Epidemiol. Infect. (2012), **140**, 1366–1371. © Cambridge University Press 2011
doi:10.1017/S095026881100207X

SHORT REPORT
Characterization of staphylococci contaminating automated teller machines in Hong Kong

M. ZHANG[1], M. O'DONONGHUE[2] AND M. V. BOOST[1]*

[1] *Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China*
[2] *School of Nursing, The Hong Kong Polytechnic University, Hong Kong SAR, China*

(*Accepted 17 September 2011; first published online 19 October 2011*)

SUMMARY

Environmental staphylococcal contamination was investigated by culture of 400 automated teller machines (ATMs). Isolates were characterized for antibiotic and antiseptic susceptibility, carriage of antiseptic resistance genes (QAC genes), and *spa* types. MRSA, which was similar to local clinical isolates, was present on two (0·5%) of the 62 (15·5%) ATMs that yielded *Staphylococcus aureus*. QAC genes were more common in coagulase-negative staphylococci (*qac*A/B 26·0%, *smr* 14%) than *S. aureus* (11·3% *qac*A/B, 1·6% *smr*). QAC-positive isolates had significantly higher minimum inhibitory concentrations/minimum bactericidal concentrations to benzalkonium chloride and chlorhexidine digluconate. QAC gene presence was significantly associated with methicillin and tetracycline resistance. Survival of staphylococci, including MRSA, on common access sites may be facilitated by low disinfectant concentrations, which select for disinfectant-tolerant strains, while co-selecting for antibiotic-resistance determinants. Disinfection procedures should be performed correctly to help prevent spread of resistant pathogens from reservoirs in the community.

Key words: Disinfectant, enterotoxin, environment, MRSA, *qac*A/B.

Methicillin-resistant *Staphylococcus aureus* (MRSA), for many years an important hospital-associated pathogen, has recently emerged in the community, leading to increased public concern about the risks of infection, including from contamination of the environment. Although other body sites may be colonized, the most important niche for *S. aureus* is the anterior nares and colonization at this site increases the risk for clinical infection [1]. However, investigations of community-associated MRSA (CA-MRSA) outbreaks have failed to isolate the strain from the nares of any of the infected subjects, leading to suggestions that CA-MRSA may transmit through sharing or contact with common items. This is supported by reports of clusters of CA-MRSA infection in athletes, military recruits and prisoners who share common facilities [2]. However, there are also isolated infections in patients who do not participate in sports or belong to these at-risk groups.

Several studies have demonstrated that although cases of CA-MRSA have increased rapidly, rates of MRSA colonization in non-healthcare workers in the community remain low. Sampling of household contacts of MRSA-colonized patients frequently yields low rates of other colonized subjects [3] and, thus, the

* Author for correspondence: Dr M. V. Boost, Associate Professor, Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China.
(Email: htmboost@polyu.edu.hk)

possibility of contracting the organism from an environmental source must be considered. Many items in the community are touched by numerous people during the day and may act as sources of infection. 'Contaminated objects, surfaces, and items' is one of the 'Five Cs' of CA-MRSA transmission developed by the Centers for Disease Control and Transmission [1]. Automated teller machines (ATMs) can be contaminated by *S. aureus*, frequently present on the fingers due to nose picking or touching the nasal area. We previously reported a relatively higher isolation rate of *S. aureus* from ATMs than from other commonly accessed items in the environment [4]. Presence of *S. aureus* on a cash machine has also been reported in the UK [5] and recent news articles in UK have also described contamination of ATMs.

The importance of decontamination by cleaning in order to reduce the risk of MRSA/*S. aureus* infection has long been stressed, and aggressive environmental cleaning is a key component of the successful 'search-and-destroy' policy to minimize MRSA infection in The Netherlands. Since the outbreaks of SARS in Hong Kong in 2003, disinfection of commonly contacted items in the community such as ATMs and lift buttons has been performed. This practice was re-enforced after the recent H1N1 influenza epidemic. However, there is concern about emerging resistance to antiseptics used as decolonization and decontamination agents. Staphylococcal strains harbouring genes that increase resistance to quaternary ammonium compounds and other disinfectants (QAC genes), including *qac*A/B and *smr*, have elevated minimum bactericidal concentrations (MBCs) to antiseptics [6] and have been demonstrated in clinical isolates of MRSA [7]. Although the elevated MBCs remain below the in-use concentrations, the significance of antiseptic-resistance gene carriage remains unclear. It has been suggested that elevated MBCs may allow persistence of organisms when cleaning is not performed correctly, increasing infection risks from the environment. The distribution of QAC genes in *S. aureus* and coagulase-negative staphylococci (CNS) has been investigated in clinical isolates, with most focus on MRSA [7]. However, little is known about the frequency of these genes in isolates from the community environment. Both *S. aureus* and CNS were investigated, as the QAC genes are plasmid mediated and can therefore transfer between species.

In this study, we investigated the frequency of staphylococcal contamination of the environment, using ATMs as a marker, and determined the prevalence of QAC genes in these contaminating staphylococci. Biocide susceptibilities of strains with QAC genes were determined.

A minimum sample size for ATMs of 202 was estimated for a cross-sectional study based on a *S. aureus* contamination rate of 20% and an assumed 5% carriage rate of QAC genes in *S. aureus* and CNS with 3% error and 95% confidence intervals. Samples were collected from 400 terminals situated throughout Hong Kong between May 2009 and May 2010. The cash-points were identified and allocated to one of five geographical areas, each having about the same number of terminals. Forty machines, eight from each area, were sampled each month by swabbing three designated buttons, including the 'enter' button, of each ATM with saline-moistened transport swabs (Transwabs, Medical Wire & Equipment, UK) and cultured within 2 h of collection.

Swabs were placed into 5% salt brain heart infusion broth (Oxoid Ltd, UK). After 24-h incubation, broths were subcultured onto *S. aureus* selective agar (SA Select, BioRad, USA), a chromogenic agar which selects for growth of all staphylococci and allows for their differentiation by colony colour. Plates were incubated for 24 h. All organisms with staphylococcal morphology were identified by Gram stain, catalase and coagulase tests, and confirmed as staphylococci by 16S rRNA genes. *S. aureus* was confirmed by a positive Staphaurex Plus test (Murex Biotech, UK) and presence of the *fem*A gene. *S. epidermidis* was differentiated from other CNS by PCR [8]. The presence of Panton–Valentine leucocin (PVL) was investigated by PCR.

Susceptibility to a range of antibiotics was determined by disc diffusion for all *S. aureus* isolates. Vancomycin minimum inhibitory concentration (MIC) was determined by broth dilution. The presence of the *mec*A gene was determined for all isolates. For *mec*A-positive isolates, the SCC*mec* type and *spa* type was determined.

Plasmid DNA, extracted by a modified alkaline lysis method, with addition of lysostaphin and lysozyme (Sigma-Aldrich, USA), was used to perform PCR for detection of *qac*A/B, *smr*, *qac*G, *qac*H, and *qac*J as previously described [9, 10]. The reaction master mix consisted of: 100 ng of extracted plasmid DNA, 1·0 ml reaction buffer, 1·5 mM $MgCl_2$, 0·2 mM dNTPs, 0·2 μM of each primer and 0·5 U *Taq* polymerase in 15 μl total volume (Promega Corp., USA). Amplification was performed using 30 cycles of

https://doi.org/10.1017/S095026881100207X Published online by Cambridge University Press

30 s denaturation at 95 °C, 30 s annealing at 53 °C (*qac*A/B, *smr*), or 50 °C (*qac*G, H, J), and one min extension at 72 °C. The PCR products were visualized by electrophoresis in 2% agarose and compared with those amplified from control *S. aureus* strains: TS77 (*qac*A/B), L20 (*smr*), RN4220 (pSK265, *qac*G), RN4220 (pSK265, *qac*H), and RN4220 (pSK265, *qac*J).

The MICs and MBCs of benzalkonium chloride (BC) and chlorhexidine digluconate (CHG) (Sigma-Aldrich Ltd) were determined in duplicate by broth microdilution with concentrations ranging from 0·5–256 mg/l for both QAC gene-positive and gene-negative isolates. The lowest concentration totally inhibiting growth after 24-h incubation at 37 °C was considered the MIC. The MBCs were calculated as the concentration producing a 99·9% kill and determined after addition of Dey–Engley broth as a neutralizing reagent.

Statistical analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., USA). Association of categorical variables was determined by χ^2 or Fisher's exact tests. Mann–Whitney *U* test was used to compare the MBC and MIC results.

S. aureus was present on 15·5% (62/400) of ATMs, two isolates (0·5%) being MRSA. Of these, one harboured SCC*mec* type IVa, and was *spa* type t091 and the second, SCC*mec* type V and *spa* type t1081. Almost all (95·3%) ATMs were colonized with CNS, of which 67·2% of strains were *S. epidermidis*. *mec*A was present in 15·3% (60/381) of CNS isolates. There was no significant association between location and month of sampling with rate of contamination.

In *S. aureus* isolates, resistance was observed to penicillin (87·1%), cefoxitin (3·2%), chloramphenicol (3·2%), ciprofloxacin (3·2%), trimethoprim–sulphamethoxazole (6·5%), gentamicin (8·1%), clindamycin (6·5%), tetracycline (22·6%), imipenem (1·6%), fusidic acid (33·9%) and erythromycin (29%). All isolates were susceptible to linezolid and vancomycin. Rates of resistance were similar to those for nasal *S. aureus* isolates from Hong Kong, except for fusidic acid resistance which was somewhat higher [6]. None of the isolates harboured genes for PVL production.

Prevalence of both *qac*A/B and *smr* was significantly higher in CNS than *S. aureus* [CNS: 26·0% and 14% (two strains harboured both genes); *S. aureus* 11·3% and 1·6%; $P<0·05$]. Only two *S. aureus* and one CNS harboured *qac*G, while *qac*J was present in one CNS isolate. No sample harboured *qac*H. *qac*A/B was significantly associated with the presence of *mec*A ($P<0·001$) and was present in both MRSA strains, but in only 8·3% (5/60) of methicillin-sensitive *S. aureus* isolates ($P=0·01$).

Resistance to tetracycline was significantly more frequent in QAC-positive (50%, 5/10) than QAC-negative (17·3% 9/52) *S. aureus* ($P=0·024$). There was no association between other antibiotics and QAC genes.

Biocide MICs and MBCs were performed for all *qac*A/B ($n=7$, 2 being MRSA) and *smr* (1) positive *S. aureus* and 58 CNS (20 *qac*A/B only, 20 *smr* only, and 18 with both *qac*A/B and *smr*), and for 40 gene negative controls (20 each *S. aureus* and CNS). The MIC/MBC range, MIC_{50}/MBC_{50}, and MIC_{90}/MBC_{90}, are shown in Table 1. Isolates with QAC genes had higher mean MICs and MBCs to both BC and CHG, with a wider range of MICs and MBCs, than isolates without ($P<0·05$).

This is one of the first reports of isolation of MRSA from an inanimate surface in a community environment. Until recently, the role of the environment in the spread of MRSA was not recognized, and most national and other guidelines provide few details on environmental decontamination regimens. The emergence of CA-MRSA has focused more attention on sources of the organism within the community, with most emphasis on identifying human and animal colonization. Other studies have reported MRSA from restricted non-healthcare sites such as fire stations and university computers [11, 12], but as contacts with these areas are limited, the potential for spread is reduced. Recently, typical hospital-associated MRSA were isolated from handrails of public buses in Portugal [13]. Our study showed that 0·5% of sites examined were MRSA-contaminated. This low rate was to be expected, since, although CA-MRSA infections are frequently reported in Hong Kong [14], nasal colonization with MRSA remains at >1·0% in non-healthcare exposed persons [6], although it may be present at other body sites. However, ATMs are accessed by numerous individuals over the course of a day and a contaminated machine could act as a source of the organism resulting in contaminated hands and potentially lead to colonization of the subject. In contrast to the Portuguese isolate [13], the two MRSA isolates in our study were typical CA-MRSA, and both types have been reported in clinical isolates in Hong Kong [14]. Our earlier study, which showed that ATMs were frequently contaminated with *S. aureus*, did

https://doi.org/10.1017/S095026881100207X Published online by Cambridge University Press

Table 1. *Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) to BC and CHG of* S. aureus *and CNS isolates with and without QAC genes*

			MIC BC				MBC BC			
Strain	QAC genes	*N*	Range	MIC_{50}	MIC_{90}	*P* value*	Range	MIC_{50}	MIC_{90}	*P* value*
S. aureus	*qac*A/B	7	4–8	8	8	0·001	8–32	16	32	0·048
	smr	1	8	—	—	0·095	16	—	—	0·571
	Negative	20	2–4	4	4	—	4–32	8	32	—
CNS	*qac*A/B	20	4–8	4	8	<0·001	4–64	8	64	0·004
	smr	20	2–16	4	8	0·003	4–64	8	32	0·013
	*qac*A/B & *smr*	18	1–8	4	8	0·001	8–64	16	64	<0·001
	negative	20	0·25–8	2	4	—	1–32	4	16	—
Total	*qac*A/B	27	4–8	4	8	<0·001	4–64	8	64	0·005
	smr	21	2–16	4	8	0·020	4–64	8	32	0·068
	*qac*A/B & *smr*	18	1–8	4	8	0·004	8–64	16	64	<0·001
	Negative	40	0·25–8	2	4	—	1–32	8	16	—
			MIC CHG				MBC CHG			
Strain	QAC genes	*N*	Range	MIC_{50}	MIC_{90}	*P* value*	Range	MIC_{50}	MIC_{90}	*P* value*
S. aureus	*qac*A/B	7	2–4	4	4	0·041	4–16	16	16	0·219
	smr	1	4	—	—	0·095	32	—	—	0·095
	Negative	20	2–4	2	2	—	4–16	8	16	—
CNS	*qac*A/B	20	2–4	4	4	<0·001	2–16	8	8	0·047
	smr	20	2–4	2	4	0·001	4–32	8	32	0·003
	*qac*A/B & *smr*	18	1–4	2	4	0·003	1–32	8	32	0·048
	Negative	20	0·25–2	2	2	—	1–32	4	16	—
Total	*qac*A/B	27	2–4	4	4	<0·001	2–16	8	16	0·244
	smr	21	2–4	2	4	0·001	4–32	8	32	0·026
	*qac*A/B & *smr*	18	1–4	2	4	0·018	1–32	8	32	0·636
	Negative	40	0·25–4	2	2	—	1–32	8	16	—

BC, Benzalkonium chloride; CHG, chlorhexidine digluconate; QAC, quaternary ammonium compound; CNS, coagulase-negative staphylococci.

* Comparison to isolates without QAC genes, χ^2 test.

not yield MRSA [4]. This may be due to the small sample size or changes in selective and enrichment methods.

Both *S. aureus* and CNS harboured QAC genes, although the rate was higher in CNS. However, as these genes are plasmid-mediated, there is potential for spread between staphylococcal species. The isolates from ATMs had a significantly higher rate of positivity of QAC genes than isolates of staphylococci from healthy subjects not employed in healthcare reported in our recent study [6]. In that study, *qac*A/B was present in 12·5% of isolates and *smr* in 7%, compared to 23·9% and 12%, respectively, in the current study (*qac*A/B: OR 1·9, 95% CI 1·3–2·7, $P=0·001$; *smr*: OR 1·8, 95% CI 1·1–2·9 $P=0·016$). This higher rate suggests that QAC gene positive strains may have a survival advantage in the environment, especially if low levels of disinfectant residues are present on ATMs. Since the SARS outbreak in 2003 in Hong Kong, many items, including ATM pads, lift keys, and public telephones, are regularly cleaned with disinfectant, although the frequency and thoroughness varies between individual sites. The current sample was collected immediately after the first isolations of H1N1 in Hong Kong in April 2009, when the frequency of cleaning was once more enhanced. Such frequently contacted items in the environment could act as a source for dissemination in the population of strains positive for QAC genes. *qac*G, H and J, which were identified in isolates from food and animal sources, remain rare in human staphylococcal isolates.

The presence of QAC genes has been associated with increased antibiotic resistance and the possibility

https://doi.org/10.1017/S095026881100207X Published online by Cambridge University Press

of co-selection suggested, in particular with respect to methicillin resistance [7]. Both MRSA isolates in our study carried QAC genes. Association between tetracycline-, gentamicin- and erythromycin-resistance determinants and the presence of QAC genes has been reported previously [15], as both types of genes may be present on the same plasmid. These associations were reflected in higher rates of tetracycline resistance in QAC-positive isolates in this study. The co-existence of *mec*A and QAC genes may contribute to the survival of methicillin-resistant strains in the environment, by allowing their persistence in areas with low-level antiseptic residues, thereby increasing the risk of spread of MRSA in the community. Use of biocides increases exposure of organisms to residues of disinfectants, in turn increasing selective pressure for carriage of QAC genes. In this study, isolates harbouring QAC genes had reduced susceptibility to BC and CHG compared to gene-negative strains.

As use of an ATM is generally not perceived as a risky behaviour, many people would not consider themselves as 'contaminated' and be willing to prepare or consume food without washing their hands after contact with a machine. In addition, spread by fingers to the nose increases the risk of nasal colonization with such strains and their later dissemination to close contacts and contamination of food during preparation.

Although environmental cleaning may have an important role to play in control of infection, if disinfectant agents are used to reduce this risk, it is essential that this is done in accordance with guidelines covering correct in-use concentration and with regular rinsing of wiping cloths. Failure to adhere to correct practice results in low efficiency of disinfection and possible selection of more disinfectant-tolerant organisms. The potential for these more resistant organisms to harbour other virulence factors increases the need for both proper disinfection procedures and surveillance of organisms in the community.

ACKNOWLEDGEMENTS

The study was funded by a grant from the The Hong Kong Polytechnic University (grant no. YG97). The authors are grateful to Mr Fred Wong for technical assistance and thank Professor K. Hiramatsu and Dr J. Bjorland for donations of control strains.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Miller LG, Diep BA.** Colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Diseases* 2008; **46**: 752–760.
2. **David MZ, *et al.*** Predominance of methicillin-resistant *Staphylococcus aureus* among pathogens causing skin and soft tissue infections in a large urban jail: risk factors and recurrence rates. *Journal of Clinical Microbiology* 2008; **46**: 3222–3227.
3. **Zafar U, *et al.*** Prevalence of nasal colonization among patients with community-associated methicillin-resistant *Staphylococcus aureus* infection and their household contacts. *Infection Control and Hospital Epidemiology* 2007; **28**: 966–969.
4. **Boost M, *et al.*** *Staphylococcus aureus*: what are the levels of contamination of common-access environmental surfaces? *Infection Control and Hospital Epidemiology* 2008; **29**: 194–196.
5. **Otter JA, French GL.** Bacterial contamination on touch surfaces in the public transport system and in public areas of a hospital in London. *Letters in Applied Microbiology* 2009; **49**: 803–805.
6. **Zhang M, *et al.*** Prevalence of antiseptic resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonizing nurses and the general population. *Journal of Hospital Infection* 2011; **78**: 113–117.
7. **Smith K, Gemmell CG, Hunter IS.** The association between biocide tolerance and the presence or absence of genes among hospital-acquired and community-acquired MRSA isolates. *Journal of Antimicrobial Chemotherapy* 2008; **61**: 78–84.
8. **Martineau F, *et al.*** Development of a PCR assay for identification of staphylococci at genus and species levels. *Journal of Clinical Microbiology* 2001; **39**: 2541–2547.
9. **Bjorland J, *et al.*** Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. *Journal of Clinical Microbiology* 2005; **43**: 4363–4368.
10. **Noguchi N, *et al.*** Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qac*A/B and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. *Journal of Medical Microbiology* 2005; **54**: 557–565.
11. **Roberts MC, *et al.*** Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from fire stations in two northwest fire districts. *American Journal of Infection Control*. Published online: 17 February 2011. doi:10.1016.ajic.2010.09.008.
12. **Kassem II, *et al.*** Public computer surfaces are reservoirs for methicillin-resistant staphylococci. *ISME Journal* 2007; **1**: 265–268.

13. **Simoes RR, *et al.*** High prevalence of EMRSA-15 in Portuguese public buses: a worrisome finding. *PLoS One* 2011; **6**: e17630.
14. **Ho PL, *et al.*** Community-associated methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*: skin and soft tissue infections in Hong Kong. *Diagnostic Microbiology and Infectious Disease* 2008; **61**: 245–250.
15. **Sidhu MS, *et al.*** Frequency of disinfectant resistance genes and genetic linkage with beta-lactamase transposon Tn552 among clinical staphylococci. *Antimicrobial Agents and Chemotherapy* 2002; **46**: 2797–2803.

https://doi.org/10.1017/S095026881100207X Published online by Cambridge University Press