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Original Article

Oxygen nipple and nut (Christmas tree) adaptor contamination rates and decontamination with disinfecting wipes

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Abstract

Objective: Different manufacturers recommend different levels of disinfection for oxygen nipple and nut adaptors, also known as Christmas-tree adaptors (CTAs). We aimed to determine the bacterial contamination rates of CTAs before and after clinical use and whether disinfection wipes effectively eliminate bacteria from CTAs.

Methods: CTAs were swabbed for bacteria directly from the shipment box or after use in a medical intensive care unit to determine levels of contamination. CTAs were also inoculated in the laboratory with a variety of bacteria and disinfected with either 0.5% hydrogen peroxide (Oxivir 1) or 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol (Super Sani-Cloth), and the effectiveness of each wipe was determined by comparing the bacterial recovery before and after disinfection.

Results: CTAs exhibit low levels of bacterial burden before and after clinical use. Both disinfecting wipes were effective at removing bacteria from the CTAs.

Conclusions: Low-level disinfection of CTAs is appropriate prior to redeployment in the clinical setting.

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Oxygen nipple and nut adaptors, also known as "Christmas-tree adaptors" (CTAs) because of their design, allow small oxygen tubing to connect easily to oxygen flowmeters. CTAs do not come into direct contact with patients, and they are classified as noncritical devices according to the Spaulding classification system.[1] Several products are available, with varying disinfection recommendations by the manufacturer. Yale New Haven Health currently uses CTAs manufactured by Teleflex (Wayne, PA). Previously considered single use only, in September 2018, the Teleflex CTA disinfection requirements were updated by the manufacturer to require either to soak them in 70% isopropyl alcohol for 30 minutes, to steam sterilize them by autoclave, or to "utilize hospital validated cleaning protocol(s)" (Teleflex, letter to customers dated September 28, 2018). However, in March 2019, the hospital-validated cleaning protocol option was removed (Teleflex, letter to customers dated March 4, 2019). Alternatively, the disinfection instructions for CTAs manufactured by MES (Seguin, TX) allow for decontamination with a "hospital-grade disinfection" wipe.[2] Given that CTAs are noncritical and that similar products often have different disinfection recommendations, we decided to evaluate the effectiveness of low-level disinfection for Teleflex CTAs. We chose to study 2 different hospital-approved disinfectant wipes, 0.5% hydrogen peroxide (Oxivir 1, Diversey, Fort Mill, SC) or 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol (Super Sani-Cloth, Professional Disposables International, Woodcliff Lake, NJ). Both products are indicated for disinfecting hard, nonporous plastic surfaces and for killing a variety of potentially pathogenic microbes.[3,4] Additionally, we assessed the bacterial contamination of CTAs as shipped directly out of the box and after use in an intensive care unit.

Methods

CTA collection

Unused CTAs were collected in Ziploc-style specimen bags directly from a box on 3 different dates prior to patient use. After use, CTAs were collected in the same type of bags during a 2- month period from a convenience sample of patient rooms in a 28-bed medical intensive care unit immediately after patient discharge but prior to room cleaning. All CTAs were cultured the same afternoon, immediately after transport to the research laboratory. We did not record the length of stay or other clinical information for patients occupying rooms from which the CTAs were removed. In total, 17 CTAs were cultured directly out of the box and 23 were cultured after patient use.

Bacterial culture and identification

All studies were performed in a research laboratory that routinely performs bacterial cultures. For each CTA, a sterile swab was

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dipped in sterile phosphate-buffered saline (PBS) and wiped over the entire outside surface. The swab contents were inoculated directly to a Lysogeny Broth (LB) agar plate, which was incubated at 37 °C for 48 hours. The negative control consisted of 100 µL PBS inoculated to a LB agar plate under similar conditions. Colony-forming units (CFU) were recorded, and bacteria of similar phenotypes were picked and transferred to 5% sheep blood agar plates (Remel, Lenexa, KS) for identification by the Yale New Haven Hospital Clinical Microbiology Laboratory using the Vitek-2 matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF MS).

Low-level disinfection of CTAs

Next, we evaluated the ability of 2 different disinfectant wipes, 0.5% hydrogen peroxide (Oxivir1) or 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol (Super Sani-Cloth), to remove bacteria from the CTAs. Both products are registered by the US Environmental Protection Agency (EPA) to be effective against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp.[5] The CTAs were inoculated from a suspension of overnight growth of 1 of the following environmental organisms recovered from CTAs from patient rooms: *Micrococcus luteus*, *Staphylococcus epidermidis* or *Bacillus megaterium*. Additionally, we tested 2 pathogenic respiratory clinical isolates: *S. aureus* and *Pseudomonas aeruginosa*.

To estimate the initial bacterial inoculum, the overnight suspension of each bacteria was serially diluted in LB, inoculated to an LB agar plate, and incubated at 37 °C overnight. The CFU count was then recorded. Next, 30 µL of each overnight bacterial suspension was added to the CTA surface. The inoculum was spread with a sterile swab and dried for 15 minutes. The *B. megaterium* grew poorly and was susceptible to desiccation, and fewer bacteria were recovered after 15 minutes of drying. Therefore, for *B. megaterium*, the CTAs were inoculated with a sterile swab dipped directly in the overnight suspension and dried for 7 minutes instead of 15 minutes. In all cases, no visible liquid was present prior to reculturing the CTA surface.

We then estimated how many bacteria remained after drying, prior to disinfection. The top half of the dried, contaminated CTA was wiped with a sterile cotton swab dipped in LB. The swab was then cut and submerged in a microcentrifuge tube with 1.0 mL LB and vortexed for 30 seconds to release bacteria from the swab. The vortexed LB was serially diluted, the dilutions were inoculated to LB agar plates incubated at 37 °C overnight, and the colonies were then counted. Alternatively, the contaminated surface was wiped directedly with a sterile swab dipped in LB and the swab used to inoculate an LB plate. Most often, this latter method was semi-quantitative, producing a lawn of bacteria that confirmed a minimum inoculum of at least $>10^3$ (data not shown).

The CTAs were then disinfected with either 0.5% hydrogen peroxide or 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol wipes by applying the wipes for 5–10 seconds, followed by a contact time determined by the manufacturers' instructions (ie, 1 minute for hydrogen peroxide or 2 minutes for tetra-ammonium chloride/isopropyl alcohol).[3,4] The CTAs were then dried for a total of 15 minutes prior to reswabbing for bacteria. For *B. megaterium*, this duration was reduced to 7 minutes because of the previously described death during drying. The bottom half of the CTA was then recultured with a sterile swab dipped in Dey/Engley (D/E) neutralizing broth (Hardy Diagnostics, Santa Maria, CA) in the area that was not swabbed prior to disinfection. This swab was used to inoculate D/E agar plates that were incubated at 37 °C as described for the previous procedure. Bacterial growth was recorded before and after disinfection for each method from the same CTA. All experiments were performed in triplicate on 3 separate CTAs for each bacterial strain for all conditions tested.

Results

CTAs have a low bacterial burden

Bacterial cultures from CTAs taken directly out of boxes prior to patient use on 3 different days demonstrated that 9 of 17 (53%) were contaminated with a low number of environmental organisms (range, 0–13 CFU) (Table 1). The organisms recovered are generally considered nonpathogenic to immunocompetent hosts (Table 1). We recovered bacteria from 8 of 23 CTAs (35%) removed from patient rooms in our medical intensive care unit (range, 1–8 CFU). *Enterococcus faecium* was the only organism recovered that is considered a traditional hospital-acquired pathogen (Table 1). Coagulase-negative staphylococci were the most common organisms recovered from CTAs after patient use, and coagulase-negative staphylococci and *Bacillus* spp were most commonly recovered from CTAs directly out of the box prior to patient use (Table 1). Moreover, 5 microbial isolates were not identified, presumably because they are not in the Vitek 2 MALDI-TOF MS database and were not pursued further.

Disinfection wipes eliminate bacteria on CTAs

We chose isolates previously recovered from CTAs for disinfection experiments as well as 2 organisms found commonly in the hospital environment with the potential to cause respiratory infections: *S. aureus* and *P. aeruginosa*. The estimated inoculum prior to drying was 7.2×10^4–3.9×10^7, depending on the bacterial strain, with 1.6×10^2–5.7×10^5 recovered after drying (Table 2). This measurement may underestimate the actual degree of CTA bacterial contamination because not all bacteria were likely removed from the CTA surface by the cotton swab and not all bacteria on the cotton swab were released into the D/E media with vortexing. After disinfection, we did not grow bacteria from 28 of 30 CTAs. We recovered 1 CFU of *B. megaterium* after wiping with 0.5% hydrogen peroxide and 1 CFU of *S. epidermidis* after using 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol wipes (Table 2). There was an estimated minimum 3 log reduction for all bacteria except for *B. megaterium* where only a 2 log reduction was found due to the low bacterial recovery after surface drying prior to disinfection. Separate experiments on a subset of the above isolates were also performed with LB as the recovery media rather than D/E neutralizing broth. Interestingly, all CTAs showed no growth after disinfection except for one inoculated with *M. luteus*, from which we recovered 31 CFU (data not shown). In retrospect, this 0.25% tetra-ammonium chloride with 44.50% isopropyl alcohol wipe was the first of a previously opened container, and it was not fully saturated with disinfectant. Nevertheless, it still achieved an estimated 3 log 10 reduction: the inoculum for this experiment was 2.5×10^4.

Discussion

Several different recommendations for CTA disinfection are available, depending on the manufacturer (Teleflex, letters to customers[2]). For example, Teleflex recommends soaking the CTA in 70% isopropyl alcohol for 30 minutes or performing steam

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Table 1. Bacterial Contamination of Christmas-Tree Apparatuses (CTAs)

CTA	CTAs with Bacteria, No. (%)	Total CFU/CTA	Bacterial Contamination, No. (%)			Bacteria Recovered[a]
			0 CFU	1–3 CFU	>3 CFU	
Directly from box (ready to use) (n = 17)	9 (53)	Avg, 1.13 Median, 0 Range, 0–13 CFU	8 (47)	7 (41) Range, 1–2 CFU	2 (12) Both with 13 CFU	*Bacillus circulans, B. firmus, B. megaterium, B. cereus* *Microbacterium flavescens* *Micrococcus luteus* *Paenibacillus provencensis* *Staphylococcus capitis, S. epidermidis*
Removed from patient room (after use) (n = 23)	8 (35)	Avg, 2.05 Median, 1 Range, 0–8 CFU	15 (65)	5 (22) Range, 1–3 CFU	3 (13) Range, 4–8 CFU	*Enterococcus faecium* *M. luteus* *Paracoccis yeei* *S. capitis, S. epidermidis, S. hominis*

[a]5 bacteria were not identified by MALDI-TOF MS and 1 did not grow after subculture.

Table 2. Christmas-Tree Apparatus (CTA) Disinfection of Select, Inoculated Bacteria With Disinfectant Wipes*

Organism	Estimated Inoculum	Estimated Recovery From CTA After Drying	Recovery After 0.25% Tetra-ammonium Chloride With 44.50% Isopropyl Alcohol Disinfection, CFU	Recovery After 0.5% Hydrogen Peroxide Disinfection, CFU
Bacillus megaterium	7.2×10^4	1.6×10^2	0, 0, 0	1, 0, 0
Micrococcus luteus	1.38×10^6	2.5×10^3	0, 0, 0	0, 0, 0
Pseudomonas aeruginosa	9.6×10^6	1.6×10^4	0, 0, 0	0, 0, 0
Staphylococcus aureus	3.9×10^6	5.7×10^5	0, 0, 0	0, 0, 0
S. epidermidis	3.9×10^7	3.9×10^5	1, 0, 0	0, 0, 0

[a]Each strain was tested in triplicate for each wipe.

sterilization, but these are both resource-intensive processes. In contrast, MES recommends "hospital grade" disinfection wipes.[2] Previous literature report varying effectiveness of different types of disinfectant wipes to eliminate microbes, including resistant bacteria, from a variety of surfaces.[6-9] For example, 0.5% hydrogen peroxide wipes were effective at disinfecting blood-pressure cuffs, but these wipes did not effectively disinfect telemetry leads.[7] Nandy et al[6] tested 6 different commercial wipes for disinfecting pulse oximeter sensors, with bleach-based products being the most effective. Alternatively, hydrogen peroxide wipes were highly effective at reducing the bacterial burden on multiple different surfaces in dental offices.[9] Therefore, our goal was to determine whether commercially available disinfectant wipes could be effectively used for low-level disinfection of CTAs that do not come into direct contact with patients. Our data demonstrate low levels of Teleflex manufactured CTA bacterial contamination before and after clinical use in the ICU setting. The most common organisms we recovered, coagulase-negative *Staphylococcus* and *Bacillus* spp are consistent with other surveys of the hospital environment.[7] Both disinfectant wipes tested were efficacious in removing much larger bacterial burdens (10^{2-5}) than we recovered from the CTAs (10^1). On 1 CTA, we recovered a significant number of bacteria (31 CFU) after disinfection. We hypothesize that this was due to using a partially dry wipe that was the first out of a used container. This finding reinforces the need to use fully saturated wipes and the importance of fully closing the top of the wipe container. One limitation of the study is that we did not test nor wipe down the inside of the CTA because it is more difficult to thread the wipes through the hole. A second is that we did not record how long the used CTAs were deployed in patient rooms prior to removal for bacterial testing. However, this study demonstrates that CTAs have low levels of contamination with bacteria that predominately are not likely causes of healthcare-associated infections and are readily eliminated by disinfectant wipes. Furthermore, the recovery of *E. faecium* from a CTA after clinical use supports the manufacturers' recommendations that CTAs be disinfected prior to redeployment.

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Conflicts of interest. All authors report no conflicts of interest related to this article.

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