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Contamination rate in open-system filling adapters in operating room anesthesia work area in India: An *in-vitro* study

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Abstract
Objective: To evaluate the rate and type of contamination in open-system adapters used for filling the anesthetic agent sevoflurane across hospitals in India.
Methods: Nineteen reusable adapters used for filling sevoflurane were collected from nine hospitals in India and evaluated for microbial contamination. Samples were collected by flushing the inner lumen and swabbing the outer surfaces of adapters and dispatched to a central laboratory in sealed sterile tubes/containers for culture and microbial identification.
Results: Out of 19 swab samples collected from the outer surfaces, 11 (57.9%) were found to be contaminated/colonized. Out of these, 5 (26.3%) were found to be contaminated with Gram-positive cocci belonging to the *Staphylococcus* species, and 6 (31.6%), with Gram-negative organisms *Pseudomonas aeruginosa* (5 [26.3%]), and *Burkholderia cepacian* (1 [5%]). Out of 19 rinse samples from the inner lumens, 8 (42.1%) were found to be contaminated, 2 (10.5%) with Gram-positive cocci belonging to *Staphylococcus* species and 6 (31.6%) with Gram-negative organisms *Achromobacter denitrificans* and *Aeromonas enteropelogene* (one (5.3%) sample each), and *Pseudomonas aeruginosa* and *Ralstonia pickettii* (2 (10.5%) samples each).
Conclusion: Reusable open-system filling adapters are susceptible to contamination with nosocomial pathogens; therefore, single-use adapters to inhibit contamination and prevent hospital-acquired infections should be encouraged.

Keywords: filling adapters, contamination, infections, micro-organisms

Introduction

Hospital-acquired infections are a major cause of preventable diseases and are responsible for a substantial increase in patient morbidity, mortality, and healthcare costs [1]. Microbial contamination of operating rooms is an important contributor in precipitating the incidence of hospital-acquired infections [2]. Anesthesia machines are known reservoirs of bacterial species, potentially contributing to hospital-acquired infections [3-5].

Bacterial contamination of anesthesia machines has been an infection control concern since the 1950s [6]. The anesthesia work area, including the anesthesia machine, vaporizer dials, adjustable pressure limiting valve, fluid warmers, supply cart, and computer keyboard and mouse, are examples of components that have the potential to get contaminated [7]. Out of these, adjustable pressure limiting valves, gas flow meters, and agent vaporizer dials are reported to be common reservoirs for enterococci and other pathogenic bacterial species [1-4, 8, 9].

Enterococci and *Staphylococcus aureus are* major human pathogens and cause of a wide range of clinical infections, both in community-acquired as well as hospital-acquired settings [10, 11]. Infections caused by microbes of the genus *Enterococcus* (particularly *E. faecalis* and *E. faecium*) in hospitalized patients include urinary tract infections, bacteremia, intra-abdominal infections, and endocarditis [11]. Challenges in the management of diseases caused by enterococci are complicated by their limited susceptibility to antibiotics, because of both inherent and acquired antibiotic resistance [12, 13]. *S. aureus*, if allowed to enter the bloodstream or internal tissues, can cause a variety of potentially serious infections [10]. It is a leading cause of bacteremia and infective endocarditis, hospital-acquired infection of surgical wounds, as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [14, 15]. Moreover, treatment of infections caused by *S. aureus* is a challenge due to the emergence of multi-drug resistant strains such as methicillin-resistant *S. aureus* (MRSA) [10].

Bacterial transmission in the anesthesia work area has been identified as the root cause of 30-day postoperative infections, affecting approximately 16% of patients undergoing surgery [8]. However, despite adequate knowledge of nosocomial infections, processes of decontamination and sterilization of equipment are often overlooked [16] Additionally, anesthesia machine design makes routine sterilization and decontamination of equipment a difficult task in daily practice [1]. Baillie *et al*. performed two cross-sectional studies to assess bacterial contamination on anesthesia machines before and after a simple intervention and

found that surfaces most commonly touched by anesthetists during induction of anesthesia, like ventilator bags, vaporizer dials, and flow control knobs, were the most contaminated [9].

Studies have confirmed that the anesthesia work area is crucial in transmitting infections to patients during and after surgical procedures [1-4, 8, 9]. Additionally, the source of contamination could also be the transfer of organisms from patients to the respiratory system machines [2, 3]. The best way to prevent such contaminations is by discarding some equipment or apparatus after single use. For instance, trachea tubes, hose pipes, airway, filling adapters, and other rubber parts could be used once and replaced before each surgical procedure [1, 9]. As current practices of decontamination and disinfection between procedures are frequently ineffective, residual contamination remains an important source of cross-infection [17].

Open-system filling adapters used to fill vaporizers with anesthesia drugs are often reused in operating rooms without disinfection [18]. Likewise, in some hospitals, there are no dedicated adapters for each bottle, and the same adapter might get used multiple times for weeks [18]. A study by Percin *et al*. evaluating the contamination rates of open-system filling adapters found that 30% of adapters were contaminated with bacteria like coagulase-negative staphylococci (CoNS), coryneform bacilli, and Bacillus species, thereby indicating the contamination risk of open-filling adapters in operating rooms [18].

In India, the use of both open- and closed-filling adapter systems is prevalent in operating rooms. Here, we present results from a study planned with the objective to evaluate the contamination rates in open-system filling adapters and to identify the micro-organisms causing contamination/colonization in adapters collected from different hospitals across India.

Methods

Study Plan

This study was conducted between May 2020 and August 2020. A total of 19 reusable adapters used for filling inhalational anesthetic agent sevoflurane were collected from 9 hospitals across 7 cities in India and used as a test product for evaluating microbial contamination. The samples were collected by flushing the inner lumens and swabbing of the outer surfaces of the adapters. The samples were dispatched to the central laboratory of Metropolis Healthcare Limited, Mumbai, India, in sealed sterile tubes/containers for culture and microbial identification. Sample processing was done as per standard operating procedures of Metropolis Healthcare Limited and by following the study protocol.

Methodology

The samples were collected from outer surfaces of the filling adapter with sterile swabs moistened with sterile 0.9% sodium chloride solution while adhering to aseptic precautions. The outer surfaces were swabbed from all around. The swabs were then transferred to sterile tubes with Amies transport medium. For inner surfaces, 10 mL of sterile saline was flushed into the lumen of the filling adapter using a sterile syringe and transferred to a sterile container. The lids of the sterile containers were then sealed to prevent leakage of rinsed saline from the containers. The swabs and sterile containers were labeled accurately and transported to the laboratory at temperatures of 2-8°C.

At the central laboratory, rinsed saline in the containers were centrifuged for 10 minutes, and the sediments were inoculated using sterile loops onto sterile blood agar, chocolate agar, MacConkey agar, and Sabouraud dextrose agar to assess the growth of microorganisms.

The collected swabs were also separately inoculated onto sterile blood agar, chocolate agar, MacConkey agar, and Sabouraud dextrose agar. The culture plates were incubated at 35°C for 24 to 48 hours. If no growth was observed at the end of the 48 hours, results were documented as *no growth*. If microbial growth was observed on the culture media, conventional tests were performed, and/or automated mass spectrometry microbial identification system Vitek MALDI-TOF (Vitek MS) was used to identify the isolated organisms.

The evaluation report was generated by Metropolis Healthcare Limited within 10 days from the date of sample collection. In addition to this, a compiled study report was also generated by Metropolis Healthcare Limited at the end of the study.

Statistical Analysis

The percentage of filling adapters with microbial contamination and the type of micro-organisms causing contamination of these adapters were reported as numbers and percentages. All assessments are presented using descriptive statistics.

Results

The species of bacteria found in the swabs collected from outer surfaces and the rinses collected from the inner lumens of the adapters are presented in Table 1. Out of 19 swab samples collected from the outer surfaces, 11 (57.9%) were found to be contaminated/colonized. Out of these, 5 (26.3%) were found to be contaminated with Gram-positive cocci belonging to *Staphylococcus* species, and the remaining 6 (31.6%) samples showed the presence of Gram-negative organisms; *Pseudomonas aeruginosa* and *Burkholderia cepacian* species were found in 5 (26.3%) and 1 (5.3%) sample, respectively.

Out of 19 rinse samples collected from the inner lumens, 8 (42.1%) were found to be contaminated/colonized. Two (10.5%) samples were found to be contaminated with Gram-positive cocci belonging to *Staphylococcus* species, and 6 (31.6%) samples were colonized with Gram-negative organisms like *Achromobacter denitrificans* and *Aeromonas enteropelogene* (isolated from 1 [5.3%] sample each) and *Pseudomonas aeruginosa* and *Ralstonia pickettii* (isolated from 2 (10.5%) samples each).

Discussion

Probable transmission of organisms in enclosed hospital settings like operating rooms is of concern [19]. Generally resistant to antibiotics, hospital-acquired infections cause substantial morbidity and mortality in susceptible populations [20-22]. Pathogenic microorganisms are known to survive on anesthesia machines [19, 23]. Even in the absence of direct contact, there is a probability that colonizing organisms are carried between anesthesia machines in operating rooms and patients [9]. Accelerated cleaning practices may reduce bacterial burden, but are ineffective in eliminating them [19, 23]. Moreover, standardized and routine cleaning practices fail to achieve the desired level of

decontamination, thus putting patients at significant risk of cross-contamination [1].

The results of the present study confirm the presence of potentially pathogenic bacteria on anesthesia equipment across hospitals in India. Eleven (57.9%) out of Nineteen swab samples and 8 (42.1%) out of Nineteen rinse samples were found to be colonized. The contamination rate of open-system filling adapters reported in our study is comparatively higher than the 30% contamination rate reported by Percin *et al* [18]. This difference can be attributed to great variability in decontamination practices and unsatisfactory infection control practices of operative settings in India [17]. The microbes found on swabs and rinses in our study included potentially pathogenic bacteria such as *S. aureus*, Gram-negative as bacteria such as *Aeromonas enteropelogene*, and nonfermenting Gram-negative bacilli (NFGNB) like *Achromobacter denitificans, Burkholderia cepacia, Pseudomonas aeruginosa,* and *Ralstonia pickettii***.**

S. aureus is a key human pathogen and a cause of a wide range of clinical infections, including a growing number of healthcare-associated infections [14]. Studies have shown that *S. aureus* phenotypes are frequently detected and transmitted in anesthesia work areas [3, 9, 24]. Moreover, multidrug-resistant strains, like MRSA, are omnipresent and are frequently being isolated from humans [25]. Nasal colonization with MRSA is a risk factor for severe MRSA sepsis following surgery [26]. Additionally, asymptomatic colonization with *S. aureus* is associated with a greater risk of wound infection [9]. Any surface that is colonized by *S. aureus* may also be colonized by MRSA and other drug-resistant pathogens [27]. Being associated with high rates of antibiotic resistance, MRSA represents a major challenge, and a cause for a significant increase in morbidity, mortality, and overall healthcare costs [28]. Prevention of *S. aureus* related infections remains a challenge given that the pathogen can survive on dry surfaces for long periods of time; only effective decontamination measures can be relied upon to prevent its colonization in the anesthesia work area [9, 10, 17].

NFGNB have emerged as important healthcare-associated pathogens [29]. In the hospital environment, they have been isolated from instruments and equipment, and from the skin of healthcare workers [29]. Recognized as nefarious multidrug-resistant organisms, NFGNB are found to survive and persist in antiseptic solutions, intravenous fluids, irrigation fluids, and detergent solutions within the hospital environment [30]. These organisms are associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator-associated pneumonia, wound infection, and osteomyelitis [31]. While *Pseudomonas aeruginosa* remains the most clinically prevalent species among NFGNB, other species, mainly *Acinetobacter baumannii*, have emerged as important nosocomial pathogens [32]. *Stenotrophomonas maltophilia* and *Burkholderia cepacia* are commonly isolated from intensive care unit patients and patients with cystic fibrosis [32].

Studies have demonstrated that Gram-negative organisms are commonly encountered in and transmitted from anesthesia work areas [4, 9, 24, 33]. The presence of Gram-negative organisms such as *Achromobacter denitificans, Burkholderia cepacia, Pseudomonas aeruginosa,* and *Ralstonia pickettii* found in our study have been previously shown to colonize adapters and have the ability to thrive in the hospital environment because of their inherent resistance to commonly used antibiotics and disinfectants solutions [30]. Due to high intrinsic resistance to various commonly used antimicrobials, these organisms represent the difficult-to-treat entities, contributing to increased morbidity as well as mortality in hospital settings [30, 34].

Because decontamination procedures are generally ineffective in eradicating pathogenic contamination, it is assumed that the anesthesia work area setting and hygiene practices followed by anesthesia providers together contribute to healthcare-associated infections [8]. Munoz *et al*. referred to this as "fecal patina in the anesthesia work area", and described it as "coating of enteric organisms that are not only restricted to the patient's skin, but also spreads to surrounding health care environment, that are touched, and contaminated by patients and health care providers" [35].

Early and recent findings strongly indicate that intraoperative bacterial transmission arising from the anesthesia work area occurs rapidly and at a significant scale and is related to increased patient morbidity and mortality [2-4, 8, 9, 24, 33, 36]. The presence of bacterial colonization and contamination of nosocomial origin in filling adapters in our study, indicates the high probability of cross-contamination between patients, especially in busy operating rooms. Therefore, it is important that the load of bacterial contamination is minimized in anesthesia work areas to make them safe for both healthcare providers and patients and to reduce the risks of hospital-acquired infections in operating rooms.

Though important, but healthcare providers' hygiene, environmental disinfection, and decontamination of anesthesia machines and related equipment cannot be sufficient for reducing the burden of pathogens in the anesthesia work area. To lessen the burden of pathogens in the operating room, it is desirable to use disposable/single-use apparatus to prevent contamination and cross-contamination of anesthesia machines during surgery. There is an increasing trend in the Western world toward the use of disposable or single-use equipment/apparatus to tide over this problem [23].

Table

Table 1: Types of pathogens obtained from swab samples from outer surfaces and rinse samples from inner lumens of adapters

Pathogens Isolated, n (%)	Swab (N = 19)	Rinse (N = 19)
Achromobacter denitrificans	-	1 (5.3)
Aeromonas enteropelogenes	-	1 (5.3)
Burkholderia cepacia	1 (5.3)	-
Staph hominis	2 (10.5)	1 (5.3)
Staph epidermidis	3 (15.8)	1 (5.3)
Ralstonia pickettii	-	2 (10.5)
Pseudomonas aeruginosa	5 (26.3)	2 (10.5)
No growth	8 (42.1)	11 (57.9)

Conclusion

The finding of our study suggests that the cleaning/decontamination of anesthetic equipment might be inadequate in preventing the spread of potentially pathogenic bacteria. The result of the present study illustrates that the surfaces and interiors of reusable open-system filling adapters are susceptible to contamination with nosocomial pathogens, and

highlights the importance of using single-use/disposal adapters to inhibit colonization/contamination and to prevent the spread of hospital-acquired infections. To the best of our knowledge, this is the first study designed to evaluate bacterial contamination events arising from the anesthesia work area in operating theatres in India. We hope that evidence from this study will encourage regulatory authorities to develop and establish policies and procedures on the use of anesthetic equipment to prevent the spread of potentially pathogenic bacteria in the hospital setting.

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