Long Term Reduction of Bacteria on Surfaces in Public Buses

ABSTRACT

Use of public transport may serve as a vehicle for the transmission of infectious disease. The goal of this study was to assess bacterial loads on high touch areas within municipal buses and assess the use of a new coating comprising silicon-oxide bonds and titanium-oxide bonds provided by Allied BioScience, Inc on the long term suppression of bacterial numbers on high touch areas within the buses. Public buses were tested on selected sites for heterotrophic bacteria. The most contaminated sites were the driver's compartment and the fare box. One group of busses was then treated with the disinfectant and another was not. After 30 days statistically significantly fewer bacteria where present on the treated buses.

KEYWORDS
Public transportation, bacteria, fomites, buses, hygiene, disinfection

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INTRODUCTION

A route of transmission of cold, flu, diarrhea and other common infections is through contact with surfaces contaminated with infectious microorganisms (pathogens) (Boone and Gerba, 2007). Contamination occurs by settling of droplets from coughs and sneezes onto surfaces, and by touching of surfaces with hands contaminated with pathogens. The pathogens then contaminate the hands of the next person who touches the same surface, and when they bring their hands to their eyes, nose, or mouth infection can result. Mass transportation systems create an environment in which large numbers of persons on a daily basis share space and interact with surfaces found within system vehicles. A recent study in the United Kingdom demonstrated an increase of respiratory infections (colds and flus) to persons if they had ridden in a bus or streetcar five days previously (Troko et al., 2011).

Application of disinfectants on surfaces has been shown to reduce absenteeism and illness in schools (Bright et al., 2010). Unfortunately surfaces have to be disinfected on a regular basis to be effective. This is difficult in mass transportation when large numbers of individuals may be using the same vehicle in a day. Surfaces may become recontaminated throughout the service day of the vehicle. Treatment of surfaces with a product that could reduce the microbial load on a continuous basis would be ideal in these situations.

This study was designed to assess the effectiveness of a coating comprising silicon-oxide bonds and titanium-oxide bonds in suppressing the number of bacteria on surfaces within a public bus.

MATERIALS AND METHODS

In a recent study done at a public bus company, forty buses out of 220 were sprayed with a new product as a test. From these 40, seven buses were selected at random as an "experimental" group that was treated with materials that form a coating comprising silicon-oxide bonds and titanium-oxide bonds obtained from Allied Bioscience, 100 Crescent Court, Suite 450 Dallas, TX. Another seven buses, selected from the180 busses that were not sprayed, were selected at random as a "control" group. All busses received only routine cleaning at the end of the work day. Routine cleaning consisted of general sweeping, removal of trash and wiping down railings and other surfaces with a commercial detergent. Prior to any treatment, both groups of buses were tested for heterotrophic bacteria on various surfaces in order to establish a baseline profile of each bus. All buses were given a four-digit code as not to reveal the treated from the untreated buses. In an average day each bus transported approximately 400 persons.

Surface samples were taken at five locations in each of the fourteen busses for heterotopic bacteria: entry railing, fare box, driver compartment, interior railing, and seat back. Samples were taken at the end of the working day after the bus returned to the transit facility but before they were cleaned by night maintenance workers. Samples were collected in all of the busses before the intervention and then 30 days later.

Sites were sampled with a Spongestick (3M, St. Paul, MN) containing a neutralizing broth to neutralize any disinfectant that may have been on the sampled area. Approximately 150 cm^2 of the surface was sampled at each selected location in the bus. All samples were inserted in individual bags that were labeled with a random number code. This procedure was used to prevent workers in the microbiology laboratory from knowing which samples belonged to which buses, thus establishing a blind study. Once the laboratory provided the culture results, the codes were used to assign values to the appropriate buses and locations within those buses. The numbers of heterotrophic bacteria (HPC) were determined on R2A media (Difco, Sparks, MD) using the spread plate method. Samples were diluted using physiological saline for assay of dilutions. All dilutions were assayed in duplicates. The agar plates were then incubated at room temperature (~24 ^oC) for five days and the resulting colonies of bacteria counted.

The bacterial concentrations used to compare the treated vs. untreated measurements for the different locations in the buses proved to have a distribution other than normal (i.e. a bell shaped distribution curve); and hence the bacterial concentrations were transformed using log base 10 (i.e. 100 = 2, 1,000 = 3, etc.). The log base 10 transformed bacterial concentrations used to compare treated vs. untreated measurements proved to be normally distributed, with similar variances and without outliers which are the conditions necessary to conduct analysis of variance (ANOVA). Analysis of variance was performed on the log base 10 transformed data using the *F* statistic and a two sided rejection region of 5% (Ott, and Longnecker. 2001)

RESULTS

The number of bacteria per 150 cm^2 ranged from 40 to 1,480,000 colony forming units (CFU) on the surfaces tested from all the buses before the intervention. Arithmetic and geometric means including standard deviations of bacteria concentrations on the areas tested in the buses are shown in Table 1. The statistical analysis (ANOVA) indicated that there was no statistical difference in the numbers of bacteria in the busses that were selected for treatment and those that were not at the beginning (baseline data) of the study with a *p*-value of 0.315. After 30 days, representing an average bus use by a total of 12,000 passengers during the study period, the same buses were resampled (Table 2). The number of bacteria on the surfaces in the treated buses was significantly less than that in the untreated buses (*p*-value = 0.005). On average there were 93% fewer bacteria on the surfaces in the treated buses vs. the untreated buses based on geometric mean and 62% based on arithmetic mean.

The goal of this study was to demonstrate if there was a significant difference between the bacterial load in the bus interior of the treated and untreated buses. The number of samples obtained at each individual location within the vehicle was not chosen to be able to demonstrate significance at each individual sampled site. However, with the exception of the entry railing, the bacterial burden at all treated sites was reduced as compared to the untreated sites (Table 3). The greatest difference between treated and untreated buses in bacteria numbers was in the driver's compartment where there were fewer than 99.8% bacteria in the treated busses. This difference was highly significant (*p*-value = 0.007).

DISCUSSION AND CONCULSIONS

Use of public transport (trains, planes, buses, ships) has been shown to play a role in the transmission of infectious diseases. The most studied have been cruise ships which have had to deal with large recurring outbreaks of norovirus (Wikswo et al., 2011). Containment of passengers for several days on the same transport makes such transmission more easily documented than commuters on airplanes and buses. Still air travel has been shown to present a risk of norovirus and respiratory infection among the passengers (Thornley et al., 2011). Studies of trains and buses suggest that transmission of respiratory infections can occur (Mohr et al., 2012), but data is limited largely to tuberculosis, since it is more likely to be diagnosed. However, a recent study in the United Kingdom demonstrated an increase of respiratory infections (colds and flus) to persons if they had ridden in a bus or streetcar five days previously (Troko et al., 2011). Luksamijarulkul et al. (2004) found elevated levels of bacteria (>550 m^3) in buses in Thailand. We are not aware of any previous published studies on the occurrence of microorganisms on surfaces in buses in the United States.

Total bacterial numbers or heterotrophic bacteria on hard surfaces are used as a general measure of the hygienic quality of public surfaces (Reynolds et al., 2005) and the effectiveness of cleaning and disinfection of interventions (Bright et al., 2010). Reynolds et al. (2005) found detectable levels of protein on 61% of, and bodily fluids (urea, hemoglobin, mucus/sweat) on 41% of armrests/handles in public busses. Viruses and bacteria that cause respiratory infections and gastroenteritis can be transmitted by contact

with contaminated bodily fluids. Since hundreds of people may be expected to use the bus throughout the day, contamination of surfaces throughout a bus can be expected.

The greatest number of bacteria was found to be on the fare box, entrance railing and the driver's compartment. Both the fare box and entrance railings were probably the most touched areas by passengers. Drivers are present throughout the operation of the bus continually interacting with surfaces within the driver's compartment. Although somewhat isolated from the passenger's transmission of infectious organisms on the surfaces, drivers' exposure could occur during breaks and shift changes.

At the beginning of the study there was no statistical difference between levels of bacteria in the buses selected for study. However, the concentration of bacteria was significantly less in the interior of the treated vs. untreated buses after 30 days of use. On average there were 93% fewer bacteria on the interior surfaces of the treated buses in comparison to the same surfaces of the untreated busses. The greatest reductions occurred in the driver's compartment and the least on the entrance rail. The large amount of surface friction from hand contact to the entrance rail may be the reason for no difference at this site compared to the others within the bus. This suggests that this site may need to be treated differently than the other sites within the bus. Although not always statistically significant, lower concentrations of bacteria were found at all interior sites of treated buses when compared to the untreated buses.

The results of this study demonstrate that reduced levels of bacteria still occur in heavily used public buses 30 days after treatment with materials that form a coating comprising silicon-oxide bonds and titanium-oxide bonds. The product's effectiveness varied from site to site probably reflecting the degree of contact with that site by passengers. Reapplication of the product at more regular frequencies at high touch sites is probably necessary to keep bacterial numbers lower at these sites.

In conclusion, this study demonstrated that application of materials that form a coating comprising silicon-oxide bonds and titanium-oxide bonds to public buses resulted in significantly lower levels of bacteria after 30 days as a result of a onetime application.

REFERENCES

Boone SA, Gerba CP. 2007. Significance of fomites in the spread of respiratory disease and enteric viral disease. Appl. Environ. Microbiol. 73:1687-1696.

Bright, KR, Boone SA, Gerba CP. 2010. Occurrence of bacteria, influenza A and norovirus on elementary classroom surfaces. J. School Nursing. 26:33-41.

Foster HA, Ditta IB, Varghese S, Steele A. (2011) Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. Appl. Microbiol. Biotechnol. 90: 1947-1868.

Isquith AJ, Abbott EA, Walters PA.1972. Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride. Appl. Microbiol,. 24:859-863.

Luksamijarukul, Sundhiyodhin V, Luksamijarulkkul S, Kaewboonchoo C. 2004. Microbial air quality in mass transport buses and work-related illness among bus drivers of Bangkok mass transport authority. J. Med. Assoc. Thai. 87:697-703.

Mohr O, Askar M, Schnik S, Eckmanns T, Krause, Poggense G. 2012. Evidence for airborne infectious disease transmission in public ground transport – a literature review. Euro Surveill. 17(35):20255.

Ott, R. Lyman and Michael Longnecker. 2001. "An introduction to statistical methods and data analysis". 5th edition. Wadsworth Group: DUXBURY, 51 Forest Lodge Road, Pacific Grove, CA 93950 USA.

Reynolds, KA, Watt PM, Boone, SA, Gerba CP. 2005. Occurrence of bacteria and biochemical markers on public surfaces. Intl. J. Environ. Hlth. Res. 15:225-234.

Thormley CN, Emsile NA, Sprott TW, Greening GE, Rapana JP. 2011. Recurring norovirus transmission on an airplane. Clin. Infect. Dis. 53:515-520.

Troko, J. et al. 2011. Is public transport a risk factor for acute respiratory infection? BMC Infectious Diseases. 11:16. Doi:10.1186/1471-2334-11-16.

Wikswo, ME, Cortes J, Hall, AJ, Vaughan G, Howard C, Gregoricus N, Cramer EH. 2011.

Disease transmission and passenger behaviors during a high morbidly norovirus outbreak on a cruise ship, January 2009. Clin. Infect. Dis. 52:1116-1122.

Table 1
Average number of bacteria per 150 cm^2 in treated vs. untreated buses at baseline (before treatment of experimental buses)

Sample Type	Sample Size (N)	Arithmetic Mean	Standard Deviation	Geometric Mean	Standard Deviation of Log_{10} Transformed Measurements
Treated	35	57,114	254,392	783	1.13
Untreated	35	5,584	13,842	1,336	0.75

Table 2
Average number of bacteria per 150 cm^2 in treated vs. untreated buses after 30 days

Sample Type	Sample Size (N)	Arithmetic Mean	Standard Deviation	Geometric Mean	Standard Deviation of Log_{10} Transformed Measurements
Treated	35	867,754	2,563,567	5,870	1.69
Untreated	33*	2,285,438	4,391,445	83,588	1.58

*data for two sites were not available

Table 3
Average number of bacterial per 150 cm^2 at specific tested sites in treated and untreated buses

Sampled Site	Sample Type	Sample Size (N)	Geometric Mean	Percent Reduction	*p*-value
All Locations in Each Bus	Treated	35	5,870	93.0	0.005
	Untreated	33	83,588		
Drivers Compartment	Treated	7	815	99.8	0.007
	Untreated	6	364,738		
Entrance Railing	Treated	7	151,053	0.0	0.832
	Untreated	7	91,451		
Seat Backs	Treated	7	687	97.8	0.071
	Untreated	7	31,022		
Interior Railing	Treated	7	2,265	88.1	0.222
	Untreated	7	19,024		
Fare Box	Treated	7	36,356	88.2	0.253
	Untreated	6	308,280		