

# Investigation of Bacterial Pathogens on 70 Frequently Used Environmental Surfaces in a Large Urban U.S. University

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## Abstract

After reports of increased severity of bacterial infections from community institutions, a broad spectrum of 70 surfaces was sampled for potential bacterial pathogens in the morning and afternoon of one day per week over three consecutive weeks in a large U.S. university. Surfaces included public telephone mouthpieces, water fountain drains, student computer keyboards and desks, and buttons on elevators, vending machines, and photocopiers. A total of 420 samples was obtained. Bacterial counts were high on telephone mouthpieces, up to 168.8 colony-forming units (CFUs)-cm<sup>2</sup> of surface area. *Stenotrophomonas maltophilia* was isolated from 60% of fountain drains. Ninety percent of the keyboards showed positive bacterial cultures in the afternoon sampling. *Staphylococcus aureus* was identified on keyboards, telephone mouthpieces, and an elevator button. No *S. aureus* were methicillin-resistant. The swab sampling method reduced bacterial counts to less than or equal to 2.0 CFU-cm<sup>2</sup> on keyboards and telephone mouthpieces. Disinfectants for possible use in cleaning of telephones, water fountain drains, and keyboards are discussed.

## Introduction

Recent reports of life-threatening community-acquired bacterial infections emphasize the need to identify community surfaces that are rich in bacteria and have the potential to harbor pathogens (Libanore, Biccocchi, Pantaleoni, & Ghinelli, 2004; Romano, Lu, & Holtom, 2006). This study is the first to examine a broad array of surfaces in a large urban university in the midwestern United States. The univer-

sity location was chosen for its substantial population of primarily late-teens and early adults, within which athletes, immunocompromised individuals, and drug users can be found. In this age group, personal hygiene habits are not always optimal, and this can lead to the spread of infectious diseases (Drankiewicz & Dundes, 2003; Scott & Vanick, 2007).

Environmental surfaces in communities may receive substantial traffic. Such sur-

faces may support bacterial growth and may or may not receive adequate cleaning. Determining the number of bacteria and screening for the presence of potential pathogens on surfaces are initial steps in maintaining the health of individuals contacting those surfaces.

The first research aim of this study was to identify frequently used sites in the university where bacteria are most concentrated. The analysis was restricted to bacteria that grow on Mannitol salt agar or MacConkey agar. These two media were used for the second aim, that of identifying the presence of two potentially pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Stenotrophomonas maltophilia*. Both of these species can transfer from environmental surfaces to people. Our third aim was to test the hypothesis that bacterial counts on surfaces increase significantly across the working day. The study involved 70 surfaces and a university population of approximately 25,000 individuals.

The first pathogen of interest in this study, MRSA, is increasing in incidence in the local area. MRSA outbreaks have posed a major concern for collegiate sport teams and have been able to spread by direct contact (Romano, Lu, & Holtom, 2006). Methicillin-sensitive *S. aureus* also causes a variety of infections if the skin barrier is penetrated. In late teenagers and

**TABLE 1****Positive Culture Plate Incidence and CFUs on Mannitol Salt Agar for Surfaces in the University**

Surface Sampled	Morning Positive Culture Plates			Afternoon Positive Culture Plates		
	No. Positive Plates/ Total Plates	% of Total Number of Plates	Plate Range of CFU-cm <sup>-2</sup>	No. Positive Plates/ Total Plates	% of Total Number of Plates	Plate Range of CFU-cm <sup>-2</sup>
Telephone mouthpieces	19/30	63	0.0-27.6	9/30	30	0.0-168.8
Elevator buttons	7/30	23	0.0-9.0	9/30	30	0.0-6.4
Photocopier buttons	5/30	17	0.0-4.2	8/30	27	0.0-6.6
Computer keyboards	19/30	63	0.0-2.5	27/30	90	0.0-3.3
Student desks	6/30	20	0.0-9.6	11/30	37	0.0-1.3
Vending machine buttons	12/30	40	0.0-1.4	6/30	20	0.0-1.4

early adults at universities, such lesions from acne, cuts, or scrapes are common. MRSA arises as a result of *S. aureus* acquiring methicillin-resistance genes (Mlynarczyk, Mlynarczyk, & Jeljaszewicz, 1999). *S. aureus* survives for long periods (days or months) on surfaces such as fabrics, plastics, and Formica (Getchell-White, Donowitz, & Gröschel, 1989; Neely & Malley, 2000). The transfer efficiencies of 42% and 41% of gram-positive cocci from telephone receivers to hands and to mouths, respectively, suggest that *S. aureus* is easily transferred from surface to humans (Rusin, Maxwell, & Gerba, 2002).

The second pathogen of interest, *S. maltophilia*, can occur in tap water and faucets (Orenstein, Consolacion, Amihod, Perna, & Miller, 2006; Sakhnini, Weissmann, & Oren, 2002). It is opportunistic for immunocompromised people, for individuals with lung disorders (Denton & Kerr, 1998), and for intravenous drug abusers (Libanore, Bicocchi, Pantaleoni, & Ghinelli, 2004). It has been documented to cause nosocomial infection (Nicodemo & Garcia Paez, 2007).

## Materials and Methods

### Environmental Surfaces

Sampling of the environmental surfaces, selected for their frequent use, occurred on Thursdays across three consecutive weeks in the winter of 2007 (February 3, 10, and 17). These dates were chosen for sampling in order to gather data about the hygienic status of commonly used surfaces in the university when it is operating with maximum occupancy and with its personnel working inside the buildings. Ten each were sampled of the

following: student desktops, computer keyboards, telephone mouthpieces, water fountain drains, and buttons on photocopiers, vending machines, and elevators.

### Sampling

Each surface was sampled twice, starting at 6:15 a.m. prior to use, and then six hours later, for a total of 420 samples. A 10 cm<sup>2</sup> area of the surface (or the entire surface area if less than 10 cm<sup>2</sup>) was sampled with sterile saline-moistened polyester swabs each time. For samples from water fountain drains a moist swab was inserted 8–10 cm into the drain and the internal drain surface swabbed for three circumferences. All samples were plated onto agar plates within three hours of collection. For all surfaces, swabs were placed in 2 mL of saline, vortexed for 45 seconds, and then 0.5 mL of the sample was spread onto the agar plates. For the drain samples, serial dilutions (10<sup>-1</sup> to 10<sup>-8</sup>) were prepared in saline, and 0.5 mL of the dilutions were spread onto the agar plates. All agar plates were incubated for 36–48 hours at 35°C. The number of bacterial colony-forming units (CFUs) was counted. Bacterial counts were standardized and expressed as CFU×cm<sup>-2</sup> surface area sampled. For drains, bacterial density was expressed as CFU×mL<sup>-1</sup>.

### Identification of Bacteria

*S. aureus* was identified as Gram-positive, catalase-positive, and coagulase-positive (Bacti-Staph® kit, Remel, KS) colonies on Mannitol salt agar. *S. maltophilia* isolates were identified as lactose-fermentation-negative colonies that were oxidase-negative (Oxidase kit, Beckton Dickinson, BBL, MD) and esculin

hydrolysis-positive (Hardy Diagnostics, CA). *S. maltophilia* isolates were confirmed using the Microscan dried gram-negative ID panel (Dade Behring, CA) and testing of *S. aureus* colonies as MRSA was done using the PBP2 Test Kit (Oxoid, UK), in the Clinical Microbiology Laboratory at Children's Memorial Hospital, Chicago.

### Subsample Pilot Study

To estimate the array of bacteria obtained on the Mannitol salt agar and MacConkey agar plates used in the sampling of the environmental surfaces, a subsample of colonies from 20 plates was examined using Gram-staining and the tests described above for *S. aureus* and for *S. maltophilia*.

### Subsample Pilot Study of Impact of Swabbing on Reducing Bacterial Counts

A pilot study was performed to see if the bacterial count in the afternoon reflected both the accumulation across the day and also residual counts that remained after swabbing in the morning. To determine if the swabbing technique used in this study reduced bacterial counts to zero, the effect of swabbing surfaces was measured by sampling computer keyboards and telephone mouthpieces midafternoon on a workday. This sampling was repeated within one to two minutes and two sets (swab 1 and 2) were plated onto agar plates within two hours of collection.

### Statistical Analyses

The original data expressed as colony counts per surface area (CFU×cm<sup>-2</sup>) were analyzed because our study focused on the density of colonization of the surface at a moment in

**TABLE 2****Comparisons Between Surface Bacterial Counts (CFU·cm<sup>-2</sup>) on Mannitol Salt Agar**

Surface	Significant $\chi^2$ Differences* or Across the Day Change on Diagonal**					
	PM	EL	CO	KB	DS	VE
Phone mouthpieces	24(10)	275(35)	290(35)	277(55)	281(38)	271(32)
Elevator buttons	-	NS(13)	56(23)	NS(51)	52(24)	40(23)
Photocopier buttons	-	-	24(10)	NS(47)	39(23)	NS(26)
Computer keyboards	-	-	-	NS(28)	NS(49)	NS(51)
Student desks	-	-	-	-	NS(13)	NS(30)
Vending buttons	-	-	-	-	-	NS(13)

*Note.* Abbreviations: CO%photocopier buttons; DS%student desks; EL%elevator buttons; KB%computer keyboards; NS%not significant; PM%telephone mouthpieces; VE%vending machine buttons.

\*  $p \leq .05$ .

\*\* Degrees of freedom are shown in subscript font in brackets next to the Chi-square value. Comparisons of the bacterial counts for each surface across the day over three weeks are shown on the diagonal of the table.

time. The processing of standardized data for descriptive statistics and for comparisons by  $\chi^2$  or by Wilcoxon signed rank tests for paired data was performed using Prostat software (Version 4.0, Poly Software International, NY), with significance set at  $p \leq .05$ .

## Results

### Bacteria on MacConkey Agar

Bacteria were obtained from all 10 water fountain drains. Eighty percent of the drains contained biofilms, evidenced by visible films present on the swab. The bacterial counts for the drain samples ranged from  $1.0 \times 10^2$  to  $1.0 \times 10^5$  CFU·mL<sup>-1</sup>. *S. maltophilia* was found in 60% of the drains.

### Bacteria on Mannitol Salt Agar

Figure 1 shows the mean bacterial colony-forming units per surface area (CFU·cm<sup>-2</sup>) from sampled surfaces in the university. The highest mean bacterial counts were obtained for telephone mouthpieces. Table 1 shows the descriptive statistics for bacteria recovered on Mannitol salt agar for all surfaces except drains. Highest individual sample counts were for telephone mouthpieces. The highest proportion of plates positive for bacteria occurred for samples from computer keyboards. As reported in the first row of Table 2,  $\chi^2$  analyses showed the bacterial counts from the mouthpieces to be significantly higher ( $p < .05$ ) than those for other surfaces. The table

also shows that the counts for elevator buttons were significantly higher than all other surfaces except the telephone mouthpieces and computer keyboards. A significant increase across the day, shown on the diagonal of Table 2, occurred for the telephone mouthpieces and the photocopier buttons. *S. aureus* was observed on telephone mouthpieces, elevator buttons, and computer keyboards at individual counts of 0.2, 1.3 and 0.1 CFU·cm<sup>-2</sup>, respectively. None of these *S. aureus* samples was methicillin resistant.

### Subsampling

A subsample of 20 Mannitol salt agar plates containing "non-*S. aureus*" colonies was examined to estimate the other bacterial species present on such plates. These colonies were identified as coagulase-negative staphylococci, streptococci, and *Micrococcus* species. A further subsample of eight colonies was obtained on MacConkey agar plates from sampling of all surfaces except the water fountain drains. These colonies were all lactose-fermentation positive. No speciation of these identified bacteria was performed, since it was not the study focus.

### Effect of Swabbing on Reduction of Bacterial Counts on Telephone Mouthpieces and Computer Keyboards

Swabbing reduced the number of bacteria on telephone mouthpieces and student keyboards (Figure 2). Surfaces were sampled with a first

swab (solid bars) and then swabbed again within two minutes (open bars). The bacterial counts for most telephone mouthpieces and keyboards on the second swabbing were less than or equal to 2.0 CFU·cm<sup>-2</sup>, a statistically significant fall (Wilcoxon's  $z = 2.8$ ,  $p < .01$ ;  $z = 2.7$ ,  $p < .01$ , respectively).

## Discussion and Conclusion

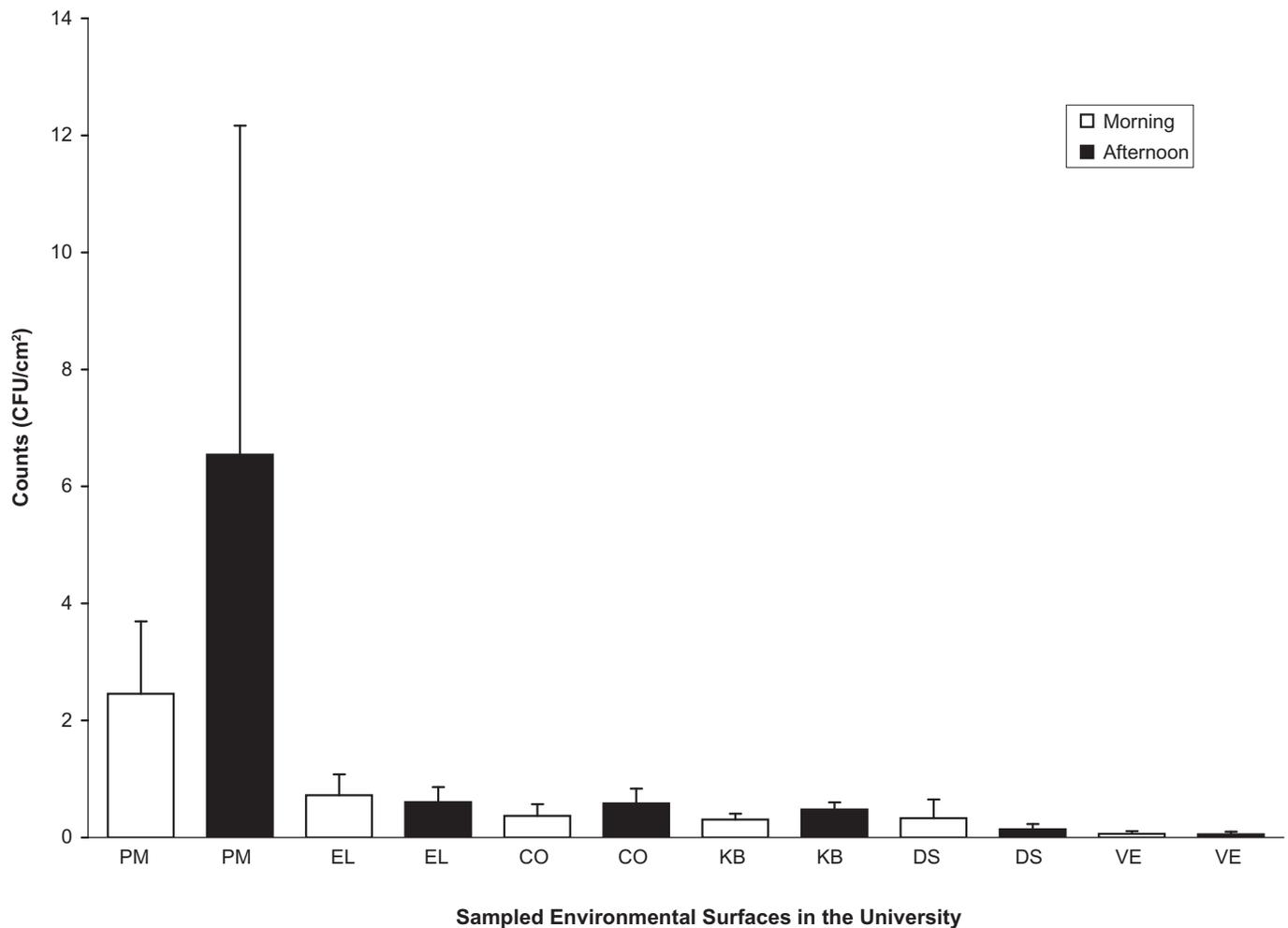
The data from this study show that in this large university community, the telephone mouthpieces showed the highest individual bacterial counts and had the highest numbers of bacteria compared to all other sampled surfaces except the drains (Table 1). It is noteworthy that in a recent study of hospital surfaces, we found that the bacterial counts on telephone mouthpieces also were higher than all other surfaces excluding sink drains (unpublished results). Additionally, a recent investigation of high school telephones reported high bacterial counts (Yalowitz & Brook, 2003). The number of bacteria on telephones may arise because of telephone use or because of the nature of the telephone surface, and moisture from exhaled water vapor from the user's mouth may support the growth of bacteria. *S. aureus* was detected on the telephone mouthpieces in the current study. A potential benefit comes from bringing cleaning expertise to bear on these devices. Cleaning telephone mouthpieces with 70% isopropyl alcohol may be appropriate (Ciragil, Gul, & Aral, 2005).

The biofilms of the university water fountain drains likely contain heterotrophic bacteria commonly found in tap water (Chaidez & Gerba, 2004). Biofilms can act as sources of infection (Costerton, Stewart, & Greenberg, 1999). The present study identified *S. maltophilia* in the biofilms from the university fountain drains. This bacterium is ubiquitous in freshwater aqueous environments; it can acquire antibiotic-resistant conferring genes, and in weakened individuals such as the immunocompromised, it can be pathogenic and even fatal (Denton & Kerr, 1998). The potential for this or other bacteria to cause an infection would be decreased by reducing the level of biofilm present. The use of hypochlorite cleaning agents has been effective for reducing bacterial counts (Rusin, Orosz-Coughlin, & Gerba, 1998).

As *S. maltophilia* is commonly found in tap water, it would be useful to determine if it is also present in the faucet aera-

# FIGURE 1

Mean Bacterial Colony-Forming Units per Surface Area of Sampled University Surface



Surfaces were sampled in the morning and in the afternoon. Vertical bars represent the standard error with  $n = 30$  samples per mean. Abbreviations: PM – telephone mouthpieces; EL – elevator buttons; CO – photocopier buttons; KB – computer keyboards; DS – desks; VE – vending machine buttons.

tors of university sink taps. Reports have shown that colonization of aerators can lead to amplification of infectious bacteria (Orenstein, Consolacion, Amihod, Perna, & Miller, 2006; Sakhnini, Weissmann, & Oren, 2002).

A pilot study revealed that swabbing significantly reduced bacterial counts obtained from a second swabbing within minutes, to a residual count of less than or equal to  $2.0 \text{ CFU} \times \text{cm}^{-2}$  on telephone mouthpieces and computer keyboards (Figure 2). These data indicated that the bacterial counts obtained from afternoon sampling of the surfaces in this study did not result solely from residual bacteria left behind after the morning sampling.

The residual count from the pilot study was used to define a “baseline.” The telephone mouthpieces were above this “baseline” in the morning sampling (Figure 1). The telephone mouthpieces and photocopier buttons showed a significant increase in bacterial counts in the afternoon (Table 2), providing some support for the study hypothesis that bacterial counts on surfaces increase significantly across the day.

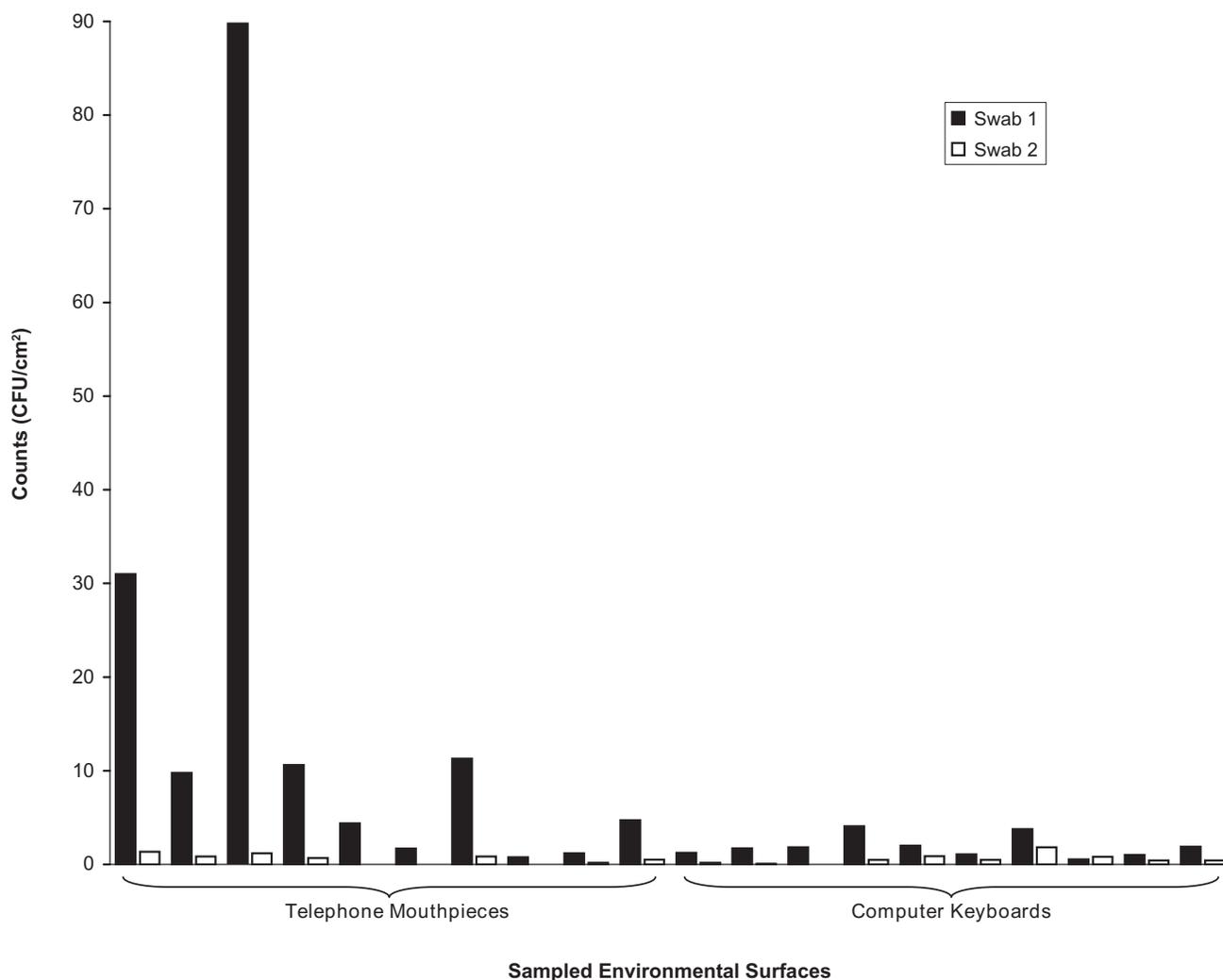
Bacterial contamination of keyboards and its potential for transfer to humans is a concern (Rutala, White, Gergen, & Weber, 2006). The keyboards in the university contained individual counts of 2.5 and  $3.3 \text{ CFU} \times \text{cm}^{-2}$ , above the “baseline” of  $2.0 \text{ CFU} \times \text{cm}^{-2}$  (Table 1). Using the swab

method, it was harder to reduce the bacterial counts on keyboards than on telephone mouthpieces (Figure 2). This may reflect the material of the keyboard. On certain surfaces, if not cleaned, potentially pathogenic bacteria can survive for days or even weeks (Kramer, Schwebke, & Kampf, 2006). Disinfectants containing chlorine, alcohol, or quaternary ammonium are effective for the cleaning of keyboards (Rutala, White, Gergen, & Weber, 2006). In studies of disinfectants used to clean environmental surfaces it was recognized that friction is also a positive factor (Muto et al., 2003; White, Dancer, & Robertson, 2007).

Over the remaining surfaces, individual sample values obtained above the “base-

## FIGURE 2

### Bacterial Colony-Forming Units per Surface Area for Repeat Swabbing of Surfaces



Telephone mouthpieces ( $n = 10$ ) and computer keyboards ( $n = 10$ ) were sampled.

line" level of  $2.0 \text{ CFU} \times \text{cm}^{-2}$  ranged from 9.6 to  $4.2 \text{ CFU} \times \text{cm}^{-2}$  (student desks and elevator and photocopier buttons). Assessment of the health risk posed requires account of the likelihood of a pathogen being present, its efficiency of transfer to and successful colonization in the host, the infectious dose for that person, and the immune status of the host. No such detailed assessment was made in the present study. Further studies are needed to determine transfer efficiencies, colonization, and infectious dose of potentially pathogenic bacteria found on surfaces such as those examined in this study.

MRSA was not found in the current study, although community-acquired MRSA car-

riage and infection are common in this city. A paper received after the completion of the present sampling offers improvements in sampling technique for MRSA, with use of methicillin contact plates to increase yields (Obee, Griffith, Cooper, & Bennion, 2007). Our present method did detect *S. aureus* on three surfaces.

Recent reports of community-acquired staphylococcal infections and the recent deaths of neonates infected by hospital-acquired bacteria, in some instances associated with sink drains, emphasize the importance of environmental monitoring for potential pathogens in selected circumstances (CHU Sainte-Justine, 2007; Mapa, 2007). Screening of commonly used surfaces in community in-

stitutions such as a large university can provide information about the general hygienic status of these surfaces. 🐛

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References on page 22

## REFERENCES continued from page 21

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