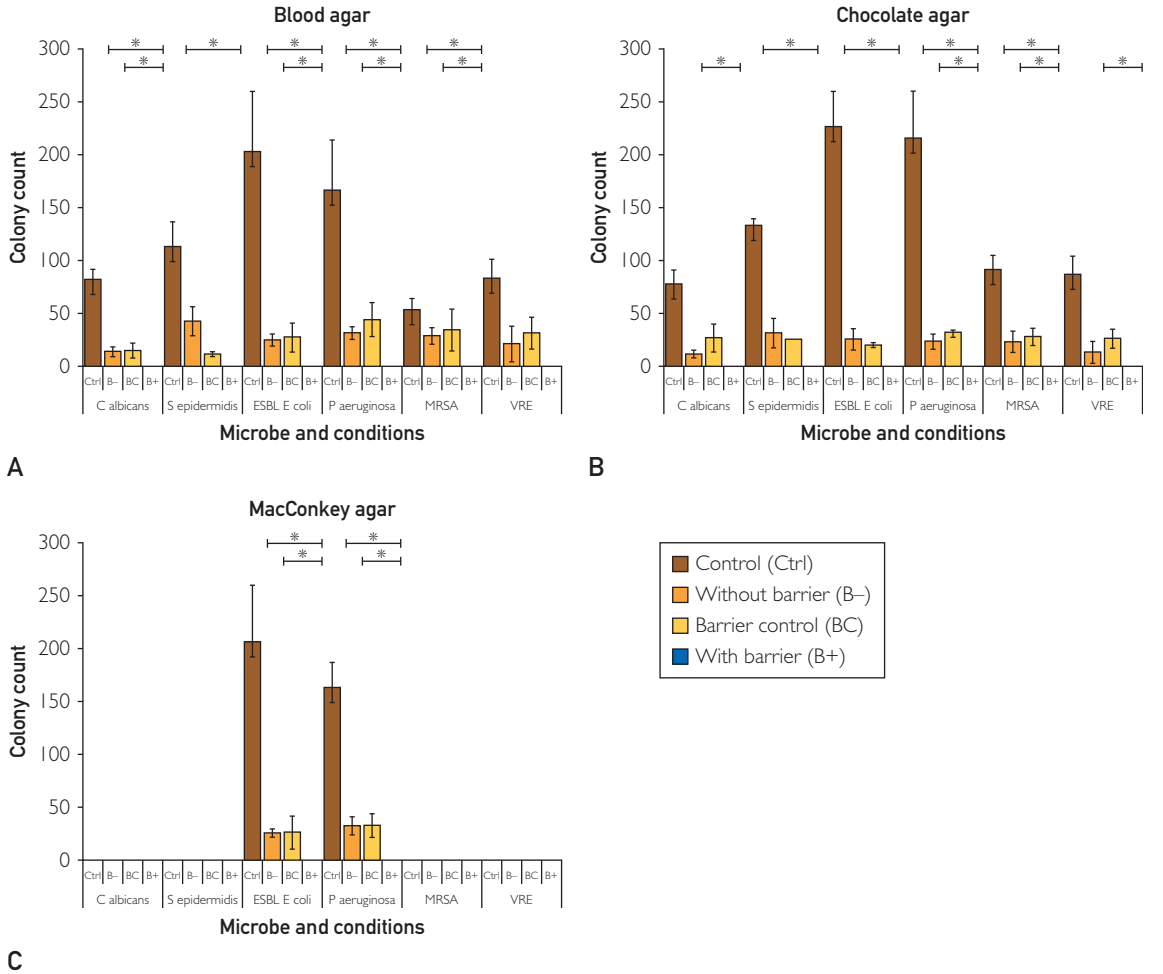

FIGURE 3. Bar charts represent mean colony counts ± standard error for microbes on stethoscope diaphragms. Colonies were counted for control (Ctrl) microorganisms (directly plated from broth), colonized diaphragms without disc barriers (B−), disc barriers colonized with microorganisms (BC), and stethoscope diaphragms with disc barriers (B+). The y-axis represents mean colony counts, and the x-axis represents the individual microbes and conditions by which diaphragms were treated. Colony counts on (A) sheep blood agar plates, (B) chocolate agar plates, and (C) MacConkey agar plates. *C albicans* = *Candida albicans*, *S epidermidis* = *Staphylococcus epidermidis*, ESBL *E coli* = extended-spectrum β-lactamase−producing *Escherichia coli*, *P aeruginosa* = *Pseudomonas aeruginosa*, MRSA = methicillin-resistant *Staphylococcus aureus*, VRE = vancomycin-resistant *Enterococcus faecium*. For B+ stethoscope diaphragms, no colonies were recovered for any microorganisms on any plate types. *Values that are statistically significant ($P \leq .05$) comparing colony counts with disc barriers (B+), without disc barriers (B−), and with barrier controls (BC) using analysis of variance with post hoc Tukey honestly significant difference.

provide an aseptic surface to reduce potential transmission of microbes that contaminate stethoscope diaphragms.

Utility of Barriers Against Anaerobes

We also tested whether barriers provided aseptic surfaces against stethoscope surfaces contaminated with anaerobic bacteria. We examined 3 separate species of *Bacteroides* in our evaluation. Each of these species grew well under anaerobic conditions on stethoscope diaphragms, but none grew in the presence of the barriers (Figure 4). Using a 1-tailed *t* test, colony counts were significantly greater ($P \leq .05$) without barriers. These results indicate that aseptic barriers may prevent transmission of anaerobic bacteria on stethoscope diaphragms.

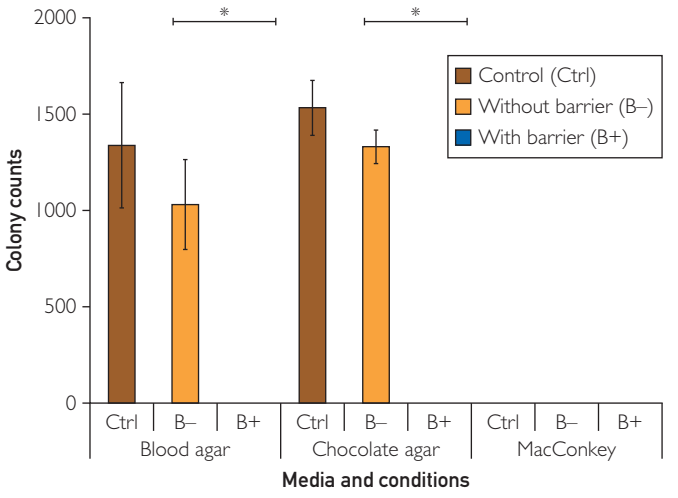

FIGURE 4. Bar charts represent mean colony counts ± standard error for *Bacteroides* species on stethoscope diaphragms. Colonies were counted for control (Ctrl) *Bacteroides* species (directly plated from broth), colonized diaphragms without disc barriers (B−), and stethoscope diaphragms with disc barriers (B+). The y-axis represents mean colony counts, and the x-axis represents media types and conditions by which stethoscopes were treated. For B+ stethoscope diaphragms, no colonies were recovered on any plate types. *Values that are statistically significant ($P \leq .05$) comparing colony counts with (B+) and without (B−) disc barriers using a 1-tail *t* test.

Evaluation of Aseptic Barriers With Human Specimens

We found that for saliva, colony counts from diaphragms without barriers were significantly greater ($P \leq .05$) than from stethoscopes with aseptic barriers (Figure 5A) for both the chocolate and blood agars. We identified the same significant ($P \leq .05$) trend when diaphragms were inoculated with stool (Figure 5B) for all media types tested. Similar results were obtained for urine (Figure 5C) and sputum (Figure 5D), on which no growth was recovered from diaphragms with barriers. However, the data for urine and sputum did not meet statistical significance secondary to the large variation that was observed in colony counts across the 5 different urine and sputum specimens tested.

In saliva, we identified only bacteria capable of growing on blood and chocolate agar plates (Figure 5A). These bacteria included various species of *Streptococcus*, *Rothia mucilaginosa*, and some oral *Neisseria* species (Table). In stool, we identified microbes that grew on all media types (Figure 5B). We found *Streptococcus gallolyticus*, various *Enterococcus* species, *Weissella cibaria*, and many *Enterobacteriaceae* family members, including *Klebsiella pneumonia*, *E coli*, *Raoultella ornithinolytica*, and *Citrobacter freundii*. Bacteria in the urine that grew on all media types (Figure 5C) included various *Enterococcus* species, *K pneumoniae*, *E coli*, and *P aeruginosa*. We also isolated bacteria from sputum-inoculated diaphragms on all media types (Figure 5D), including *Streptococcus parasanguinis*, *R mucilaginosa*, *Abiotrophia defectiva*, *Granulicatella adiacens*, *S aureus*, *M catarrhalis*, *H influenzae*, and *P aeruginosa*. These data indicate that aseptic barriers can prevent the transmission of a large variety of microbes from stethoscope diaphragms to patients.

DISCUSSION

We tested 2 separate barriers against a range of potentially transmissible antibiotic-resistant and pathogenic microbes to determine their efficacy in reducing pathogen transmission. Our results were comprehensive in that both barriers prevented the growth of anaerobes, antibiotic-resistant bacteria, yeasts, and body samples, including saliva, stool, urine, and sputum. While we specifically evaluated VRE, MRSA, ESBL *E coli*, and MDR *P aeruginosa* to demonstrate the potential role in reducing the transmission of antibiotic-resistant bacteria, we also tested other microbes in *S epidermidis*, *Bacteroides* species, and *C albicans* (Figures 3 and 4). None of these organisms grew in the presence of the aseptic barriers. We observed this same phenomenon from samples of saliva, stool, urine, and sputum in which microbes were unable to grow in the presence of barriers (Table; Figure 5). These results strongly suggest that barriers prevent the transmission of most if not all bacteria and yeasts that might contaminate stethoscope diaphragms.

Because it is now recognized that stethoscope hygiene is another weakness in hospital infection prevention practices,[13] we evaluated the role that aseptic barriers could play in reducing the transmission of microbes. We used medical-grade aseptic barriers that do not affect the subjective quality of auscultation while still providing physical protection from microbes. These barriers are

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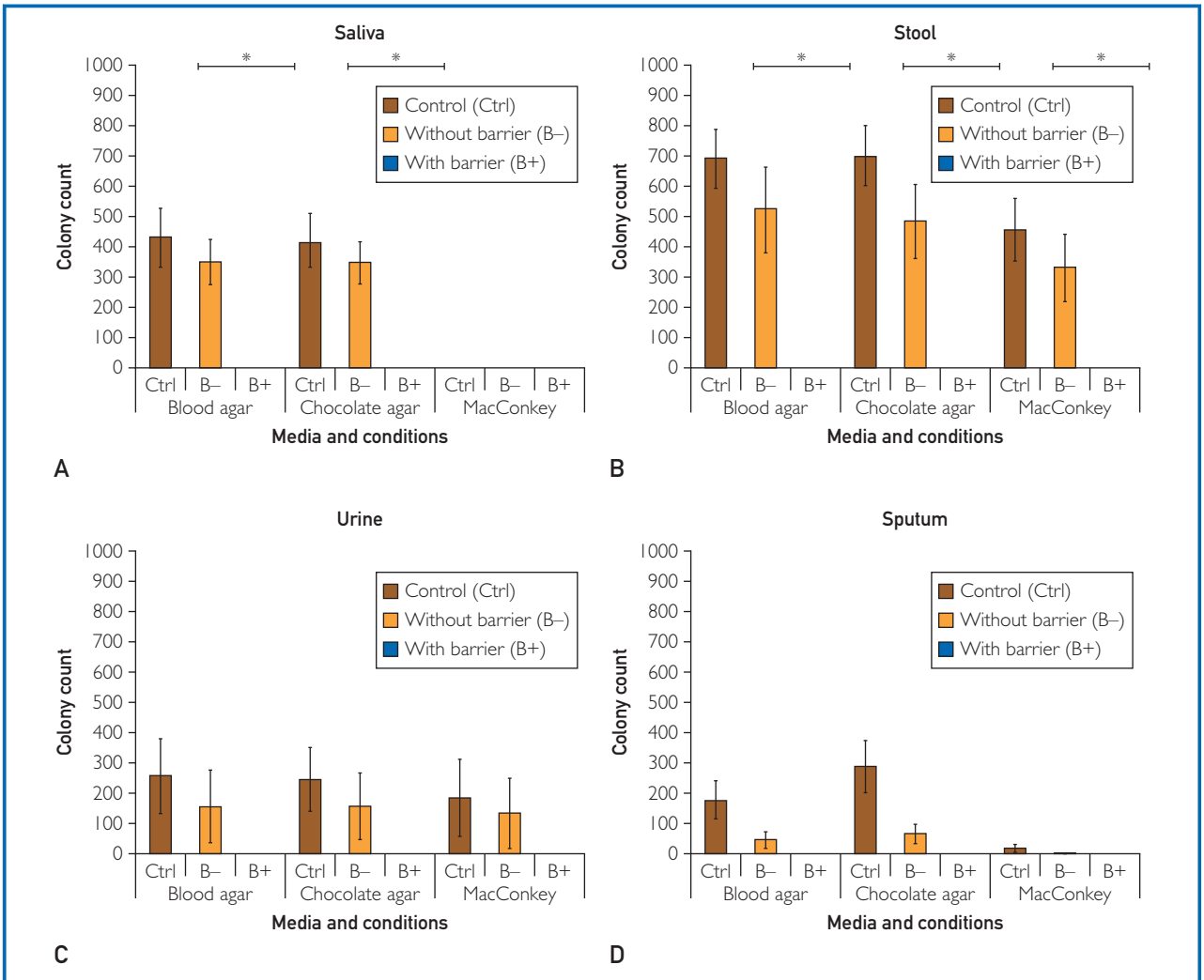

FIGURE 5. Bar charts represent mean colony counts ± standard error of bacteria from human specimens contaminating stethoscope diaphragms: (A) saliva, (B) stool, (C) urine, and (D) sputum. Colonies were counted for control (Ctrl) specimens (directly plated from the specimen), colonized diaphragms without disc barriers (B−), and stethoscope diaphragms with disc barriers (B+). The y-axis represents mean colony counts, and the x-axis represents media types and conditions by which diaphragms were treated. For B+ stethoscope diaphragms, no colonies were recovered for any specimen types on any plate types. *Values that are statistically significant ($P \leq .05$) comparing colony counts with (B+) and without (B−) disc barriers using a 1-tail *t* test.

single use and applied to the stethoscope diaphragm using a sterile hands-free dispenser just before evaluating the patient, thus ensuring hygienic patient contact similar to disposable gowns/gloves. Their potential benefits include reduced transmission of pathogenic and antibiotic-resistant microbes and potentially improved auscultation in the pulmonary and cardiac physical examination compared with the current standard of care in high-acuity settings in which single-patient stethoscopes are used.[31]

Although other solutions to stethoscope hygiene have been promoted, they have elicited ambiguous results. One study implemented visual reminders for stethoscope hygiene and alcohol-swab baskets in hospital wards and reported an increase in the rate of stethoscope cleaning from 34% to 59%.[32] Another implemented a similar protocol in addition to providing informational lectures to the medical staff and observed no compliance (0%) either before or after the intervention.[33]

TABLE. Microbes Recovered on Stethoscope Diaphragms

Saliva	Stool	Urine	Sputum
Streptococcus oralis			
Streptococcus parasanguinis			*S parasanguinis*
Streptococcus mitis			
	Streptococcus gallolyticus		
			Abiotrophia defectiva
			Granulicatella adiacens
Rothia mucilaginosa			*R mucilaginosa*
	Weissella cibaria		
			Staphylococcus aureus
	Enterococcus faecalis	*E faecalis*	
	Enterococcus avium	*E avium*	
	Enterococcus gallinarum		
Neisseria subflava			
Neisseria flavescens			
			Moraxella catarrhalis
			Haemophilus influenza
	Klebsiella pneumoniae	*K pneumoniae*	
	Escherichia coli	*E coli*	
	Raoultella ornithinolytica		
	Citrobacter freundii		
		Pseudomonas aeruginosa	*P aeruginosa*

Alternatively, stethoscope hygiene solutions have been described. One study evaluated the antimicrobial properties of a copper-alloy metal stethoscope, citing decreased levels of contamination; however, it did not mention the cost implementing such a change.[34] Another investigated a stethoscope UV-light case, but reported incomplete decontamination.[35] An additional study investigated an antimicrobial stethoscope coating.[36] Such a coating might select for resistant microorganism stethoscope colonization. Our study demonstrated the effectiveness of an aseptic diaphragm barrier without antimicrobial properties (Figure 3) to limit the formation and propagation of resistant pathogens. Because the barriers are disposable, are single-use, and work by providing a physical rather than antimicrobial barrier, they provide protection without the risk for resistant microorganisms. Although prior technologies have accomplished only a partial reduction in stethoscope contamination, our data support that the diaphragm barriers are capable of remaining aseptic against a variety of pathogens.

As we learn about the microorganisms that inhabit our bodies and environment, we find that microbes are constantly shared between individuals and their surroundings.[37] In the hospital environment, our level of concern should increase,[38] in part because many antibiotic-resistant and pathogenic microbes are in the hospital for which exposure to a susceptible host can have serious consequences. Thus, it is important to recognize the routes by which microbes are shared in the hospital and work to limit the potential for transmission.[39] Efforts to limit the transmission of MDR organisms (MDROs) include the use of contact precautions, strict hand hygiene, and wearing disposable gowns and gloves when they come into contact with patients who are colonized or infected with MDROs to limit the possibility of transfer to other patients.[40] This process is effective[41] but places a limitation on the physical examination auscultation via stethoscope. Health care providers (HPs) are provided with single-patient disposable stethoscopes when in contact with patients identified with an MDRO. Unfortunately, the audio quality of these stethoscopes is poor,[31] causing HPs to sometimes abandon their use in favor of using their own stethoscopes. This results in contamination of the HP's stethoscopes with MDROs that can be transmitted to other patients upon subsequent use.[17] In addition, one study found that single-patient stethoscopes can be colonized by MDRO bacteria such as MRSA.[22] This can put patients who are in contact precautions at significant risk for acquiring an HAI. Another important issue that contributes to the spread of pathogenic microbes is that many patients are colonized or infected with pathogens that are not yet known to their HPs.[42] Under these circumstances, HPs use their own stethoscopes and unknowingly transmit these microbes between patients, with transfer occurring when the contaminated stethoscope comes in direct contact with the patient.[17]

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CONCLUSION

Stethoscope diaphragm barriers may reduce the transmission of pathogens, including MDROs, during examinations. While most hospitals use disposable stethoscopes in the presence of MDROs, the quality of these products can significantly affect the quality of the physical examination. Single-use disposable barriers hold the promise of providing high-quality auscultation while significantly reducing the potential for pathogen transmission.

Abbreviations and Acronyms: **B−** = colonized diaphragms without disc barriers; **B+** = stethoscope diaphragms with disc barriers; **BC** = disc barriers colonized with microorganisms; **CFU** = colony-forming unit; **Ctrl** = control; **ESBL** = extended-spectrum β-lactamase–producing *Escherichia coli*; **HAI** = health care–associated infection; **HP** = health care provider; **MRSA** = methicillin-resistant *Staphylococcus aureus*; **MDR** = multidrug resistant; **MDRO** = multidrug-resistant organism; **VRE** = vancomycin-resistant *Enterococcus*

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