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Sustainable reduction of nasal colonization and hand contamination with *Staphylococcus aureus* in food handlers, 2002–2011

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SUMMARY

A longitudinal study of nasal colonization and hand contamination of food handlers with *Staphylococcus aureus* commenced in 2002 prior to the outbreak of severe acute respiratory syndrome. In the follow-up in 2003 when hygiene measures were strictly implemented, significant reductions in carriage were observed. To investigate if this change was sustained, nasal and hand carriage rates were compared between the earlier studies and a further sampling in 2011. The initial nasal carriage rate was 35% and hand contamination 41·2%, decreasing to 23·5% and 11·6%, respectively in 2003 ($P < 0{\cdot}001$). In 2011, nasal carriage was similar to 2003 (22·9%), while hand contamination dropped further to 3·7% ($P < 0{\cdot}001$). *Spa*-typing revealed 39 types in 2002 and 42 in 2011. This study reveals that the marked reduction in colonization had been sustained. This may be attributed to reduced opportunities for spread due to enhanced hygiene and reinforces its importance for control of disease.

Key words: Colonization, food handler, *S*, *aureus*.

INTRODUCTION

Nasal carriage and hand contamination of food handlers with *Staphylococcus aureus* have been implicated in food poisoning outbreaks [1, 2]. Investigation of staphylococcal food poisoning has revealed that colonizing strains of food handlers were attributable in more than 50% of cases [3]. Contamination of food with *S. aureus* and subsequent growth at ambient temperature leads to production of enterotoxins which are secreted into the food matrix [1].

It is estimated that *S. aureus* is persistently carried by 20% of healthy individuals who act as reservoirs for its dissemination, while others are occasionally colonized [4]. The percentage of transient carriers can vary affecting the overall prevalence of nasal carriage in a community at a given time.

To investigate patterns of colonization and hand contamination with *S. aureus* in Hong Kong food handlers, a longitudinal study was initiated in autumn 2002. Collection of the second round of samples commenced in spring 2003 but because of the severe acute respiratory syndrome (SARS) outbreak most of the follow-up samples were collected 6–8 months after the initial sampling which coincided with the end of the outbreak. During the SARS epidemic, improved hand hygiene practices and use of face masks and gloves were strictly implemented for food handlers to reduce viral transmission. Surveillance data indicated that there was a significant decrease in episodes of food poisoning in 2003 which was attributed to compliance with the enhanced hygiene measures [5]. Measures undertaken during the SARS outbreak

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were supplemented in early 2005 by several strategies including mandatory employment of a certified hygiene officer at all catering establishments and restaurants whose major role was supervision of hand washing and other personal hygiene practices. The hygiene officers were also responsible for providing instructions to all food handlers regarding the proper hand washing technique. In addition, government inspection of food premises was made more rigorous. However, whether the effect of the changes in food handling practices has led to a sustainable effect on nasal carriage and hand contamination has not been investigated. This study aimed to compare prevalence of *S. aureus* carriage in food handlers over a decade and investigate characteristics of the isolates.

Table 1. *Distribution of subjects and sites sampled*

	Number of food handlers recruited (number of establishments sampled)		
	2002	2003	2011
Hotel	–	–	203 (2)
Supermarket	306 (7)	264 (7)	–
College canteen	80 (5)	80 (5)	93 (2)
Centralized kitchen for hospitals*	103 (2)	58 (1)	40 (1)
Centralized kitchen for sports facility*	130 (1)	125 (1)	98 (1)
Total	**619 (15)**	**527 (14)**	**434 (6)**

* These facilities were visited on all three occasions.

METHODS

Study participants

Between mid-October and mid-December 2002, samples were collected from 619 food handlers at 15 catering establishments. Fourteen of these premises, which included supermarkets, college canteens, centralized kitchens for local hospitals and for a major sporting facility, were revisited in May/June 2003 and a further 527 samples collected. Of the participants, 499 were sampled on both occasions and those repeatedly positive for *S. aureus* were defined as persistent carriers. In 2011, 434 food handlers were recruited from six large catering establishments which included hotels, college canteens, and centralized kitchens for local hospitals and for a major sporting facility. The sample size was based on the prevalence observed in a recent study of the general public [6]. Participants in the studies of 2002 and 2011 completed a short questionnaire concerning risk factors for colonization, including demographic data, smoking, years of working experience, and foods handled. In 2011 additional questions involving family members working in healthcare, pet ownership, wearing gloves at work, and recent hospitalization or antibiotic use were added. Details of participating establishments are given in Table 1.

Ethical statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, revised in 2008. Ethical approval was obtained from the University Human Subjects Ethics Committee. Each subject was provided with a brief description of the study and gave written consent.

Specimen collection

Nasal swabs were collected from the anterior nares of study participants 1–2 hours after commencement of their shift by the research team using saline-moistened transport swabs (Oxoid, UK), placed in brain heart infusion broth (Oxoid) supplemented with 5% sodium chloride for enrichment and incubated overnight at 37 °C. In 2002 and 2003, specimens were subsequently subcultured onto mannitol salt agar (Oxoid), while for 2011 samples MRSASelect™ chromogenic agar (Bio-Rad UK Ltd, UK) was used. The use of this recently introduced chromogenic agar allowed *S. aureus* to be more easily differentiated from other staphylococci. On all sampling occasions, hand contamination was determined by fingertip impression of the dominant hand onto mannitol salt agar. Food handlers were instructed not to wash their hands prior to providing the sample. All agar plates were incubated overnight at 37 °C. Colonies resembling *S. aureus* were picked from primary selective agars and pure cultures obtained by subculture on tryptic soy agar (Oxoid). In order to assess reproducibility of the sampling methodology, sampling was repeated at one institution 2 weeks after the initial investigation of carriage rates.

Bacterial identification and characterization

All isolates were confirmed as *S. aureus* by DNase production, Staphaurex Plus latex agglutination test

(Remel, USA) and amplification of the 16S rRNA gene [7]. Methicillin resistance was detected by disc diffusion using cefoxitin and oxacillin [8] and confirmed by *mec*A detection which was performed simultaneously with SCC*mec* typing by a multiplex PCR [9]. Clonal relationships of isolates were identified by sequencing the polymorphic region of the *spa* gene [10].

Susceptibility to other antimicrobial agents; fusidic acid, quinupristin-dalfopristin, erythromycin, clindamycin, co-trimoxazole, ciprofloxacin, gentamicin, chloramphenicol and tetracycline, was determined by disc diffusion [8] except for fusidic acid susceptibility which was performed according to EUCAST guidelines [11]. All zone sizes were determined using an automatic reader, Mastascan Elite (Mast Group Ltd, UK).

Data analysis

Differences in carriage rates over time were compared by trend analysis of the total food-handler population as well as subgroups of food handlers in different types of workplace. Association of potential risk factors with carriage was determined using χ^2 test followed by multivariate analysis using a backward logistic regression model to control for confounders. Analysis was performed using SPSS software v. 19·0 for Windows (SPSS Inc., USA). A P value <0·05 was considered to be statistically significant. Characteristics of the *spa* loci of the isolates from persistent carriers were compared and the mutation rate calculated as number of mutations divided by time in months [12]. The epidemic index (E) was calculated as E = 1 – D, where D refers to Simpson's index of diversity, in order to compare diversity of *spa* types in 2002 and 2011 [13, 14]. Isolates were grouped into clonal clusters using the criteria of Ruppitsch *et al.* [15].

RESULTS

Comparison of the baseline characteristics of the subjects revealed that there were significant differences in proportions of male food handlers and food handlers who were smokers (Table 2). Smoking was found to be significantly associated with male gender (P < 0·001). The nasal carriage rate in 2002 prior to the SARS outbreak was 35% and hand contamination was 41·2%. Repetition of sampling for consistency at one institution yielded an identical nasal carriage rate (28·9%) although some of the nasally colonized participants differed between the samplings. The hand contamination rate was also similar (15·5% first sample, 17·8% second sample). This repeated estimate was not included in the overall calculation.

Table 2. *Comparison of characteristics of participating subjects in 2002 and 2011*

	Percentage of subjects		
	2002	2011	P value*
Gender			
Male	50·5	66·4	0·03
Female	49·5	33·6	
Age, years			
⩽40	38	43·5	0·47
>40	62	56·5	
Smoking			
Yes	8·9	30·4	<0·001
No	91·1	69·6	
Years of experience			
⩽5	54	44	0·21
>6	46	56	
Handling raw meat			
Yes	67	57	0·19
No	33	43	

* Pearson χ^2 test.

Comparison of nasal carriage rates in the different establishments showed there was no significant difference in the rates between institutions in 2002 ($P = 0·31$), 2003 ($P = 0·76$) and 2011 ($P = 0·86$). Stratification of workplace also showed no significant difference in nasal colonization rates (2002: $P = 0·20$; 2003: $P = 0·56$; 2011: $P = 0·86$). In the immediate post-SARS period (2003), both nasal and hand colonization rates were reduced to 23·5% and 11·6%, respectively ($P < 0·001$). In 2011, the nasal carriage rate was similar to 2003 (22·9%) while the hand contamination rate dropped further to 3·7%. Trend analysis showed this reduction to be significant ($P_{trend} < 0·001$) (Table 3). In order to determine if this trend was similar in all types of establishment, the trend analysis was repeated for each type of workplace: canteens ($P_{trend} < 0·001$); hospital kitchens ($P_{trend} = 0·06$); sporting facility ($P_{trend} = 0·009$); others ($P_{trend} = 0·02$) (Table 3). Similarly, trend analysis of hand contamination showed an overall significant reduction ($P_{trend} < 0·001$), while that of different establishment types varied ($P_{trend} < 0·001$ to 0·01) (Table 3). The percentage of both colonized and non-colonized subjects whose hands were contaminated was significantly reduced over the period (Table 4).

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Table 3. *Temporal changes of nasal and hand carriage rates by workplace*

Site		Number of carriers (%) 2002	2003	2011	P_{trend}*
Canteens	Nasal	31/80 (38·8)	16/80 (20)	19/93 (20·4)	<0·001
	Hand	60/80 (75)	3/80 (3·8)	2/93 (2·2)	<0·001
Hospital	Nasal	39/103 (37·9)	9/58 (15·5)	11/40 (27·5)	0·06
	Hand	28/103 (27·2)	3/58 (5·2)	6/40 (15)	0·01
Sport facility	Nasal	52/130 (40)	33/125 (26·4)	24/98 (24·5)	0·009
	Hand	36/130 (27·7)	10/125 (8)	3/98 (3·1)	<0·001
Others†	Nasal	95/306 (31)	66/264 (25)	45/203 (22·5)	0·02
	Hand	131/306 (42·8)	45/264 (17·1)	5/203 (2·5)	<0·001
Overall	Nasal	217/619 (35)	124/527 (23·5)	99/434 (3·7)	<0·001
	Hand	255/619 (41·2)	61/527 (11·6)	16/434 (3·7)	<0·001

* χ^2 test for trend.
† Refers to supermarkets and hotels.

Table 4. *Temporal changes in prevalence of nasal colonization and hand contamination with* S. aureus *with respect to nasal carriage status*

Nasal carriage status	Percentage of participants 2002 (*n* = 619)	2003 (*n* = 527)	2011 (*n* = 434)	Hand contamination	Percentage of participants 2002 Nasal carriers (*n* = 217) Non-carriers (*n* = 402)	2003 Non-carriers (*n* = 124) Non-carriers (*n* = 403)	2011 Nasal carriers (*n* = 99) Non-carriers (*n* = 335)
Carriers	35	23·5	22·9	Yes	53·5	17·7	5·1
				No	46·5	82·3	94·9
Non-carriers	65	76·5	77·1	Yes	34·6	9·7	3·3
				No	65·4	90·3	96·7
Overall positive (95% confidence interval)					**41·2 (37·4–45·1)**	**11·6 (9·9–15·5)**	**3·7 (2·3–5·9)**

In 2002, contact with cooked meat was found to be a risk factor for colonization (OR 1·21, 95% CI 1·0–1·46, $P = 0·044$), and contact with raw meat almost reached significance (OR 1·12, 95% CI 1·0–1·42, $P = 0·062$) (Table 5). Of the total 151 colonized workers handling meat, 82 (54%) were in contact with both cooked and raw meat. Raw meat as a risk factor for colonization was confirmed in 2011 (OR 2·7, 95% CI 1·7–4·5, $P < 0·001$) [16]. No other factors were found to be significantly associated with carriage on either occasion.

Spa-typing for 2002 and 2011 isolates revealed 39 *spa* types clustered into 13 *spa* clonal clusters (*spa* CCs) and 42 *spa* types clustered into 10 *spa* CCs, respectively. Between two and 65 isolates were present in each *spa* CC (Table 6). The epidemic index showed that there was a slight increase of *spa* type diversity over the period (2002: $E_{persistent} = 0·056$; $E_{overall} = 0·061$ *vs.* 2011: $E_{overall} = 0·052$), reflecting the absence in 2011 of 28 *spa* types observed in 2002 and 29 types newly detected in 2011. In particular, the number of *spa* CC15-associated types reduced significantly from 23% to 11% ($P = 0·046$) while *spa* CC45-associated types increased from 4% to 12% ($P = 0·004$) (Table 6).

Of the 499 participants sampled in both 2002 and 2003, 89 (17·8%) were persistently nasally colonized. The percentage of transient carriers fell from 20·2% ($n = 101$) to only 5·4% ($n = 27$) between these samplings. Eighty (89·8%) of the persistent carriers yielded an isolate with the same *spa* type on both occasions. Common *spa* types were t189 (18·8%), t084 (12·5%), t091 (7·5%), t437 (7·5%) and t034 (6·3%). The remaining

Table 5. *Analyses of risk factors for nasal carriage with* S. aureus *(2002)*

			Univariate analysis		Multivariate analysis	
Variable	Carriers (%)	Non-carriers (%)	*P* value	OR (95% CI)	*P* value	aOR (95% CI)
Gender						
Male	95 (38·9)	149 (61·1)	0·08	–		
Female	110 (31·9)	235 (68·1)				
Smoking						
Yes	18 (34·6)	34 (65·4)	0·58	–		
No	81 (30·7)	183 (69·3)				
Age, years						
⩽40	99 (44·2)	125 (55·8)	0·08	–		
>40	110 (30·1)	255 (69·9)				
Years of experience as a food handler						
⩽1	18(40·0)	27 (60)	0·104	–		
2–3	52 (28·7)	129 (71·3)				
4–5	32 (34·0)	62 (66)				
⩾6	103 (38·2)	167 (61·8)				
Handled raw meat regularly						
Yes	114 (38·4)	183 (61·6)	0·062	1·38 (0·98–1·94)		
No	91 (31·1)	202 (68·9)				
Handled cooked meat regularly						
Yes	119 (38·5)	190 (61·5)	0·044*	1·42 (1·01–1·99)	0·023	1·49 (1·06–2·11)
No	86 (30·6)	195 (69·4)				
Handled vegetables regularly						
Yes	109 (36·7)	188 (63·3)	0·33	–		
No	96 (32·9)	196 (67·1)				
Handled dairy or creamy desserts regularly						
Yes	41 (36·6)	71 (63·4)	0·67	–		
No	164 (34·3)	314 (65·7)				
Handled bakery items regularly						
Yes	64 (37·2)	108 (62·8)	0·42	–		
No	141 (33·7)	277 (66·3)				
Handled rice, congee or noodles regularly						
Yes	80 (35·7)	144 (64·3)	0·69	–		
No	125 (34·2)	241 (65·8)				
Handled salad regularly						
Yes	35 (41·7)	49 (58·3)	0·15	–		
No	170 (33·6)	336 (66·4)				

OR, Odds ratio, aOR, adjusted odds ratio; CI, confidence interval.
* The difference is statistically significant.

nine subjects had either a change or a mutation in the colonizing strain (Table 7). *Spa* types seen in transient carriers were similar to those of persistent carriers. The mutation rate of *spa* locus in persistent carriers is one mutation per 223 months.

There was a higher prevalence of methicillin resistance in nasal isolates in 2011 (1·2%) compared to 2002 (0·6%) and 2003 (0·8%) but this did not reach statistical significance. The majority of isolates were SCC*mec* types IV or V. However, there was a notable change to a predominance of one *spa* type, t1081, in 2011. Characteristics of these isolates are shown in Table 8. All isolates of persistent carriers were methicillin sensitive.

Resistance to ciprofloxacin increased significantly over the study period from 1·4% to 7% ($P = 0{\cdot}012$), whereas susceptibility to erythromycin, clindamycin, gentamicin, tetracycline, and chloramphenicol remained unchanged. All nasal isolates collected were sensitive to linezolid.

Table 6. *Comparison of* spa *clonal clusters and types of* S. aureus *nasal carriage strains in 2002 and 2011*

		spa type (no. of isolates)†		
spa cluster (*n*)*	MLST	2002	2011	*P* value‡
Cluster A (55)	CC15/ST7/ST568	t2932 (3), t2949 (1), t796 (3), **t091 (13)**, t1190 (1), t11518 (1), t085 (6), t5864 (1), **t084(15)**	t7568 (1), **t091 (7), t084 (3)**	0·046
Cluster B (20)	CC45/ST291	t050 (3), t026 (3), **t1081 (2)**	t937 (1), **t1081 (8)**, t5598 (1), t1857 (1), t4981 (1)	0·004
Cluster C (43)	ST30/ST239	t1239 (3), t012 (4), t12778 (2), **t338 (12), t021 (8)**	t030 (1), t037 (2), t253 (1), t584 (1), t2868 (1), t8917 (1), **t338 (5), t021 (1)**	n.s.
Cluster D (9)	CC5	t7738 (2)	t002 (4), t179 (1), t668 (1), t688 (1)	n.s.
Cluster E (9)	ST6/ST8	**t701 (2)**	**t701 (4)**, t304 (3)	n.s.
Cluster F (16)	CC1	**t127 (7)**	**t127 (8),** t527 (1)	n.s.
Cluster G (3)	ST88	t786 (1)	t2592 (1), t4016 (1)	n.s.
Cluster H (20)	ST541	t571 (3), **t034 (13)**	t3625 (1), **t034 (3)**	n.s.
Cluster I (65)	ST188/ST12	**t189 (43)**, nt1§ (1), t8139 (1)	**t189 (17)**, t213 (2), t888 (1)	n.s.
Cluster J (7)	ST1619	t616 (5), t364 (2)	None	n.s.
Cluster K (3)	ST72	t3092 (1)	t148 (1), t401 (1)	n.s.
Cluster L (4)	no data	t2459 (3)	nt2§ (1)	n.s.
Cluster M (2)	ST25	t3232 (1)	t401(1)	n.s.

MLST, Multilocus sequence type; CC, clonal cluster; ST, sequence type; n.s., non-significant.
* Defined by visual analysis as described by Ruppitsch *et al.* [15].
† Bold font represents *spa* types present in both 2002 and 2011.
‡ Comparison of number of isolates in the *spa* CC (χ^2 test).
§ nt = new type nt1, repeat succession: 07-23-12-21-17-254; nt2, repeat succession: 23-34-34-16-34-33-13.
The remaining isolates were singletons not belonging to any of the clusters above, 51 in 2002 and 10 in 2011.

DISCUSSION

Our study revealed that the prevalence of nasal carriage and hand contamination with *S. aureus* decreased remarkably between 2002 and 2003 and that this reduction appears to have been sustained as evidenced by rates observed in 2011. Although nasal colonization rates can vary temporally, the change does not usually reach statistical significance, as was observed in a 9-month longitudinal follow-up of carriers, in which the prevalence of *S. aureus* varied by only 2% [13]. The repeated sampling at one site showed no variation in the nasal carriage rate and the difference in the hand contamination rate did not reach significance ($P = 0{\cdot}76$). This suggests that there was an extraneous factor which affected the carriage rate between years in our study. Baseline characteristics of participants were similar except for the proportion of male food handlers and the increase in smokers. These were found to be related as there is a strong association of smoking with male gender. Neither of these factors was found to be associated with colonization. There was a wide variety of establishments employing food handlers sampled on each occasion, but comparison of nasal colonization rates showed no significant difference between places of employment. In the hospital kitchens included, staff in the kitchens had no contact with either patients or healthcare workers, which may have reduced, although not totally eliminated, environmental pressures typical of hospitals. In the case of the sports facility, once again there was no direct contact between food handlers and sports participants and they each had separate bathroom and changing facilities. Trend analysis showed a significant reduction in both hand and nasal contamination/colonization over the decade of study and this significant trend was detected in almost all of the individual groupings of food handlers. The one exception, i.e. hospital kitchen workers, which was very near to significance ($P_{trend} = 0{\cdot}06$), may be attributable to small sample size.

We attributed the considerable decrease in both nasal and hand colonization rates to the enhanced hygiene practices implemented in the food industry

Table 7. *Characteristics of altered* spa *types in persistent carriers*

Sample	*spa* type	Repeat succession*	Comments
FL31	NID t571	23-434-34-254-434-12-23-02-12-23† 08-16-02-25-02-25-34-25	Change of colonizer strain
FW13	NID t364	07-23-12-21-17-254 04-34-17-32-17-23-24	Change of colonizer strain
JC79	NID t021	16-02-279-425-25-34-34-25 15-12-16-02-16-02-25-17-24	Change of colonizer strain
KO64	NID t437	23-12-34-34-12-12-12-23-02 04-20-17-20-17-25-34	Change of colonizer strain
JC112	t008 t12821	11-19-**12**-21-17-34-24-34-22-25 11-19-**178**-21-17-34-24-34-22-25	Mutation Glu→Lys (GAA→AAA)
FL10	t034 t571	08-16-02-25-02-25-34-**24**-25 08-16-02-25-02-25-34-25	Deletion of 24 bp repeat
FW54	t338 t1190	15-21-16-02-25-17-24 07-23-21-17-34-34-34-34-33-34	Change of colonizer strain
SS23	t4864 t091	35-25-17-24 07-23-21-17-34-12-23-02-12-23	Change of colonizer strain
KO61	t189 t2196	07-23-12-21-17-34 04-34-22-25	Change of colonizer strain

NID, Not in database.
* Bold font indicates change of *spa* repeats.
† Upper row denotes *spa* type for the isolate in 2002 and lower row for that in 2003.

Table 8. *Methicillin-resistant* S. aureus *strain characteristics*

Year	Specimen ID	*spa* type	SCC*mec*	Resistance profile
2002 (*n* = 4) (0·6%)	KO02	t189	II	ERY, DA, SXT, GEN, CHL, TET
	JC51	t338	IV	ERY, TET
	NH38	t437	V	ERY
	LP32	t091	V	TET
2003 (*n* = 4) (0·8%)	FW42	t084	IV	ERY, CIP, TET
	FW53	t701	V	ERY, DA, CIP, TET
	FW59	t701	V	ERY, DA, CIP, TET
	HS01	t437	V	ERY, DA, CIP, TET
2011 (*n* = 5) (1·2%)	JC16	t1081	IV	ERY, CIP, GEN, TET
	JC03	t1081	IV	CIP, TET
	JC23	t1081	V	ERY, CIP, TET
	GM133	t1081	V	FD, CIP, TET
	JC07	t4981	V	CIP, TET

CIP, Ciprofloxacin; CHL, chloramphenicol; DA, clindamycin; ERY, erythromycin; FD, fusidic acid; GEN, gentamicin; TET, tetracycline.

during and after the SARS epidemic. In response to the outbreak, use of gloves and masks was strictly enforced for food handlers in Hong Kong. This was accompanied by increased emphasis on the importance of regular hand washing, with the implementation of a government-enforced hygiene supervisor scheme with a major role for monitoring hand washing practices. There were also more in-depth inspections of food premises and practices. These measures could minimize the spread to transient carriers resulting in lower colonization rates as was evidenced in the 2003 sampling in which the majority of positive

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subjects were persistently colonized. Transient carriers are most likely to become colonized as a result of transmission to their nose from their contaminated hands. This hand contamination is most likely to occur as a result of contact with work surfaces in their environment. Improved hand hygiene and use of gloves would reduce these opportunities for self-colonization. Studies have shown that persistent carriers have higher rates of skin contamination and of shedding of *S. aureus* than transiently colonized subjects [17] Interestingly, a significant decrease in nasal colonization was noted in a national survey of non-institutionalized US citizens, from 32·4% in 2001/2002 to 28·6% in 2003/2004 [18]. This may be the result of global efforts made at that time to improve hygiene for prevention of coronavirus transmission. A similar effect was observed in studies conducted in Hong Kong on subjects from the general population. A carriage rate of 35% was reported in young adults in samples collected in early 2002 [19]. A more recent study revealed that the carriage rate had fallen to 24% [20]. In the months after the epidemic in Hong Kong, telephone surveys revealed a sustainable increase in self-reported hand hygiene practices in the general public [21]. This was confirmed to be sustainable in an investigation in 2009 which revealed that 91% of the general population wash their hands more than six times per day with 46·6% washing more than 10 times per day [22]. Since 2003, although use of gloves and improvements in hand hygiene has been sustained, use of masks has decreased in the food industry except for handlers of some sensitive foods, such as sushi, and for operatives suffering from respiratory infections or rhinitis. The sustained low levels of nasal carriage and hand contamination strongly suggest that prevention of dissemination of *S. aureus* is largely a result of hand hygiene measures including use of gloves.

Although a number of risk factors for colonization were investigated, only contact with meat appeared to increase the risk. The association with raw meat became stronger in 2011 than in the earlier study (2002). This may be due to the association being obscured by more frequent transient colonization due to contamination of surfaces and equipment in the earlier study (2002) by persistent carriers. In addition, over half of workers handled both raw and cooked meat leading to considerable overlap of these exposures. We have previously reported that one *S. aureus* strain, t189, was associated with food handlers who regularly handled raw meat at work [16].

While resistance rates to most classes of antimicrobial agents remained stable, susceptibility to ciprofloxacin increased throughout the period. This may reflect the increased use of this agent in Hong Kong [23]. A similar trend was also observed in the USA [18].

Isolation of t189 as the most common *spa* type, representing 18% of isolates in both 2002 and 2011 is noteworthy because this *spa* type is rarely reported as a human nasal commensal. A large-scale cross-sectional survey of human *S. aureus* nasal carriage involving more than 30 000 participants from nine European countries [24] failed to detect t189 in methicillin-resistant strains. However, *spa*-typing was not performed on methicillin-susceptible isolates which are known to differ from methicillin-resistant strains. As mentioned above, *S. aureus* t189 was associated with exposure to raw meat [16]. Therefore, the presence of this clone in the food production chain may increase risk for its carriage.

There was a large change in circulating *spa* types between 2002 and 2011. In particular, it was observed that *spa* CC15 incidence fell while that of *spa* CC45 increased significantly over the period. Although only nine *spa* types were detected in both samples, it appears that some clones have been circulating in the region for more than a decade.

Examination of the rate of mutations in the *spa* locus revealed there had been only two mutations over the period of 445 sample months which equates to a molecular clock of one per 223 months in the *spa* locus. This mutation rate is considerably slower than those previously reported [13, 25] indicating that colonizing strains in our subjects rarely undergo mutations. A possible explanation for the observed differences in mutation rates may be variation in selective pressure on the strains in the studies. The subjects in the previous studies were regularly exposed to healthcare environments where antimicrobial agents are regularly used. They were either cystic fibrosis patients or healthcare workers while in our study subjects were food handlers working in the community where the selective pressure is less.

The prevalence of methicillin resistance in food handlers was comparable to that observed in the general public [6], indicating that food handling may not be a risk factor for methicillin-resistant *S. aureus* (MRSA) carriage. This was in contrast to our previous finding that butchers handling raw meat exhibited a fivefold increased risk for colonization with MRSA [26]. The risk for butchers may be increased because they are additionally responsible for primary cut-up of whole carcasses, including the snout which is the site most likely to be colonized in the slaughtered animal.

CONCLUSION

The decrease in colonization rates following the SARS epidemic may be attributed to reduced opportunities for spread to transient carriers due to use of protective measures accompanied by improved hand hygiene. After the epidemic, the reinforcement of use of gloves and improved hand hygiene together with use of masks in the presence of respiratory symptoms has resulted in a sustainable lower nasal colonization level accompanied by greatly reduced incidence of hand contamination. This reinforces the importance of intensive hygiene education and interventions for the control of communicable diseases.

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DECLARATION OF INTEREST

None.

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