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SHORT COMMUNICATION

Public computer surfaces are reservoirs for methicillin-resistant staphylococci

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The role of computer keyboards used by students of a metropolitan university as reservoirs of antibiotic-resistant staphylococci was determined. Putative methicillin (oxacillin)-resistant staphylococci isolates were identified from keyboard swabs following a combination of biochemical and genetic analyses. Of 24 keyboards surveyed, 17 were contaminated with staphylococci that grew in the presence of oxacillin (2 mg l^{-1}). Methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA), -*S. epidermidis* (MRSE) and -*S. hominis* (MRSH) were present on two, five and two keyboards, respectively, while all three staphylococci co-contaminated one keyboard. Furthermore, these were found to be part of a greater community of oxacillin-resistant bacteria. Combined with the broad user base common to public computers, the presence of antibiotic-resistant staphylococci on keyboard surfaces might impact the transmission and prevalence of pathogens throughout the community.
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The prevalence of bacterial infections in humans is increasing (Eguia and Chambers, 2003; Weber, 2005) and has been shown to result in part from transmission of pathogens from the hospital setting to the community and vice versa (Eguia and Chambers, 2003; Hidron *et al.*, 2005; Lescure *et al.*, 2006). Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant nosocomial infectious threat, prompting several studies that have identified MRSA reservoirs in the hospital setting including bed rails, countertops, floors, bed linens, tables and blood pressure cuffs (Boyce *et al.*, 1997; Blythe *et al.*, 1998). Because of frequent dermal contact by numerous users, one reservoir of interest is computer keyboards, which have been shown to harbor MRSA in the hospital setting (Bures *et al.*, 2000; Devine *et al.*, 2001; Neely *et al.*, 2005; Wilson *et al.*, 2006). Of increasing concern, however, is the role of keyboards in the non-hospital environment as pathogen reservoirs. Because the asymptomatic carriage of MRSA in humans is increasing (Creech *et al.*, 2005; Hidron *et al.*, 2005) along with the occurrence of community-associated MRSA infections (Purcell and Fergie, 2005), it follows that the ubiquitous sharing of public computers by a broad user base might facilitate increased transmission and prevalence of MRSA throughout the community. Therefore, we targeted computer facilities in a metropolitan university to determine the prevalence of MRSA contamination of computers that feature a broad student and staff user base. Since our initial surveys suggested that keyboard surfaces were contaminated not only with MRSA, but also with mixed assemblages of antibiotic-resistant staphylococci, we additionally investigated the prevalence and structural complexity of oxacillin-resistant bacterial communities.

The surfaces of 24 computer keyboards in three, open-access, student computer facilities were sampled for bacteria with culture collection and transport swabs (Fisher Scientific, Inc., Pittsburgh, PA, USA) during operating hours featuring normal student/staff traffic. A control, 'field blank' swab that was briefly exposed to the air in each computer facility was also collected. Swabs were incubated (48 h at 35°C, with shaking) in tryptic soy broth (TSB) supplemented with oxacillin (2 mg l^{-1}) (Jonas *et al.*, 2002). Oxacillin has replaced methicillin as the current agent of choice for selecting bacteria that are resistant to penicillinase-stable penicillins. However, the designation 'methicillin-resistant' is still widely used and readily interchangeable with 'oxacillin-resistant' (Clinical and Laboratory Standards Institute, 2006). Seventeen of the enrichments exhibited turbid growth. Enrichments of field blank swabs resulted in no growth. An aliquot (100 μl)

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from each turbid culture was inoculated onto Baird-Parker agar (Remel Inc., Lenexa, KS, USA), a selective medium used to isolate putative *Staphylococcus* spp (Baird and Lee, 1995). Forty-five colonies exhibiting morphology common to *Staphylococcus* spp (convex, shiny, black colonies) were randomly selected from among all positive enrichments. Biochemical testing showed that all isolates were catalase-positive, while 10 were also coagulase-positive. DNA from each colony was isolated and used in a multiplex PCR analysis (Figure 1a) that targeted the 16S rRNA gene of *Staphylococcus* spp (Mason *et al.*, 2001), *femB* (Jonas *et al.*, 2002) and *mecA* (Jonas *et al.*, 2002). The targeted *femB* sequence is *S. aureus*-specific and encodes a protein crucial for peptidoglycan crosslinking, while *mecA* has been established as a standard marker for confirming methicillin resistance in staphylococci (Clinical and Laboratory Standards Institute, 2006).

Results revealed that five keyboards were contaminated with *S. aureus*, two of which contained MRSA (Table 1). rep-PCR (Del Vecchio *et al.*, 1995) fingerprints of the MRSA isolates ($n=6$) indicated the presence of two readily distinguishable genotypes, each contaminating each of the two keyboards. Since humans are known to harbor clonal populations of MRSA (Leski *et al.*, 1998), this result suggests the unique host origin of each MRSA type. Although typical risk factors that predispose keyboards of clinical computers to MRSA contamination (e.g. narrow user base with high potential exposure to MRSA) were not present in the current study, the proportion of keyboards that harbored MRSA was comparable to that reported for computers in clinical settings (Devine *et al.*, 2001; Fellowes *et al.*, 2006). This finding not only highlights the role of non-clinical computers as MRSA reservoirs, but also raises public health concerns when considering the large number of public computers available in the community (for example Internet cafes, public libraries and university campuses).

To establish the identity of isolates that were not identified as *S. aureus* or MRSA following initial analyses, a duplex PCR analysis that targeted two portions of the 16S rRNA gene specific to (i) *Staphylococcus* spp and (ii) *S. epidermidis* was performed (Martineau *et al.*, 1996). These analyses revealed that 10 keyboards were contaminated with *S. epidermidis*, 5 of which harbored *mecA*-contain-

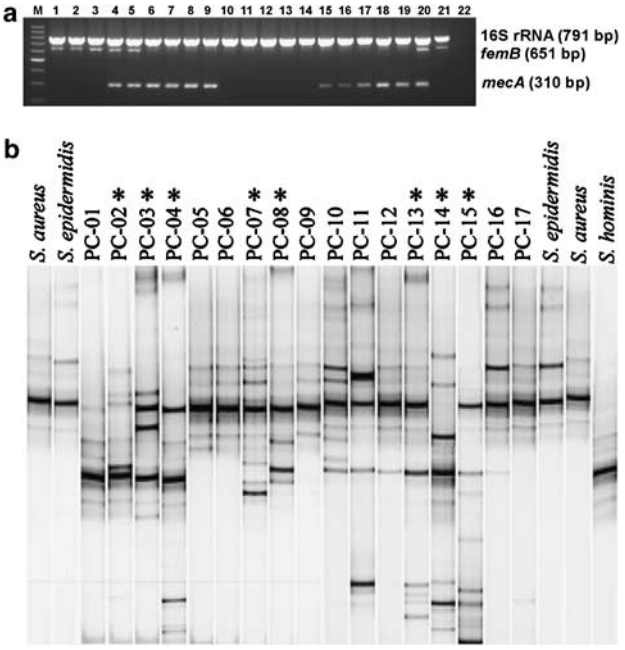

Figure 1 (**a**) Representative results of multiplex-PCR analysis targeting the 16S rRNA gene of *Staphylococcus* spp, *femB* and *mecA*. The isolates represented were chosen from among five different computer keyboards. Legend: M, 100 bp DNA size ladder; lanes 1–3, *mecA*$^-$ *S. aureus*; lanes 4, 5, MRSA; lanes 6–9 and 15–19, *mecA*$^+$ *Staphylococcus* spp (later identified as MRSE as described in text); lanes 10–14, *mecA*$^-$ *Staphylococcus* spp; lane 20, MRSA-positive DNA control; lane 21, *mecA*$^-$ *S. aureus*-positive DNA control; lane 22, negative DNA control (no template). (**b**) DGGE fingerprint of the 16S rRNA gene isolated from bacterial cultures growing in media supplemented with oxacillin (2 mg l^{-1}). Lanes marked '*' exhibited additional bands that were not diagnostic of *S. aureus*, *S. epidermidis* and *S. hominis* (see positive control lanes) indicating the presence of a potentially complex oxacillin-resistant bacterial community on each personal computer keyboard (PC-'XX').

Table 1 Summary of PCR-based detection of *S. aureus*, *S. epidermidis* and *S. hominis* (including oxacillin-resistant strains; MRSA, MRSE and MRSH) isolated from computer keyboards

Staphylococci strain	*Gene*				*No. of computers colonized (of 24 swabbed)*	*No. of isolates (52 total)*
	S. 16S rRNA[a]	*SE 16S rRNA*[b]	femB	mecA		
S. aureus	+	−	+	−	3	4
MRSA	+	−	+	+	2	6
S. epidermidis	+	+	−	−	10	22
MRSE[c]	+	+	−	+	5	15
MRSH[c]	+	−	−	+	2	5

+, positive detection of gene; −, negative detection of gene.
[a]*Staphylococcus* spp-specific 16S rRNA marker.
[b]*S. epidermidis*-specific 16S rRNA marker.
[c]Identity confirmed following DNA sequencing.

ing, methicillin-resistant *S. epidermidis* (MRSE) (Table 1). Because *S. epidermidis* has been shown to comprise a significant proportion of the coagulase-negative staphylococci associated with humans (Ben Saida *et al.*, 2006), the isolation of the bacterium from the keyboards was not surprising. However, increased virulence in *S. epidermidis* resulting from the acquisition of methicillin resistance has been recognized (Blum and Rodvold, 1987) and further reinforces the role of keyboards as pathogen reservoirs.

MRSA and MRSE were found to co-contaminate three keyboards, suggesting that the bacterial contamination of the keyboards might not be limited to simple bacterial assemblages, but rather resistant communities of considerable complexity. We assessed this complexity by performing denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.*, 1993) of DNA directly isolated from the TSB/oxacillin cultures described above. DGGE fingerprints revealed the presence of complex, oxacillin-resistant bacterial communities on eight of the keyboards (Figure 1b). This complexity was also confirmed by sequencing (192 bp of the 16S rRNA gene; Muyzer *et al.*, 1993) the DNA of seven, oxacillin-resistant (*mecA*-positive) staphylococci colonies collected from two of the culture-positive keyboards. BLAST analysis of these sequences revealed 100% similarity to *S. hominis* and *S. epidermidis* (Table 1) (GenBank accession numbers DQ831715–DQ831721). These results support the conclusion that the oxacillin-resistant bacterial community contaminating individual keyboards was not limited to MRSA and/or MRSE but was complex and included, for example, *S. hominis* (MRSH), a known nosocomial pathogen (Chaves *et al.*, 2005).

To our knowledge, the isolation of a mixed assemblage of oxacillin-resistant pathogens, including MRSA, MRSE and MRSH, from public, non-hospital fomites, has not been previously reported. The significance of this finding concerns not only the isolation of pathogens from public computer keyboards, but also the potential of pathogen transmission to numerous computer users. The frequent occurrence of *mecA* in isolated staphylococci (Table 1) combined with the complexity of the bacterial community contaminating the keyboards (Figure 1b) also raises concerns surrounding the potential for interspecies transfer of genes conferring antibiotic resistance. This has been shown to occur among staphylococci, including *S. aureus* and *S. epidermidis* (Forbes and Schaberg, 1983; Hanssen *et al.*, 2004), and could effectively increase the diversity and number of antibiotic-resistant pathogens in this public reservoir.

Overall, our findings highlight the necessity for public awareness to hygiene following the use of public facilities and warrant further investigation into the epidemiology of community-associated MRSA and other staphylococci. The factors driving the contamination of the keyboards are unknown. However, the widespread nasal carriage of staphylococci by humans (Graham *et al.*, 2006) likely facilitated the contamination via (i) hand-to-mouth or hand-to-nose contact while using the keyboard, and/or (ii) poor hand-washing habits (American Society for Microbiology, 2005). Although we established that public computers are reservoirs of oxacillin-resistant pathogens, the duration of bacterial survival and transmission rate to humans in community settings remains unknown. Therefore, subsequent steps to limit the spread of antibiotic-resistant pathogens throughout the community should include efforts to not only increase awareness of appropriate hygiene and decontamination strategies, but also to elucidate the ecology of bacteria contaminating community fomites.

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