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BRIEF COMMUNICATION

Detection of antibiotic resistance genes associated with methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci in hospital air filter dust by PCR

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Abstract Dust from the pre-filters of stand-alone hospital isolation room air cleaners was tested by PCR for the presence of antibiotic resistance genes associated with methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci. Resistance genes for three classes of antibiotics (*aac*(6′)-*aph*(2″), *erm*A, and *mec*A) were detected in multiple samples, indicating the presence of genetic material and likely cells of multidrug-resistant MRSA and other staphylococci in hospital air and that stand-alone air cleaners can reduce airborne levels of these contaminants. Screening for *van*A was negative. Our results further suggest that dust may serve as an important reservoir of genetic elements that can confer drug resistance.

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1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) can be dispersed from people and inanimate hospital surfaces, leading to environmental contamination and potentially contributing to the transmission of hospital-acquired infections (Dancer 2008; Cimolai 2008). To control the spread of MRSA, colonized or infected individuals may be placed in isolation and managed using contact precautions (Coia et al. 2006). Isolation rooms may be equipped with stand-alone air cleaning devices (SACDs) intended to remove infectious particles from the air. There is limited information regarding the airborne prevalence of multi-resistant MRSA in the hospital setting. Several reports have isolated these organisms from air and settled dust (reviewed by Gilbert et al. 2010). This finding might be expected in light of the shedding of these bacteria on skin scales and the potential for squames to become airborne; however, the significance is uncertain in terms of aerosol transmission of these agents or exposure risk to maintenance workers handling the filters.

There is little evidence on the efficacy of SACDs in removing drug-resistant bacteria from air. Lastly, few studies to date have directly examined antibiotic resistance genes in environmental samples. To address

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these knowledge gaps, we investigated the presence of selected antibiotic resistance genes associated with MRSA in airborne dust captured by SACDs in isolation rooms using a culture-independent PCR-based approach. Genes were chosen based on the availability of published primer sequences and also on the prior knowledge of the prevalence of resistance phenotypes in the facilities tested.

2 Materials and methods

Bulk samples of captured dust ($n = 15$) were collected from the pre-filters of in-service SACDs from various departments (emergency, general medicine, critical care, nursery, and chest clinic) of four urban tertiary care hospitals in Toronto, ON, Canada, between November 2008 and March 2009 (Table 1).

2.1 Devices tested

Two basic configurations of SACDs were sampled. The first consisted of a floor-standing device with a return air grille near the floor. The second was a wall-mounted device with an air return on the unit's underside. In both cases, dust was sampled from pre-filters located behind return air grilles. Pre-filters are coarse particle filters used to extend the lifetime of the more expensive HEPA filters. We sampled from pre-filters for two reasons: (1) Pre-filters are the front elements of the particle capture process in SACDs. The larger particles with which bacteria are most commonly associated are captured in greater quantities than on HEPA filters; and (2) Pre-filters are easily accessible without the use of specialized tools. Transfer of particles to filters by skin contact is unlikely because the filters are covered by protective grilles.

The devices tested included the HEPA-CARE (HC) 800F and 1000V (Abatement Technologies, Fort Erie, Ontario, Canada); NQ 400 (NQ Industries, Rocky Hill, CT); and the HEPA-NET II WM-600 (Qualitair) (wall-mounted). Both are floor-standing devices with flow rates that are variable and subject to user control, ranging 100–750 CFM for the HC 800F and 100–900 CFM for the HC 1000V. The pre-filter

Table 1 Antibiotic resistance genes detected in dust from the pre-filters of isolation room SACDs

Sample	Hospital	Department	SACD model	Genes detected				
				aac(6′)-*aph*(2″)	*erm*A	*mec*A	*van*A	PVL
01	A	Chest clinic	HC 800F	+	+	+	–	–
02	A	General medicine	HC 800F	–	–	+	–	–
03	A	Emergency	WM-600	–	–	+	–	–
04	B	Critical care	HC 800F	–	–	+	–	–
05	C	General medicine	NQ 400	+	+	+	–	–
06	C	General medicine	NQ 400	–	+	+	–	–
07	C	General medicine	NQ 400	+	+	+	–	–
08	C	General medicine	NQ 400	+	+	+	–	–
09	C	Level 2 nursery	NQ 400	–	–	+	–	–
10	C	Level 3 nursery	NQ 400	+	–	+	–	–
11	C	Emergency	NQ 400	+	+	+	–	–
12	D	(Unknown)[a]	HC 1000V	+	–	+	–	–
13	D	(Unknown)[a]	HC 800F	+	–	+	–	–
14	D	(Unknown)[a]	HC 1000V	+	–	+	–	–
15	D	(Unknown)[a]	HC 1000V	+	–	+	–	–

The models of SACD sampled were HEPA-CARE (HC) 800F and 1000V (both are floor-standing), Abatement Technologies; NQ 400, NQ Industries (wall-mounted); HEPA-NET II WM-600, Qualitair (wall-mounted)

SACD = stand-alone air cleaning device

[a] All units from hospital D were in storage on-site during the time of sampling, but had been previously and exclusively employed in isolation rooms in the hospital

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of the HC 800F is a cellulosic ASHRAE MRV8-rated pleated filter treated with EPA-registered antimicrobial; and the first-stage pre-filter of HC 1000V first stage consists of a 1 inch untreated woven polyester fiber pad. The NQ 400 is a wall-mounted device that operates at a flow rate of 400 CFM and uses a 102 mm 25–30% efficiency ASHRAE G4-rated pre-filter. The HEPA-NET II WM-600 is a wall-mounted unit with two user-selected operating flow rates of 400 and 600 CFM. This device is not equipped with a pre-filter, and samples were taken directly from the surface of the HEPA filter.

All devices were operated intermittently according to clinical indications of the need for aerosol precautions. No data were available to establish whether carriers of MRSA or MSSA had been seen in the rooms tested. However, given the prevalence of these bacteria in the community, it is expected that this is a frequent occurrence. As well, no records were kept of the usage frequency of the devices; however, all devices were reported to be used regularly and maintained in accordance with the manufacturer's recommendations, including the regular replacement of pre-filters, where applicable.

2.2 Sampling procedure

Samples were collected from filter surfaces by plucking portions of accumulated dust matt with sterile forceps and placing it into sterile polypropylene. The goal of filter sampling was to collect a "random sample" as a basis to construct a snapshot of the aerosol distribution of selected drug resistance. Here, we use "random" in the clinical sampling sense to indicate a sample collected at an arbitrary time point without prior preparation. No effort was made to quantify the dust loading on the filter or the bulk amount collected. Samples were processed within 4 h of collection.

DNA was extracted from 10–20 mg aliquots of dust by using the FastDNA SPIN® kit for soil (MP Biomedicals, Solon, OH) and a FastPrep®-24 homogenizer (MP Biomedicals), following the manufacturer's instructions, with the exception that samples were initially incubated in 978 µL of sodium phosphate buffer (50 mM, pH 7.2) for 15 min at room temperature to hydrate dust and facilitate extraction. The following drug resistance genes were amplified using specific flanking primers: *aac*(6′)-*aph*(2″) (aminoglycoside resistance), *erm*A (macrolide–lincosamide–streptogramin B resistance), *mec*A (methicillin resistance), and *van*A (glycopeptide resistance) (Clark et al. 1993; Geha et al. 1994; Lina et al. 1999a; Martineau et al. 2000). An additional PCR assay was carried out to detect the Panton–Valentine leukocidin (PVL) gene using the primers *luk*S-*PV* and *luk*F-*PV* (Lina et al. 1999b). This gene encodes a virulence factor associated with community-acquired MRSA strains (CA-MRSA). PCR was performed in a 25 µL final volume containing 1× PCR buffer (Qiagen, Mississauga, ON, Canada), 200 µM (each) deoxynucleoside triphosphate (Fermentas Life Science, Burlington, ON, Canada), 3 pmol of each primer, 0.75 U of Taq DNA polymerase (Qiagen, Mississauga, ON, Canada), and 1 µL of DNA template. Each set of PCR reactions included control DNA from clinical isolates either possessing or lacking the gene being assayed. Reaction products were detected by agarose gel electrophoresis followed by ethidium bromide staining and visualization under UV light.

3 Results

Four drug resistance genes and PVL were assayed by PCR in dust samples from hospital SACDs. The *mec*A gene was detected in all dust samples from all hospitals (Table 1). The *aac*(6′)-*aph*(2″) gene was detected in 10/15 samples from 3/4 hospitals. The *erm*A gene was detected in 6/15 samples from 2/4 hospitals. The *aac*(6′)-*aph*(2″), *erm*A, and *mec*A genes were detected concurrently in 5/15 samples from 2/4 hospitals. The *van*A and *luk*S-*PV*/*luk*F-*PV* genes were not detected in any sample.

The detection of *mec*A in all samples indicates that methicillin-resistant staphylococci such as MRSA commonly become airborne in hospital isolation room air. The presence of *aac*(6′)-*aph*(2″) and *erm*A together with *mec*A in some samples strongly suggests the airborne transmission of multidrug-resistant strains of MRSA (Lina et al. 1999a; Martineau et al. 2000). The absence of *van*A in our sample set supports the view that bacteria carrying this gene, which include vancomycin-resistant enterococci (VRE) and uncommon strains of MRSA, are rare in hospital air (Sievert et al. 2008). Similarly, the absence of *luk*S-*PV* and *luk*F-*PV* indicates that

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PVL-positive CA-MRSA strains are currently uncommon in the air where we sampled.

4 Discussion

Our use of a culture-independent approach to bacterial detection based on PCR amplification of specific antibiotic resistance genes precluded definitive identification of the host bacteria. Nevertheless, there are strong, regular taxonomic associations of the genes that we examined with bacteria that are thought to be primarily contact-transmitted, namely *Staphylococcus* spp., including *S. aureus* and coagulase-negative staphylococci (Geha et al. 1994; Lina et al. 1999a; Martineau et al. 2000). Our results suggest that airborne transmission of resistant strains of these bacteria may be underestimated. As well, the extent to which dust itself may serve as a reservoir for not only living drug-resistant bacteria but also genetic elements capable of conferring drug resistance may be underappreciated. The use of PCR targeting specific antibiotic resistance genes permitted the detection of host bacteria independent of culturability, including those rendered inert by environmental stressors, such as dessication of viable cells following their deposition on the filter media due to the ongoing airflow through the SACD. By utilizing SACD pre-filters as air samplers coupled with a culture-independent detection approach, our method allowed longer-term, integrative assessment of airborne bacteria irrespective of viability or culturability.

The presence of antibiotic resistance genes in SACD pre-filter dust both demonstrates the airborne presence of drug resistance genes of contact-transmitted pathogens and verifies the function of SACDs in reducing the airborne bacterial burden in the health care setting. The recovery of resistance genes was unrelated to the specific model of SACD investigated. MRSA can persist for months on dry surfaces, implicating dust as a potential source of infectious airborne bacteria (Dancer 2008). Our observation is consistent with the report of Stanley et al. (2008), who cultured coagulase-negative staphylococci from air filters in the ventilation system of a public building despite the ongoing air flow that would be expected to reduce cell viability over time. It is possible that MRSA as well as multidrug-resistant MRSA strains may similarly remain viable for lengthy periods in dust trapped on filter surfaces; however, given the cell stress imposed by desiccation during SACD use, active growth of MRSA in filter dust is unlikely.

Hospital ventilation systems have been implicated in outbreaks of MRSA, raising the possibility that SACD pre-filters may represent a possible source of MRSA exposure in HVAC maintenance workers, particularly in the health care setting (Kumari et al. 1998). The re-entrainment of captured bacteria may also present a hazard to health care workers and patients. For example, Kumari et al. (1998) found that a pressure reversal during an HVAC shutdown was responsible for the contamination of hospital ward air with MRSA. A more comprehensive study of SACDs is warranted to determine the potential for these systems to act as reservoirs for nosocomial agents and to provide guidance in defining safe maintenance practices.

The frequency and abundance of antibiotic resistance genes in environmental samples remains largely unknown. Otter et al. (2007) reported the culture-independent detection of *mec*A in environmental surface samples using a commercial real-time PCR kit developed for nasal swabs. However, they found the method to have low specificity compared to a culture-based approach. PCR-based methods typically require less time than conventional culture-based methods, and they can simultaneously detect multiple antibiotic resistance genes. However, culture-independent methods cannot distinguish viable bacteria from cell-free DNA and nonviable or viable-nonculturable bacterial cells. A direct comparison of culture- and PCR-based methods for the detection of resistant bacteria in dust would further inform the suitability of the latter in the analysis of environmental samples.

5 Conclusion

Genes encoding resistance to several antibiotic classes were detected by PCR in dust samples collected from the pre-filters of isolation room SACDs. This confirms that (1) multidrug-resistant genes and likely cells of MRSA and other staphylococci are episodically present in hospital air; (2) SACDs can reduce airborne levels of these bacteria; (3) dust may serve as a reservoir for genetic elements capable of conferring drug resistance.

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