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## The prevalence and source of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community in Hong Kong

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### SUMMARY

Although reports of isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from patients admitted from the community have increased, few studies have investigated colonization of healthy subjects. This study aimed to determine community levels of MRSA in Hong Kong. Nasal swabs from a cross section of young adults and family units were cultured for MRSA. Antibiotic sensitivities and risk factors for carriage were determined and clonal relationships were investigated by pulsed-field gel electrophoresis (PFGE). Overall carriage was low (1·4%), and associated with health-care exposures (OR 13·56, 95% CI 1·11–165·21). Subjects working in health care yielded multi-resistant MRSA strains, but isolates from non-hospital-exposed subjects were methicillin-resistant only. Strains indistinguishable by PFGE were carried by subjects working together, and some spread to other contacts was observed. MRSA colonization in the community is rare in Hong Kong and is largely associated with working in health care. Community-acquired staphylococcal infections may be treated with agents effective against methicillin-sensitive strains.

### INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has long been recognized as an important cause of hospital-acquired infection [1]. Levels of antibiotic resistance in *S. aureus* are high in Hong Kong [2] and MRSA is an important cause of nosocomial infection in Hong Kong hospitals [3, 4]. More recently MRSA has been reported as a community pathogen and hospital outbreaks have been shown to have originated in the community [5–7]. Although the occurrence of MRSA is regularly monitored within hospitals, there are relatively few surveys of its prevalence in the community, except in nursing homes for the elderly [8, 9]. A hospital-based study conducted

in the United States, reported that 40% of adults suffering from MRSA infection had acquired the organism prior to admission [10]. Furthermore, a recent report of the prevalence of MRSA in the intensive care units (ICU) in Hong Kong hospitals indicated that subjects admitted directly from the emergency department had a mean carriage rate of MRSA of 2·7% [11]. However, many patients being admitted tend to come from long-term care facilities, are re-admissions or are transferred from other hospitals, and thus, are not truly representative of the general community. In the United Kingdom, a recent study investigated carriage of MRSA in the general community and found that the prevalence was low (1·5%) [12]. This frequency was similar to that obtained from studies performed in the United States, Portugal and Italy, all of which reported carriage rates of less than 1% [13–15]. Although the importance of the community as a source of MRSA infections has not been

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determined, this change in pattern of occurrence may require modifications in hospital infection control programmes. We report here the results of a community study performed in Hong Kong.

## PARTICIPANTS AND METHODS

### Sample population

The community prevalence of MRSA in Hong Kong has not previously been determined. Estimates elsewhere have reported prevalence rates ranging from 0.1 to 2%, but carriage rates of up to 17% have been reported from selected populations [9]. A sample size of 202 would estimate 5% population prevalence within 3% with 95% confidence. To be within 2%, a sample size of 456 would be required. Subjects were recruited from two sources.

#### *Group A: Young adults*

Young adults were represented by students attending the Hong Kong Polytechnic University. Comparison of the socioeconomic backgrounds of the student population, undertaken by the University Student Affairs Office has shown that the mix of students enrolled closely matches that of the general population, as reported in the latest census (2001). All ( $n=460$ ) students registered in the second year of courses in Nursing, Biomedical Science, Hospitality Management, and Tourism were invited to participate in the study. Subjects received an information sheet about the survey and completed a consent form and a short questionnaire. Information collected included demographic details, hospital admissions in the past 12 months, recent antibiotic use (in the past 6 weeks), and contact with health-care facilities. Both nursing and biomedical science students attended local hospitals as part of their course requirements.

#### *Group B: Family study*

One hundred families of first-year students of the same courses, who were randomly selected from the student list, were invited to participate in the study. At this stage, nursing and biomedical science students had not been in contact with health-care facilities as part of their study requirements. All family members living at home were requested to participate in the study. They received similar information as Group A and were asked to complete a questionnaire, which included additional questions concerning contact with

health-care facilities in the past 4 weeks, and whether the subject was receiving long-term treatment.

The study was approved by the Human Subjects Ethics Committee of the Hong Kong Polytechnic University.

### Microbiological methods

A nasal swab was collected from the right nostril of each participant using a transport swab (Medical Wire and Equipment Co. Ltd, Bath, UK). All swabs were transported to the laboratory for culture within 18 h of collection. Specimens were inoculated on Columbia agar supplemented with 5% horse blood agar (BA) and mannitol salt agar (MSA; Oxoid Ltd, Basingstoke, UK), and the swabs were then placed in cooked meat broth with 5% salt for enrichment of *S. aureus*. BA and MSA plates were examined after 24 and 48 h and colonies resembling *S. aureus* were tested using Staphaurex for detection of coagulase (Murex Diagnostics Ltd, Dartford, UK). At least five colonies from each plate with isolates confirmed as *S. aureus*, were tested for resistance to methicillin by culture on oxacillin resistance screening agar base (ORSAB; Oxoid). Mannitol fermenting strains (producing blue colonies) were confirmed as methicillin resistant by agar dilution tests with oxacillin and results were interpreted as the minimum inhibitory concentration of oxacillin for the strain. All MRSA strains were tested for susceptibility to tetracycline (30 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), vancomycin (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), rifampicin (5 µg), chloramphenicol (30 µg) and trimethoprim-sulphamethoxazole (1.25/23.75 µg) using disc sensitivity testing on Mueller-Hinton agar. Results were interpreted using National Committee for Clinical Laboratory Standards (NCCLS) guidelines [16] except for fusidic acid for which the British Society for Antibiotic (BSAC) guidelines were used [17].

### Molecular typing

All strains were compared by pulsed field gel electrophoresis (PFGE) using a previously described method [18]. Two or three discrete colonies from a Columbia agar plate were grown in brain heart infusion broth (Oxoid) for 4 h at 37 °C. Following centrifugation, the cells from the resulting pellet were mixed in a 1:1 ratio with low-melting-point agarose (Bio-Rad Inc.,

Table 1. Characteristics of MRSA isolates from young adults (Group A)

Isolate no.	Programme of study	Antibiotic within the last 6 weeks	Resistant to*
1	Nursing	No	P, Ox, E, Da, Cip, GN, Te
2	Nursing	Yes	P, Ox, K, E, Da, Cip, GN, Te, W
3	Nursing	No	P, Ox, K, E, Da, Cip, GN, Te, W
4	Nursing	No	P, Ox, E, Da, Cip, GN, Te
5	Nursing	No	P, Ox, K, E, Da, Cip, GN, Te, W
6	Biomedical science	No	P, Ox, E, Da, Te

\* P, penicillin G; Ox, oxacillin; E, erythromycin; Da, clindamycin; Cip, ciprofloxacin; K, kanamycin; G, gentamicin; Te, tetracycline; W, trimethoprim.

Veenendaal, The Netherlands). Agarose plugs were prepared using Bio-Rad casting forms (Bio-Rad) and were incubated with lysostaphin (Sigma Chemical Co., St Louis, MO, USA). The cells were lysed in a buffer containing 1% sodium dodecyl sulphate and 1 mg/ml proteinase K (Bio-Rad). Plugs were washed six times for 30 min in 10 mM Tris-HCl plus 1 mM EDTA and stored at 4 °C. Following digestion of the DNA with *Sma*I (Bio-Rad), PFGE was performed in a 1% agarose gel in a 0.5× Tris borate EDTA buffer using a Bio-Rad CHEF Mapper at 14 °C, with a running time of 22 h, and a voltage of 6 V/cm at an angle of 120°. Gels were stained with ethidium bromide and photographed under UV light. Results were interpreted as described by Tenover et al. [19] and isolates with identical restriction patterns were considered to be the same strain of *S. aureus*.

## RESULTS

### Response rates and participants

#### Group A

In total, 331 students (72%) agreed to participate in the study and were sampled for carriage of MRSA. Eighty-four participants (25%) were male and all subjects were of Chinese ethnicity. All subjects were in the age range 19–23 years and were healthy, none was receiving long-term medical treatment. Seventy-four subjects (22.3%) had received antibiotics in the previous 6 weeks, with a further 13 (3.9%) being unsure of the medication they had received. A total of 187 (56%) were in courses related to health care and had visited a hospital in the past 4 weeks. Two subjects (0.6%) had been admitted to hospital for more than 2 days in the last 12 months.

#### Group B

Ninety-two families (92%) agreed to participate in the study and a total of 322 subjects were sampled for carriage of MRSA. Two hundred and four participants (63%) were female and all subjects were of Chinese ethnicity. Ninety-five per cent of subjects (304/322) were in the age range 11–60 years, with only seven aged 10 or under and eleven over 60 years. Fifty-five respondents (17%) were in an occupation related to health care. Only 13 participants (3.9%) reported a hospital admission over 2 days in the previous 12 months, although 56 (17.3%) had received antibiotics in the previous 4 weeks. Twenty-one per cent of respondents (70/322) had contact with a hospital in the preceding 4 weeks, 4% (14) with a nursing home, 24.5% (79) with a public or private clinic and 0.6% (2) with an ICU. Nine subjects (2.7%) were receiving long-term treatment.

## MICROBIOLOGY

#### Group A

Two specimens yielded no bacterial growth. In total 113 samples (34%) grew *S. aureus*, of which six (5.3%) were methicillin resistant. This is a prevalence of 1.7% (95% CI 0.4–3.0). All MRSA strains were multi-resistant, and three sensitivity patterns were observed (Table 1).

#### Group B

All swabs yielded growth, of which 73 (23%) were from subjects nasally colonized with *S. aureus*. Three of these isolates (4.1%) were MRSA, giving an overall prevalence of 0.9% (95% CI 0.89–0.91). Two of these MRSA strains were resistant to penicillin and methicillin only (Table 2).

Table 2. Characteristics of MRSA isolates from families (Group B)

Isolate	Sex	Age (years)	Occupation in health care	Contact with health care	Hospital admission*	Resistant to†
A	M	25	Yes (nursing home)	Yes	No	P, Ox
B	M	20	No	No	No	P, Ox
C	F	20	Yes (hospital)	Yes	Yes	P, Ox, Da, E, Te

\* For over 2 days in last 12 months.

† P, penicillin; Ox, oxacillin; Da, clindamycin; E, erythromycin; Te, tetracycline.

### Characteristics of MRSA carriers

All carriers in Group A were students of health-care programmes and had spent time in either the wards or clinical pathology laboratories of district hospitals, and, considered alone, the carriage rate in this group was 3.2%. All MRSA carriers were female and none had recently been admitted to hospital. One subject had received antibiotics recently.

Of the three carriers in Group B, two were male and two worked in health-care facilities (Table 2). Other family members of these carriers were not colonized by MRSA, although several were nasally colonized by *S. aureus*. A multi-resistant strain was isolated from a hospital worker who had been recently admitted to hospital (Table 2). All carriers were aged between 20 and 30 years, and none had received antibiotics in the past 6 weeks.

### Pulsed field gel electrophoresis

Of five strains isolated from nursing students, three were identical by PFGE (Fig.). The sixth health-care isolate from a biomedical science student had a different antibiotic sensitivity pattern and was not typed. This student had not had contact with nursing students.

The three community strains all appeared different from each other and from the strains harboured by the nursing students (Fig.).

### Risk factors for carriage of MRSA

In the young adult group, students of health-care programmes were more likely to become colonized with MRSA (Fisher's exact test,  $P=0.035$ ). Subjects colonized with MRSA were compared with other subjects in their sample and those colonized with methicillin-sensitive *S. aureus* (MSSA). An occupation related to health care (OR 13.56, 95% CI 1.11–165.21) was the only factor shown to be associated with carriage of MRSA using the  $\chi^2$  test (Table 3).

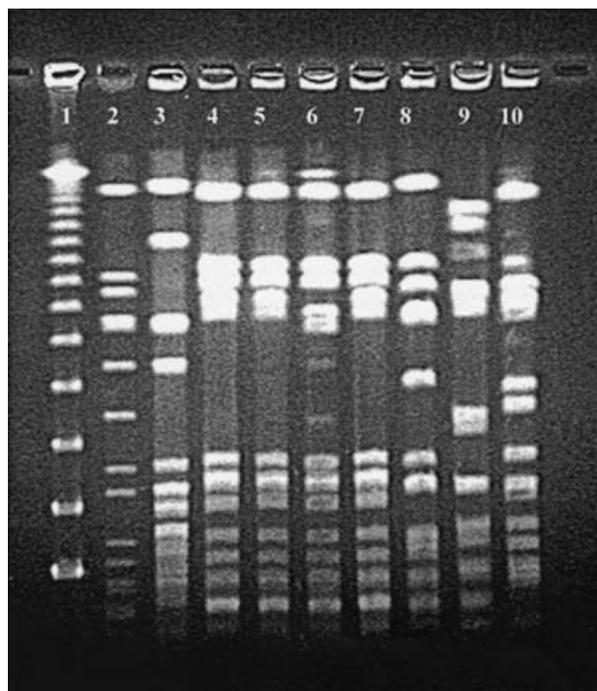


Fig. Pulsed field gel electrophoresis of MRSA strains. PFGE of *SmaI* digests of strains. Lane 1, molecular size marker; lane 2, control strain of *S. aureus*; lane 3, strain from nursing student 1; lane 4, strain from nursing student 2; lane 5, strain from nursing student 3; lane 6, strain from nursing student 4; lane 7, strain from nursing student 5; lane 8, community strain A; lane 9, community strain B; lane 10, community strain C.

Recent use of antibiotics was not associated with MRSA colonization of subjects from the healthy community. Interestingly, in the family study, those who had visited a doctor in the last 4 weeks were more likely to carry MSSA than MRSA (OR 0.73, 95% CI 0.63–0.84).

### DISCUSSION

The point prevalence of MRSA nasal colonization for young adults was 1.7% and for families 0.9%. These

Table 3. Risk factors for colonization with MRSA in carriers of *S. aureus*

Factor	Number ( <i>n</i> = 73)	OR	95% CI
Male sex	30	0.33	0.03–3.85
Occupation related to health care	11	13.56	1.11–165.21
Hospital admission over 2 days in the past 12 months	1	1.50	0.67–3.34
Taken antibiotics in the past 4 weeks	11	0.99	0.98–1.00
Contact with hospital in last 4 weeks	15	2.0	0.17–23.67
Contact with nursing home in last 4 weeks	6	6.5	0.50–84.68
Visit to doctor in last 4 weeks	19	0.73	0.63–0.84
Under long-term treatment	4	0.94	0.89–1.00
Family size > 3	47	0.17	0.02–2.03

levels of carriage are similar to those reported in community studies elsewhere [12–15]. The chosen sample site of the anterior nares has been demonstrated to have the highest sensitivity to detect carriage in the absence of infected wounds [20]. Contact with health-care facilities was recorded for all carriers in the young adults group and two out of three in the family group. This suggests that much of the colonization may well have occurred within a health-care facility and is not true community carriage. Investigation of risk factors showed that contact with health care in the case of the students, and occupation associated with health care in the family study were the only ones associated with colonization with MRSA. It would appear that transmission outside of the hospital remains rare in Hong Kong.

Long-term colonization with MRSA has been demonstrated in patients discharged from hospital [20, 21]. In recent years, Hong Kong has followed a trend to reduce the length of hospital stay of patients, and this may help to reduce chances for colonization, as longer stays have been associated with carriage of resistant strains [22]. If fewer patients are colonized during hospitalization, this will tend to reduce those bringing resistant strains back into the community.

Other workers have noted that exposure of students to the hospital environment increases colonization with antibiotic-resistant strains [23]. However, these workers did not report MRSA colonization. Investigation of the hospital attachments of the colonized students in this study revealed that two of the subjects, isolate nos. 4 and 6, carried strains with identical PFGE patterns and had worked on the same ward during their hospital placement. The other three carriers were in the same tutorial group, but had different hospital placements, and one of these also carried the

same strain. The remaining two strains from nursing students and the strain from the biomedical science student appeared to have a different source, as they differed by more than two bands. This suggests that colonization of at least two subjects occurred within the hospital, spreading to a further classmate in the community setting.

It is interesting to note that in the family study, there was no spread of MRSA to other family members even though some were nasal carriers of *S. aureus*. Other studies have observed colonization of other family members [24, 25] and even pets [26]. The factors which affect colonization are still poorly understood [27, 28], although the role of lipoteichoic acid [29, 30] and surface-associated proteins in the staphylococcal cell wall [31, 32], as well as those binding to nasal mucin [33] have been investigated. It would appear that in the hospital setting, the more susceptible strains tend to be replaced by their resistant counterparts, although it has been suggested that persistent carriage may have a protective effect against the acquisition of other strains [34]. Replacement may not occur in the home in the absence of selection pressure, or colonization may need exposure to a higher infective dose as may occur in the hospital environment. Although recent antibiotic use was reported, there was no association between taking of antibiotics and colonization with MRSA in the community observed in this study. Interestingly, MSSA carriage was more likely in those with a recent visit to a general practitioner.

Two of the MRSA strains carried by participants in the family study were susceptible to all drugs except  $\beta$ -lactams. These are more typical of community strains reported elsewhere [5, 35]. One of these carriers, subject B had no reported contact with health-care facilities. This may represent a true community

strain, and this may also apply to the strain from subject A, as isolates from nursing homes in Hong Kong frequently have this susceptibility pattern (M. V. Boost & M. M. O'Donoghue, unpublished results). PFGE showed that the family strains had no relationships to each other and were from different sources. The third subject may have been colonized in the hospital as the sensitivity pattern was similar to that seen in the strains from one of the colonized students.

Interestingly, carriage of MRSA was not seen in older subjects in the family study, although carriage rates of *S. aureus* tended to be higher in the 40–60 years age group (24–25%), than in those aged 21–30 years (17%). The older age group may be expected to have had more contacts with health-care facilities. This is in contrast to the findings of a UK study in which carriage of MRSA was found in older subjects [12]. Our study might suffer from some sample bias, as the number of elderly subjects was low because some did not wish to have specimens collected. In contrast to the UK study, the younger subjects were more likely to be cooperative and a high response rate was achieved for this group (72%).

Increasing concern about MRSA in the community has led to recommendations for surveillance of carriage levels [6, 36]. This study has shown that community colonization with MRSA remains low in Hong Kong, and patients admitted directly from the community are unlikely to be colonized with multi-resistant MRSA, unless they are health-care workers. It may be worthwhile to screen health-care workers for MRSA colonization on admission. It is unlikely that community-acquired infections are caused by MRSA strains and, therefore, may still be safely treated with conventional agents effective against methicillin-sensitive strains in Hong Kong.

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