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## Research Fund for the Control of Infectious Diseases

Research Dissemination Reports

Commissioned studies by the University of Hong Kong

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研究成果報告

香港大學委任研究

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Respiratory Infectious Diseases
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# EDITORIAL

After the outbreak of severe acute respiratory syndrome (SARS) in Hong Kong in 2003, the *Research Fund for the Control of Infectious Diseases* (RFCID) was established to encourage, facilitate and support research on the prevention, treatment and control of infectious diseases, in particular emerging infectious diseases, so as to formulate policies. Over the course of 5 years, researchers in the University of Hong Kong completed a portfolio of basic, epidemiological, public health and clinical research on a diverse range of potentially emerging and re-emerging infectious diseases, including SARS, influenza, tuberculosis, and antibiotic-resistant bacteria. Evidence-based knowledge generated from these projects has helped in health policy formulation and health care services delivery. In this issue, a representative selection from the portfolio is presented. Three projects are highlighted owing to their contribution to knowledge on emerging and re-emerging pathogens and their impact on patient care.

SARS was the first newly emergent communicable disease epidemic of the 21st century, eventually infecting 8098 individuals around the world, of whom 774 died. Leung et al[1] used an integrated dataset including information from all 1755 reported local cases to generate the definitive epidemiological parameters of the epidemic. Predictors of SARS-related mortality were also determined. The analysis provides a summary of the time-course and patient locations during the outbreak in Hong Kong and underscores the value of having a centralised systematic data registry in place, so as to deal with SARS or any other emerging or re-emerging infectious disease epidemic.

The evolution of a pandemic human influenza strain from reassortment of human influenza virus genes with those from avian influenza H5N1 is of importance for public health. In a telephone survey of 986 Chinese adults, Fielding et al[2] determined population knowledge of risk and estimated degree of influenza hazard from live poultry sales at the height of the 2004 Asian avian influenza epidemic. The general public perceived the risk of buying live chickens as moderate. Buying live poultry was strongly predicted by the erroneous belief that cooking is the best means of protection from avian influenza. Cooking protects from infection by eating, but not from infection through contact prior to eating. This study has implications for public health groups seeking to increase preventive practices to control possible avian influenza outbreaks.

AmpC beta-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of the *Enterobacteriaceae* where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and beta-lactamase inhibitor and beta-lactam combinations. Ho et al[3] studied the production of AmpC and extended-spectrum beta-lactamases (ESBLs) in blood isolates of *Enterobacter* spp isolated during 2000 to 2002 in two general regional hospitals. They found that application of standard criteria designed for ESBL detection in *Escherichia coli* and *Klebsiella* spp would lead to many false-positive results in *Enterobacter* spp. Modifying the screening conditions increased test specificity. This study is important locally, as it shows that the prevalence of ESBL among *Enterobacter* spp in Hong Kong is high and that their beta-lactamase content is diverse.

We hope you will enjoy this selection of research dissemination reports. Electronic copies can be downloaded from the Research Fund Secretariat website (**http://www.fhb.gov.hk/grants**). Researchers interested in the funds administered by the Food and Health Bureau may visit the website for detailed information about application procedures.

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# Detection and characterisation of extended-spectrum beta-lactamases among blood stream isolates of *Enterobacter* species in Hong Kong

**Key Messages**

1. Extended-spectrum beta-lactamase (ESBL) resistance in *Enterobacter* spp may be under-recognised.
2. Detection methods for ESBL resistance in *Enterobacter* spp may need to be modified.

## Introduction

Among *Enterobacteriaceae*, production of extended-spectrum ß-lactamases (ESBLs) is increasingly recognised as a cause of resistance to expanded spectrum cephalosporins. In large hospitals, this resistance mechanism is found in 10-40% of *Klebsiella* spp and *Escherichia coli*. Less is known about the incidence of ESBL in *Enterobacter* because ESBL phenotypic screening of *Enterobacteriaceae* is commonly performed in the clinical laboratories only for *E coli* and *Klebsiella* spp. Detection of ESBL in *Enterobacter* spp is more complicated because high-level AmpC production interferes with tests that rely on synergism between clavulanic acid and a third-generation cephalosporin. Thus, recent studies have addressed this by using cefepime to replace the third-generation cephalosporins that were conventionally used. With this modification, a general hospital in Greece reported ESBL rates of 25% and 58% during 1998-1999 in a consecutive series of *Enterobacter cloacae* and *Enterobacter aerogenes* isolates, respectively.[1] Similarly, blood isolates of *Enterobacter* spp tested in a Korean tertiary hospital during 1994–2001 had an overall ESBL prevalence of 43%.[2] These findings highlight the potential for overlooking high ESBL rates in *Enterobacter* spp. In our study, we examined the production of AmpC and ESBL in blood isolates of *Enterobacter* spp collected over a 3-year period at two regional hospitals in Hong Kong.

## Aims and objectives

To study the production of AmpC and extended-spectrum beta-lactamases in *Enterobacter* spp, with a view to improve surveillance of this type of emerging antibiotic resistance mechanisms.

## Methods

### *Bacterial strains, identification, susceptibility testing and patient data*

Blood isolates of *Enterobacter* isolated during 2000-2002 in two general regional hospitals were evaluated. Duplicate isolates were excluded by the first isolate per patient method. The VITEK GNI system was used for bacterial identification. For accurate identification at the species level, the glucose oxidation test was carried out as described. Antibiotic susceptibilities were tested by the disc diffusion method using Mueller–Hinton agar and interpreted according to the NCCLS.[3]

### *Detection of ESBL and AmpC*

Three methods were used to detect ESBL production: modified double-disc synergy test, combined disc method, and the three-dimensional extract test.

### *Characterisation of ESBL-producing strains*

Analytic IEF and filter mating were performed by standard methods. Beta-lactamase genes (TEM, SHV, CTX-M) were amplified and sequenced using class-specific primers.[4] The subset of ESBL-producing *Enterobacter hormaechei* was examined further by pulsed-field gel electrophoresis.

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## Results

The ESBLs were identified in nine isolates (7%), including seven of 39 (18%) *E hormaechei*, one of 27 (4%) *E aerogenes* and the only *E intermedius* strain. The *E intermedius* strain was positive only in the three-dimensional extract test but not in the other two tests. The other eight strains were positive in all three tests. No ESBL was detected in other species, including non-hormaechei members of the *E cloacae* complex (n=61), *E agglomerans* (n=7), *E gergoviae* (n=4) and *E sakazakii* (n=1). For the detection of ESBL, the NCCLS screen method lacked specificity (63-72%). Discrimination could be improved using cefepime at a cut-off of ≤25 mm (sensitivity 90%, specificity 94%). The ESBL content included five different CTX-M enzymes (CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-24 and a novel CTX-M-2-like ß-lactamase), SHV-12 (n=2) and unidentifiable ESBLs with a pI of 7.7 or 7.9 in two strains. The seven ESBL-producing *E hormaechei* were genotyped by pulsed-field gel electrophoresis and were found to be unrelated to each other. In three of the CTX-M-producing strains, ISEcp1-like elements, including promoters for the ß-lactamase gene, were found.

## Discussion

The simple application of NCCLS criteria designed for ESBL detection in *E coli* and *Klebsiella* spp lead to many false-positive results in *Enterobacter* spp, in contrast to specificities of 97 to 99% reported for screening of *E coli* and *Klebsiella* spp.[5] In our collection of *Enterobacter* spp, it appeared to be possible to increase the test specificity by including cefepime in the initial screening. The high diversity of ESBLs among *Enterobacter* spp in Hong Kong is intriguing. In addition to having CTX-M and SHV classes of ESBL, each ß-lactamase combination in the nine strains was distinct. For the SHV class of ESBL, only SHV-12 was present among these strains. Elsewhere, this ESBL is known to exist among *E aerogenes* (in Italy) and among *E cloacae* (in Beijing, Taiwan, Korea and Thailand).[6] In agreement with previous studies, we demonstrated that four of the five isolates carrying *bla*CTX-M genes were able to transfer the ESBL phenotype in mating experiments, suggesting the spread of *bla*CTX-M genes by conjugative plasmids.[6] Interestingly, similar ß-lactamase combinations involving SHV-12, CTX-M-9, CTX-M-13 and CTX-M-14 were known to exist among *Enterobacteriaceae* collected from a hospital in Guangzhou. Millions of passengers travel between Guangzhou and Hong Kong annually. Our finding thus indicates a probable cross-boundary spread of ESBL-producing organisms or their determinants. The IS*Ecp1*-like element observed upstream of the *bla*CTX-M genes in the present study may involve in the translocation and dissemination of these ß-lactamase genes.[7]

## Conclusions

The prevalence of ESBL among *Enterobacter* spp in Hong Kong is high and that their ß-lactamase content is diverse. Our finding adds to the increasing recognition of CTX-M enzymes in the Far East and further emphasises the need for screening ESBL in clinical isolates of *Enterobacter* spp.

The detection of ESBL in *Enterobacter* spp could be enhanced by: (1) inclusion of the glucose oxidation test for more accurate speciation of *E hormaechei*, and (2) inclusion of cefepime as an indicator in the ESBL screen.

## Acknowledgement

This project forms part of a series of studies commissioned by the Food and Health Bureau of the Hong Kong SAR Government and was funded by the Research Fund for the Control of Infectious Diseases. The results of this study have been reported in the following publication:
Ho PL, Shek RH, Chow KH, et al. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of *Enterobacter* spp. in Hong Kong, 2000-2002. J Antimicrob Chemother 2005;55:326-32.

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# Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus*

**Key Messages**

1. Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains with diverse genetic backgrounds are emerging in Hong Kong.
2. Intra-familial spread of community-associated MRSA is common.

## Introduction

*Staphylococcus aureus* is a common cause of community- and health care–associated skin and soft-tissue infections (SSTIs), pneumonia, and bacteraemia. Traditionally, methicillin-resistant *S aureus* (MRSA) infections are confined to individuals with established risk factors, for examples: nursing home residents, hospitalised individuals, patients undergoing operations, persons with indwelling medical devices. Since the 1990s, there are increasing reports of MRSA infections in healthy individuals from the community without established risk factors.[1] By means of genotypic studies, most community-associated MRSAs are found to be genetically distinct from the health care–associated MRSA. With few exceptions, the former strains possess the Panton-Valentine leukocidin genes and one of the novel *Staphylococcus* cassette chromosome mec elements (types IV and V). They are sensitive to most antibiotics except β-lactams. Nowadays, community-associated MRSAs are recognised to cause outbreaks in 'closed populations', such as aboriginals, contact sports athletes, inmates of correctional services, military recruits, and children attending day care centres. Recently, they have even been found to spread inside hospitals and are displacing the traditional health care–associated MRSA as the pathogens in nosocomial infections.[2]

## Aims and objectives

We evaluated the molecular epidemiology and household transmission of community-associated MRSA in patients who were reported to a monitoring system in Hong Kong from January 2004 to December 2005.

## Methods

In January 2004, a monitoring group was formed under the coordination of the Department of Health and the Centre of Infection at the University of Hong Kong, to conduct laboratory-based surveillance for community-associated MRSA. The participating microbiology network included five public and six private hospital laboratories, as well as six stand-alone community laboratories. These laboratories were estimated to provide inpatient and outpatient service to half of the 6.5-million inhabitants of Hong Kong. All participating laboratories were requested to screen the clinical information in the request forms and pay attention to MRSA isolates with a non-multiresistant antibiogram. Suspected community-associated MRSA isolates were referred to the laboratory in the Centre of Infection for molecular testing.

Culture swabs from household contacts were processed as described previously. A broth enrichment step (mannitol-salt medium; Oxoid, Hampshire, UK) was used, followed by plating onto oxacillin (6 μg/mL) blood and mannitol salt agar. The MRSA were characterised by *Staphylococcus* cassette chromosome mec typing, pulsed-field gel electrophoresis and multilocus sequence typing. Polymerase chain reaction was used to detect mecA, Panton-Valentine leukocidin, and erythromycin resistance determinants.[3-5]

## Results

From January 2004 to December 2005, 25 episodes of community-associated

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MRSA infections from seven children (aged <16 years) and 16 adults were reported; 14 were Chinese, three were Filipino, and two each were British, Nepalese, and Japanese. The mean age of the patients was 28 (standard deviation [SD], 21; range, 1-91) years. Eight episodes of SSTIs (including six furuncles/carbuncles, one infected chickenpox lesion, and one infected eczema) occurred in the 7 children; one 7-year-old child had two episodes of infection. The 17 episodes of infections in the 16 adults included one bacteraemia complicated by meningitis and 16 SSTIs (11 furuncles/carbuncles, two perianal abscesses, one deep-seated thigh infection, one infected sebaceous cyst, and one scalp abscess).

The 23 patients belonged to 21 unrelated families. Nine families declined screening. A total of 46 household members from the remaining 12 families were assessed. Two community-associated MRSA infections and four carriages were found. In households 3 and 10, more than one person was infected.

An analysis of the relationship between the 29 isolates was performed using pulsed-field gel electrophoresis. At a cut off of >80% similarity, the dendrogram divided all but one of the isolates into three pulsed-field type clusters (designated as HKU100 to HKU300) [Table]. Infections and carriages in the same families were caused by MRSA isolates with identical pulsed-field type. HKU100 was the largest cluster, with 18 isolates throughout Hong Kong. This cluster included isolates from persons of Chinese, Nepalese, Filipino, and British origins. In the multilocus sequence typing analysis, HKU100 isolates were found to belong to the ST30 (2-2-2-2-6-3-2) group. All HKU200 isolates were recovered from persons of Chinese origin. Unlike HKU100 isolates, the geographic sources of HKU200 isolates were more restricted. Four of the five isolates were recovered from patients residing in districts close to the border with mainland China. The only patient whose residential address was not close to the border had a travel history to mainland China 1 month before the onset of infection. Furthermore, the HKU200 cluster included the two isolates from household 12 with frequent travel history to mainland China. HKU200 isolates had three antibiogram patterns. They had an ST59 allelic profile (19-23-15-2-19-20-15) or its single locus variant (19-23-15-48-19-20-15, designated as ST338). All five isolates of HKU300 were recovered from one Japanese family. Interestingly, the isolates exhibited different susceptibility to gentamicin and tetracycline, resulting in three different antibiogram patterns. Three HKU300 isolates were found to have an ST8 allelic profile (3-3-1-1-4-4-3). The singleton isolate had an ST8 allelic profile.

**Table. *Staphylococcus* cassette chromosome mec (SCCmec) types and Panton-Valentine leukocidin (PVL) positivity according to dendrogram grouping**

| Dendrogram groups (no. of isolates) | *ccr* gene complex | Loci present | SCCmec type | No. of PVL positive/total |
|---|---|---|---|---|
| HKU100 (18) | 2 | D | IV | 17/18 |
| HKU200 (5) | 5 | EF | V | 5/5 |
| HKU300 (5) | 2 | DG | IVA | 0/5 |
| Singleton (1) | 2 | D | IV | 1/1 |

## Discussion

There are multiple lineages of community-associated MRSA (ST30-IV, ST59-V, and ST8-IV/IVA) in Hong Kong. These lineages differed from the major clones of MRSA that account for most health care–associated MRSAs in this locality.[5]

Our community-associated MRSA strains could have been introduced from other areas through international travel, as Hong Kong has over 10-million visitors annually. HKU200 isolates may have a link to mainland China because all were recovered from individuals whose addresses were close to the border with mainland China or with a history of frequent cross-border travel.

Community-associated MRSA infections could spread among household contacts of individuals. In each household, transmission of the same MRSA strain was confirmed by pulsed-field gel electrophoresis analysis. Although spread of MRSA among household contacts of persons with community-associated MRSA has been previously reported,[6] our study is the first to document the magnitude of this risk. Intrafamilial transmission of community-associated MRSA raises issues such as whether household screening should be routinely arranged, with a view to decolonise the carriers.

Nine (39%) of the 23 isolates from the index patients were recovered from individuals of non-Chinese origins. Therefore, certain ethnic groups in Hong Kong could be at a higher risk for community-associated MRSA infections.

## Conclusions

Multiple lineages of community-associated MRSA, including the widespread ST30-IV Southwest Pacific clone, are spreading in the Hong Kong community. Intrafamilial transmission of community-associated MRSA may remain under-recognised unless household screening is conducted. More studies should be conducted to understand the transmission dynamics of community-associated MRSA in non-familial settings.

## Acknowledgements

This project forms part of a series of studies commissioned by the Food and Health Bureau of the Hong Kong SAR Government and was funded by the Research Fund for the Control of Infectious Diseases. The results of this study have been reported in the following publications:

1. Ho PL, Cheung C, Mak GC, et al. Molecular epidemiology

and household transmission of community-associated methicillin-resistant *Staphylococcus aureus* in Hong Kong. Diagn Microbiol Infect Dis 2007;57:145-51.

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WK Tung 董偉杰
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# Community-associated methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections in Hong Kong

**Key Message**

Community-associated methicillin-resistant *Staphylococcus aureus* is an emerging cause of skin and soft tissue infections in Hong Kong, especially among certain ethnic minorities.

## Introduction

Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) has the ability to cause serious and even fatal infections (necrotising pneumonia, meningitis, pyomyositis, and severe sepsis) in healthy individuals, in addition to the more common skin and soft tissue infections (SSTIs). With few exceptions, community-associated MRSA strains possess the Panton-Valentine leukocidin (PVL) genes and one of the novel *Staphylococcus* cassette chromosome mec (SCCmec) elements (types IV and V). These isolates have been described worldwide, including outbreaks involving 'closed populations', for example, among contact sports athletes, jail inmates, military recruits, and some indigenous communities.[1] In 11 major United States cities, community-associated MRSA strains related to the USA300 clone have emerged as the most common cause of SSTI among patients presenting to emergency departments. Moreover, such infections may be more likely to spread to household members and recur than methicillin-sensitive *S aureus* (MSSA) infections.

Community-associated MRSA strains are also emerging in Asia, but their prevalence in SSTIs remains undefined. In Hong Kong, such infections may be disproportionately common among individuals from foreign families.[2,3] Pulsed-field gel electrophoresis (PFGE) and multilocus sequencing typing showed that most isolates were attributed to the ST30-HKU100 and ST59-HKU200 clones.

## Aims and objectives

To determine the burden of PVL-positive community-associated MRSA among patients presenting to the emergency department with purulent SSTI and assess potential associated risk factors.

## Methods

An enhanced surveillance for community-acquired MRSA was conducted over a 4-month period from November 2006 to February 2007. It involved the emergency departments in six regional hospitals estimated to provide service to half of the 6.6-million inhabitants in Hong Kong. Wound swabs were obtained for culture from all patients who present with purulent SSTIs of less than 7 days duration.

All MRSA and a subset of MSSA were further characterised at the laboratory in the Centre of Infection, University of Hong Kong. The SCCmec types and presence of PVL genes were determined as described.[2,3] A standardised questionnaire was used to collect patient information on demographics, history, and underlying medical conditions. Community-associated MRSA infection was defined by outpatient presentation and the absence of health care exposures (including hospitalisation, surgery, or dialysis within the past 12 months), any indwelling catheter or percutaneous device (eg urinary catheter) at the time of presentation, and residence in a nursing home.

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**Table. Epidemiologic and microbiologic characteristics of 13 Panton-Valentine leukocidin (PVL) positive community-associated methicillin-resistant *Staphylococcus aureus* infections**

| Strain | Patient sex/ age (years) | Ethnicity | *Staphylococcus* cassette chromosome mec | *agr* | Resistance pattern* | spa type | Pulsed-field gel electrophoresis† |
|---|---|---|---|---|---|---|---|
| AE-052 | M/43 | Chinese | IV | 3 | None | t019 | HKU100 |
| AE-090 | M/41 | Chinese | IV | 3 | None | t019 | HKU100 |
| AE-255 | F/8 | Chinese | IV | - | None | t019 | HKU100 |
| AE-069 | F/42 | Filipino | IV | 3 | None | t019 | HKU100 |
| AE-086 | F/32 | Filipino | IV | 3 | None | t019 | HKU100 |
| AE-088 | F/34 | Filipino | IV | 3 | None | t019 | HKU100 |
| AE-156 | M/27 | Filipino | IV | 3 | None | t019 | HKU100 |
| AE-124 | M/24 | Chinese | IV | 4 | E, CC | t437 | HKU200 |
| AE-136 | M/18 | Chinese | IV | 4 | Te | t437 | HKU200 |
| AE-260 | M/50 | Chinese | V | 4 | Te | t437 | HKU200 |
| AE-281 | M/25 | Chinese | V | 4 | E, CC, Ch, Te | t437 | HKU200 |
| AE-297 | M/48 | Chinese | V | 4 | E, CC | t437 | HKU200 |
| AE-298 | F/28 | Sri Lankan | IV | 1 | None | t008 | HKU400‡ |

* E denotes erythromycin, CC clindamycin, Te fusidic acid, gentamicin, rifampin, and tetracycline, and Ch Chloramphenicol
† Pulsed-field gel electrophoresis and spa type identical to the singleton isolate (strain 7295) in our study[3]
‡ Polymerase chain reaction negative on repeat testing

## Results

A total of 298 patients aged 2 to 97 (median, 41) years with purulent SSTIs were recruited. About 66% of patients were males, and 3% were children aged 12 years or less. About 92% of the patients were Chinese. The other ethnic groups were Filipino (n=10), Pakistani (n=5), Indian (n=3), Nepalese (n=3), Caucasian (n=1), Sri Lankan (n=1), and mixed (n=1).

*Staphylococcus aureu*s was isolated from 126 (42%) patients, including MSSA from 105 of them, MRSA from 19, and borderline oxacillin-resistant *S aureus* from two. Moreover, MRSA was isolated from 5% (13/241) of abscesses, 13% (5/40) of infected wounds, and 17% (1/6) of purulent discharges associated with cellulitis.

The PVL gene was detected in 13 of the 19 MRSA isolates, including 12 isolates from abscesses and one from cellulitis. Among the six patients with PVL-negative MRSA, four had recognised risk factors for health care–associated infection, including a history of hospitalisation in three, indwelling medical devices in two, and one each for nursing home residence and intravenous drug abuse. None were health care workers. All patients with PVL-positive MRSA represented community-associated infection. In univariate analysis, Filipino ethnicity was significantly more likely than Chinese to be infected by PVL-positive community-associated MRSA (odds ratio, 14.8; 95% confidence interval, 3.3-70.0; P<0.001). All other clinical and epidemiologic features were not predictive of PVL-positive community-associated MRSA. In addition, an analysis was conducted for the patient subset consisting of healthy young adults aged between 18 and 49 years who were Chinese or Filipinos and who met the criteria for community-associated infection. This subset comprised 83 Chinese and 9 Filipinos. The result confirmed Filipino ethnicity as a significantly risk factor (odds ratio, 10.3; 95% confidence interval, 2.2-48.6; P=0.003).

Epidemiologic and microbiologic features for the 13 PVL-positive community-associated MRSA are summarised in the Table. Four patients (AE-69, AE-86, AE-88, and AE-298) were foreign domestic workers, and in two, the symptoms occurred within a short period of their arrival for employment (3 weeks for AE-86 and 3 months for AE-69). The other patients had diverse occupations. The PFGE types for the 13 PVL-positive isolates were characteristic of community-associated MRSA (seven were HKU100, five were HKU200, and one was HKU400). All PVL-posit–associated MRSA had SCCmec type IV or V. Conversely, PVL-negative isolates had PFGE patterns and spa types (t1081 or t037), which were typical of health care–associated MRSA in Hong Kong. Their SCCmec types were III, IV, or V.

## Discussion

Community-associated MRSA is rapidly emerging in Hong Kong. Among patients with purulent SSTIs, 10% (13/125) of all *S aureus* isolates and 5% (12/241) of cutaneous abscess were attributed to PVL-positive community-associated MRSA. The figures represent a remarkable increase since the first detection of PVL-positive community-associated MRSA isolates in Hong Kong 3 years ago.

Four (31%) of the 13 PVL-positive MRSA isolates were recovered from Filipinos. This ethnic group only comprises 2% of the Hong Kong population. Filipino ethnicity was identified as a risk factor for PVL-positive MRSA infection. Because most Filipinos are domestic workers in Hong Kong, the observed association could possibly reflect poverty and inadequate access to medical services. Both PFGE and spa type confirms that the MRSA isolates among the Filipino patients were clonally related. Because the cases had no apparent epidemiologic linkage, the reason for the clonal spread is unclear. As some of these cases were

work migrants, country-to-country transmission involving MRSA carriage is a possibility.

For the treatment of purulent SSTIs, the importance of incision and drainage has been clearly established. On the contrary, the impact of an initial active antimicrobial therapy on clinical outcomes was mixed. Although earlier studies found clinical resolution for most community-associated MRSA SSTIs after drainage, regardless of whether the patient received an appropriate antimicrobial agent, one recent report clearly demonstrates a small but significant number of treatment failures after certain active antimicrobial therapies.[4]

## Conclusions

Community-associated MRSA is an increasingly important public health issue in Hong Kong. Accordingly, its notification has been made mandatory since January 2007. Active contact tracing and MRSA decolonisation with daily nasal mupirocin and chlorhexidine detergent for showers for 5 days are offered to all carriers. Future studies should address the effectiveness of this approach, and home sanitation and hand hygiene as control measures. The over-representation of Filipinos among PVL-positive community-associated MRSA infections deserves further investigation with a larger sample.

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Ho PL, Chuang SK, Choi YF, et al. Community-associated methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*: skin and soft tissue infections in Hong Kong. Diagn Microbiol Infect Dis 2008;61:245-50.

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# Epidemiology of SARS in the 2003 Hong Kong epidemic

**Key Messages**

1. The temporal and spatial evolution of the SARS epidemic in Hong Kong is described.
2. Estimates of key epidemiological distributions and their stability over the course of the epidemic are derived.
3. The characteristics of those who contracted the disease are determined including factors associated with the likelihood of mortality as a result of SARS-coronavirus infection.

## Introduction

The SARS epidemic was the first communicable disease epidemic of the 21st century, with 29 countries affected. The first human case was identified in Guangdong, China on 16 November 2002 and the last known case had a symptom onset date of 5 July 2003 in Taiwan. The disease infected 8098 individuals of whom 774 died.[1,2] Hong Kong bore a large proportion of this morbidity and mortality burden, and was the link between cases in China and other parts of the world. Of 1755 cases, 299 deaths occurred from 15 February to 31 May 2003.

To formulate public health policy, an account of the epidemiology of SARS in Hong Kong was undertaken during the outbreak.[3] The dataset has since been updated using information from all 1755 reported cases. Relaxation of parametric assumptions was allowed in the mid-epidemic analysis, in the analysis of the interval from symptoms to admission, admission to death, and admission to discharge. Furthermore, complete case data enabled analysis of predictors of SARS-related mortality using logistic regression.

## Aims and objectives

To generate and delineate the definitive epidemiological parameters of SARS-CoV, using the complete case-contact data from the 2003 Hong Kong outbreak.

## Methods

### *Sources of data*

We analysed an integrated database (SARSID) derived from the Hong Kong Hospital Authority eSARS system (a secure web-based data repository containing mostly real-time clinical data). Some data fields were collected/confirmed retrospectively via a detailed chart review (according to a standardised protocol by trained nurses) and the Department of Health's Master List (consisting mostly of questionnaires of case and case-contact data). The latter contained details on all SARS patients admitted to hospitals in Hong Kong throughout the entire epidemic. The questionnaires (exploring case and case-contact information) were administered, mostly through telephone interviews, with all SARS patients (in whom the diagnosis were confirmed by the Department of Health). The interviews were conducted mostly within 3 days (up to a maximum of 1 week) of the initial presentation. For patients who could not be contacted or were too ill to be interviewed or dead, proxy reporting was obtained from an immediate family member most familiar with the medical and contact history of the patients before infection. Data on case and contact information were collected on all 1755 SARS patients, although not all data elements were completed for all cases.

Laboratory confirmation of SARS was by: (1) reverse transcription–polymerase chain reaction (RT-PCR) for SARS-CoV and (2) serological testing for IgG against SARS-CoV. Patients were considered to have laboratory-confirmed SARS if there was: (1) a positive RT-PCR result from two or more clinical specimens, either from different sites or tested in different laboratories, obtained either from live patients or post-mortem; or (2) seroconversion by

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ELISA, immunofluorescence assay (IFA) or neutralisation assay. Paired samples for serological testing were collected at least 21 to 28 days apart. All specimens were carried out in three designated laboratories (The Chinese University of Hong Kong, The University of Hong Kong, and the Department of Health) where rigorous quality control procedures were in place.

### *Statistical analysis*

The epidemic time series of all 1755 local cases was constructed based on the date of symptom onset and infection cluster. Infection clusters were classified by probable transmission setting (institutional vs community), location (eg housing estates), occupation (eg health care workers) and workplace (eg hospitals). The age and sex distributions of SARS patients were compared with general population estimates derived from the 2001 population census.

To illustrate the geospatial pattern of disease spread, we used a geographic information system (ArcGIS and its extension modules) to construct a map of infection clusters in different districts of Hong Kong.

Empirical distributions were plotted for intervals from onset to admission, onset to death, and onset to discharge, and the mean and variance of these distributions calculated. The database contained 81 patients with one exposure to a confirmed SARS patient within 15 days (with start and end dates recorded), no travel and who were not hospitalised prior to the onset of symptoms. The relationship between the onset-to-discharge interval and patient age was tested by linear regression. A model was fitted to the onset-to-admission interval, with onset category as the independent variable. In addition, the sex-specific linear relationships between age and the variability of the onset-to-discharge and onset-to-death intervals were examined by modelling the standard deviation as a linear function in age. The resulting model was solved by the maximum likelihood method.

Logistic regression was used to identify factors significantly associated with fatality due to SARS. The following variables were tested in the model: age, sex, occupation (health care worker vs others), symptoms on presentation (typical vs atypical), infection cluster, calendar period of infection (as defined by the symptom onset date, interval from onset to admission), presence of pre-existing co-morbidities (including asthma, chronic obstructive pulmonary disease, cardiovascular disease, cerebrovascular disease, cancer, diabetes mellitus, chronic renal disease, and chronic liver disease). The ratio of the lactate dehydrogenase level to the upper normal limit was used as an indicator of disease severity on admission, and the number of days between the onset of symptoms and initiation of ribavirin. We used indicator variables to denote missing items for variables that did not have complete data coverage, ie atypical symptoms, infection cluster, and lactate dehydrogenase level.

Probability of survival/mortality curves were plotted (stratified by age) to illustrate the dependence of time-to-death for those who died. All analyses were repeated on the 1467 patients with laboratory confirmation of SARS. All statistical analyses were carried out on STATA version 8.0.

## Results

### *Laboratory confirmation of SARS status*

Of the 1755 SARS patients, 1467 (83.6%) were confirmed by laboratory: 447 seroconverted and had two or more positive RT-PCR results, 959 seroconverted only, and 61 had two or more positive RT-PCR results only. In 288 patients, laboratory confirmation was not possible for various reasons, including inadequate or insufficient specimens (n=199), negative RT-PCR and/or serology results (n=89).

### *Time*

The patient who initiated the largest transmission chain in Hong Kong and the global outbreak was from Guangdong province. He first had symptoms on 15 February 2003 and was admitted to hospital on 22 February 2003, one day after arriving in Hong Kong.[4] The development of the epidemic featured a period of exponential growth, beginning on 10 March 2003, which was further exacerbated by transmission not related to intimate personal contact (in the Amoy Gardens estate and immediate neighbourhood).[4] This was followed by a period of comparative stability throughout early to mid April, with a declining trend beginning in the week of 22 April 2003. The last case had symptoms onset on 31 May 2003 and was admitted to hospital on 2 June 2003.

### *Place*

About 49% of SARS patients were infected in clinics, hospitals or elderly/nursing homes. The superspreading event in Amoy Gardens resulted in a daily incidence of close to 100 at the height of the outbreak in late March. Spread within residential buildings accounted for 22% of all cases, mostly at Amoy Gardens. An additional 7% of all cases were classified as 'near to Amoy Gardens'. This referred to SARS patients living in the immediate neighbourhood of Amoy Gardens, who were believed to be linked to the main Amoy Gardens cluster, but not themselves residents of that housing estate. About 5% of Hong Kong cases were imported (or re-imported) from overseas or from air travel. Fewer than 10% resulted from transmission in the general community including household settings (aside from the superspreading event in Amoy Gardens). Of these, 64% (97/152) could be attributed to intra-familial or within-household spread (defined as transmission from one household or family member to another with no other known sources of an infectious contact).

There was clear clustering of cases in certain districts of the Kowloon peninsula (Kwun Tong in which Amoy Gardens is located) and the New Territories (Shatin and Tai Po districts where the Prince of Wales and Alice Ho

Mui Ling Nethersole Hospitals, sites of large nosocomial outbreaks, are located respectively), but Hong Kong Island was relatively spared. Clustering became apparent as the epidemic unfolded, with per capita incidence varying significantly between districts.[3]

### *People*

The female/male ratio among infected patients was 1.26. Compared to the age and sex distribution of the Hong Kong general population, there was a clear excess of young adults, especially females (102 out of the 254 female SARS patients aged 25 to 34 years were nurses). Moreover, there was a relative deficit of children and adolescents. Elderly men (>75 years old) were over-represented among SARS patients, as were elderly women despite to a lesser extent. Health care workers accounted for 23% of all infected persons; most of them worked in the public sector, where SARS patients were mainly cared for (in 14 designated centres). Some patients were initially admitted to other hospitals but later transferred. Nurses accounted for 52% of the 405 health care workers infected, followed by health care assistants such as orderlies (28%) and medical doctors (16%).

### *Key epidemiological parameters*

The estimated mean and variance of the incubation period was 4.6 and 15.9 days, respectively[2]; 95% of patients had the onset of symptoms within 12.5 days of infection.

Onset and admission times are both observable events. Patients were grouped by the week of clinical onset, and 11 time-periods were analysed. There were too few patients with symptom onset before 15 February 2003 for robust analysis. According to a biphasic linear model, the interval from symptom onset to admission decreased significantly during the first 5 weeks (P<0.001), but not over the last 6 weeks (P=0.27).

The respective mean and variance of the interval from symptom onset to death were 23.7 and 221.0 days, and for the interval from symptom onset to discharge were 26.5 and 194.9 days.[2] There was substantial variability in the distribution of these two intervals, with greater variance observed for the former. The variability decreased with age for the former (P=0.027), whereas the opposite was true for the latter (P<0.001). The symptom onset-to-death intervals varied significantly according to patient age, demonstrating an inverted U-shaped relationship, where those aged 50 to 59 years (especially females) had the longest mean intervals and those aged >70 years had comparatively briefer periods of illness before death. In contrast, older patients who survived were usually discharged later and this relationship appeared to be linearly related to age (P<0.001).

### *Case fatality ratios and associated predictors*

The overall case fatality ratio was 17% (299 deaths out of 1755 SARS cases). Survival was heavily influenced by both age and sex. Male SARS patients had a 50% (95% CI=7-109%) excess risk of death. Mortality increased significantly with age (P<0.001). For example, none of the female patients <30 years old died, compared to approximately 75% of males aged >70 years died. A lower case fatality was associated with health care worker status (adjusted odds ratio [OR]=0.35; 95% CI, 0.15-0.80). The minority of individuals presenting with atypical symptoms (3%) had a significantly increased risk of death (adjusted OR=2.62; 95% CI, 1.24-5.53). Similarly, the presence of pre-existing co-morbidities and greater disease severity (as inferred from higher lactate dehydrogenase levels on admission) increased the risk of death. The calendar time-period during which patients fell ill was not significantly associated with survival, nor was earlier admission after the symptom onset, or the timing of ribavirin administration. The precise infection cluster that a patient belonged to was not a significant predictor (at the 0.05 level).

Analyses based on the subset of 1467 patients with laboratory confirmation of SARS produced similar results to those of the full cohort. However, health care worker status (adjusted OR=0.64; 95% CI, 0.26-1.56) and atypical symptoms (adjusted OR=2.06; 95% CI, 0.83-5.11) were no longer significantly associated with survival at the 0.05 level for this subset of patients. We believe that the full 1755 cohort should remain the main results partly because 199 out of 288 non-laboratory confirmed cases did not have adequate or sufficient clinical specimens to be tested. Nonetheless, they fulfilled clinical and epidemiological criteria for the diagnosis of SARS prior to laboratory testing. This is different from the scenario where results of both RT-PCR and serological tests were negative. Additionally, we examined the influence of missing data on the stability of the logistic regression models (ie both the 1755 and 1467 models) through a series of sensitivity analyses. The two variables with the most numbers of missing values, namely atypical symptoms (missing items=273, 16%) and lactate dehydrogenase level on admission (missing items=242, 14%), were excluded from the regression model. The results were robust even after deletion of these two variables, as they achieved significance as well as directionality and magnitude of associations. Moreover, after multiple imputation to deal with missing data for these two variables, the regression results were again very similar to the baseline model.

## Discussion

Our findings provide a summary of the time-course and patient location of the 2003 Hong Kong SARS outbreak and the characteristics of those infected. The time-course of the epidemic was marked by an initial period of exponential growth and a decline after 6 weeks of intensive public health control measures. Significant geospatial clustering was observed, with several large clusters of SARS cases in hospitals and residential settings and a high proportion of health care workers. These observations are largely consistent with those reported for the Singapore

and Toronto outbreaks, where the hospital environment substantially amplified the risk of infection.[5-7] The pattern of infection clusters also suggests that the viral infection is of low transmissibility, except in settings of intimate contact or where significant environmental contamination occurred. It may also suggest low infectivity for some days following the onset of clinical symptoms. In addition, the risk of acquiring the infection varied significantly according to age, with relatively few cases and no deaths in children and adolescents. The reasons for this remain unclear.

One of the key aspects of infection control introduced during the epidemic was a policy of quarantine, where individuals who were possibly infected or had contact with known SARS cases were isolated for a fixed period. Definition of this period was informed by timely estimates of the time from exposure to first symptoms, ie the incubation period distribution. The analyses of the full dataset indicated that 13 days may be necessary to capture 95% of all possible cases,[2,8] compared to a period of 10 days recommended by the World Health Organization and US Centers for Disease Control and Prevention. Yet our estimation procedure adopted a parametric gamma distribution and thus implicitly assumed the possibility of very long incubation periods. In addition, owing to methodological constraints, this distribution was fitted to data on a very small subset of cases with a single exposure source with known start and end dates, and therefore the generalisability of these findings to the whole sample was unknown.

The analysis of the onset-to-admission interval showed a progressive shortening of the interval from the onset of symptoms to presentation at hospital, likely due to heightened community awareness and a high index of suspicion among health care providers as the epidemic spread.[9] Coupled with the observation that SARS almost exclusively manifested as a florid clinical syndrome requiring inpatient treatment and rarely as a subclinical or mild infection (ie with no asymptomatic carriers of the disease), it was possible to reduce the onset-to-admission interval to a minimum (ie 2 days) and this might be an effective public health control measure. It was relatively easy for those infected to recognise their illness and promptly present to the health care system. This enabled rapid isolation of infectious individuals, hence reducing the effective infectious period and thus the risk of onward transmission. However, shortening the time between first symptoms and the initiation of treatment after hospital admission did not appear to increase the probability of survival. Clinical studies of the typical course of infection in SARS-CoV patients suggested that the average peak infectiousness may occur 8 to 9 days after the onset of symptoms.[10] This pattern, which is atypical for most respiratory or gastrointestinal tract infections, implies that prompt isolation after the onset of symptoms is a very effective public health measure for this particular infection. This observation also helps to explain the large fraction of cases that occurred in health care workers in Hong Kong, Singapore, Taipei and Toronto, since they had contact with patients during their peak infectious phase.

The distributions of onset-to-death and onset-to-discharge intervals add information to the natural history of the disease process (mostly among treated patients) and underline the importance of patient age and sex in determining the course of illness. They also allow clinicians to understand the relative distributions of time to clinical outcomes, so that this SARS outbreak can be compared to future outbreaks should they occur. The lower mean and variability in the symptom onset-to-death interval distribution among the deceased elderly was likely due to their relative frailty and higher prevalence of co-morbidities. Whereas factors such as post-SARS disability and treatment complications might have led to a longer hospital stay for elderly survivors; some of these patients were hospitalised for treatment of other diseases after recovery from SARS. The modal peak of the symptom onset-to-discharge interval distribution of 21 days was, to an extent, an artefact of administrative guidelines, namely a minimum 21 days of hospitalisation, which had been in effect since early April 2003.

The estimation of epidemiological parameters and case fatality ratios during an ongoing epidemic is complicated by the open cohort problem of censoring, such that it is impossible to ascertain who will eventually die or be discharged among those still hospitalised at the time of the analysis. This is further complicated by the temporal evolution of the epidemic with incident cases continually being added to the pool of infected individuals. In this analysis of all 1755 consecutive cases in Hong Kong, the outcome was observed in all cases and hence issues regarding censoring do not apply.

Although the overall case fatality ratio was 17%, this figure masks the significant variation in case fatality by age. Male gender, more severe illness on presentation as indicated by the lactate dehydrogenase level, and the presence of pre-existing co-morbidities were significantly associated with a high case fatality in the multivariable analysis. The timing of ribavirin administration did not significantly influence clinical outcome, possibly due to residual confounding or insufficient power to detect a difference given that most patients were treated. Previous analyses of case fatality predictors have only examined small, hospital-based datasets with limited information on a comprehensive range of personal and clinical variables, yet their findings were similar to the present study with respect to the effects of age, sex, co-morbidities and high lactate dehydrogenase levels on mortality. It should be noted that even in the largest case cohort in Hong Kong, there was insufficient statistical power to examine all important factors that might have influenced case fatality.

As our study demonstrated, the appropriate methodology to identify predictors of survival (or case fatality) is through a multivariable logistic regression model with a closed cohort. In the heat of a crisis, however, observational studies based

on amalgamated datasets from different clinical settings are the only means by which treatment value can be assessed. In drawing conclusions from such analyses, bias may be present in patient choice for any given treatment, and this must be taken into account.

## Conclusions

Future research should closely examine the relative merits and drawbacks of different statistical approaches to estimating the distribution of incubation periods, since such estimates are central to public health and evolving an infection control policy. Quarantine times must take into account the extent of potential disruption to people's lives and the likely degree of compliance in different communities.

To clarify some of the unresolved issues raised in this report, more detailed analysis involving other relevant clinical factors, such recourse to non-invasive assisted ventilation or other medications and their timing, as well as longitudinal observations of clinical and laboratory parameters are needed.

Public health authorities worldwide should formulate appropriately resourced protocols for randomised controlled trials to properly evaluate the efficacy of various management strategies should SARS recur. While SARS is unlikely to return as a large epidemic across many different countries, clinical investigators need to recognise the importance of multi-national, multi-centred epidemiological studies and collaboration. This should extend to clinical trials to increase the power to detect moderate effects of treatment regimens and associated risk factors.

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# Spatial clustering of SARS in Hong Kong

## Key Messages

1. Geographic information system (GIS) can be applied during an acute infectious disease outbreak to reveal new geospatial information in addition to standard field epidemiological analyses.
2. When applied in real time during the onset and evolution of an epidemic, GIS can monitor and enhance understanding of the transmission dynamics of an infectious agent, thereby facilitating the design, implementation and evaluation of potential intervention strategies.

## Introduction

Since the SARS outbreak, progress has been made in understanding the biology, pathogenesis and epidemiology of both the disease and coronavirus. Nonetheless, much remains to be done in the development of effective therapeutic interventions and diagnostic tools of sufficient sensitivity and specificity soon after the onset of symptoms. The evaluation of key epidemiological parameters and the impact of outbreak interventions at the population level remain to be investigated. Moreover, there was evidence of clear geographic micro-clusters of SARS cases and superspreading events (SSEs) which yielded widely varying densities of infection among the districts.[1]

The application of geographic information system (GIS) in health and health care is a new approach. In particular, a wide variety of cartographic techniques for mapping and analysis of communicable disease data have become available. These enable the application of GIS to examine spatially related problems from different perspectives. In addition to descriptive mapping functions, GIS is capable of data manipulation and geostatistical analysis.

## Aims and objectives

The GIS technology was applied to map and visualise the SARS outbreak in Hong Kong. Cartographic and geostatistical methods were used to represent and analyse patterns of disease spread during the 2003 outbreak. This study also assessed the utility and limitations of GIS as a real-time disease surveillance tool.

## Methods

Both spatial and non-spatial data were used. Spatial data are geographic and represent the real world features as points, lines, or areas. SARS occurrences were depicted as points on a base map of Hong Kong. Descriptive data about the confirmed cases of SARS were derived from case-contact interviews. Associated residential address data were first cleaned and then verified before undergoing geo-referencing to enable mapping.

We analysed the SARSID integrated database which contained details on all confirmed SARS cases admitted to hospitals in Hong Kong during this epidemic (from 15 February 2003 to 22 June 2003). A total of 1709 confirmed cases (out of 1755 cases) were extracted for geo-coding and analysis. The unaccounted cases (2.6% of the total) had inconsistencies in the address entries.

Three levels of analysis were carried out: (1) elementary, (2) cluster, and (3) contextual. Simple visual inspection of the SARS incidence was conducted at the elementary level, followed by an identification of hot spots through cluster analysis. At the contextual level, the relationships between SARS and various geographical phenomena were examined. All analyses were carried out using the ArcGIS and its extension modules by the Environmental Systems Research Institute in Redlands, California.

## Results

Maps generated in elementary analysis revealed the geographic spread of SARS

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infection by residential addresses of Hong Kong. There was clear clustering of cases in certain districts of the Kowloon peninsula (such as Kwun Tong) and the New Territories (including Shatin and Tai Po), but Hong Kong Island was relatively spared.

Cluster analysis generated statistical surfaces using the kernel density method to account for SARS incidents on the date of symptom onset and a 5-day incubation period. Each of the time-series kernel maps showed SARS infection rate per 1000 inhabitants on a prototypical day during the 16-week epidemic, with darker zones emphasising disease hot spots.

Contextual analysis enabled construction of daily histograms of the number of observations by 15 classes of infection rates, which were primarily composed of inverse J-shaped curves, showing an increased concentration of SARS occurrences toward the end of March 2003. Origin-and-destination plots of disease clusters were used to explore likely or probable locations of index cases or environmental sources of infection as informed through contact tracing by public health authorities.

## Discussion

The kernel method of portraying infection rates provided a means of highlighting locations of disease risks. The related R-values and Moran's coefficients enhanced the analytical context of the point-pattern distributions. Such geospatial intelligence provided the basis for formulating our transmission dynamics model.[2] A variety of approaches ranging from a simple deterministic compartmental approach to a spatially explicit and individual-based simulation were possible in constructing the transmission dynamics model. We based our analyses on a stochastic meta-population compartmental model because the incidence of SARS varied substantially according to geographical districts.

The daily animated series of kernel maps clearly show that SARS was a highly localised disease. In contrast with influenza and measles transmitted through casual contact, the route of transmission for SARS was more compatible with close contact via heavy respiratory droplets and fomites. An alternative interpretation of the observed high degree of geospatial clustering shows that SARS was attributed to an environmental point source outbreak, as demonstrated by faulty sewage systems and the chimney effect hypothesised for the Amoy Gardens SSE. Although it is difficult to gauge retrospectively, had the GIS been available for near real-time analysis, it might have afforded more rapid contact tracing and public health interventions to prevent further large-scale environmental point source outbreaks.

Contextual analysis is a useful adjunct to the usual bio-mathematical modelling approach using reproductive numbers at different points in time throughout the SARS epidemic.[2] The origin-and-destination analysis complemented with R and Moran's I values suggested the direction of spread in a disease cluster that could be used to inform contact tracing and the design of quarantine measures. Instead of isolating entire residential districts as practised in China at the height of the SARS outbreak, these analytical approaches might have enabled better selection of such districts for quarantine.

There are limitations to the GIS technique in infectious disease epidemiology and outbreak investigation. Mapping of diseases tends to expose the 'where' but not 'why there' of the outbreak although map patterns can provide stimuli for generating hypotheses of disease causation. Moreover, newer developments that complement traditional mapping functions such as cluster and contextual analyses can be useful adjunctive investigational tools in outbreak control.

The completeness and rapid availability of necessary data is another area of concern. Conventional field epidemiological data rarely contain the full range of variables required in a GIS analysis. Unfortunately, non-standardisation of patient address formats and missing details diminish the proportion of useable cases for analyses. A number of generic problems associated with information system development must be resolved to render real-time disease monitoring and surveillance. On top of the list is standardisation of data capture documents, as well as procedures and protocols for information management. There is also an urgent need to manage delays in transferring and updating disease information to facilitate rapid analysis and audit of databases. The SARS epidemic is a clear signal that Hong Kong needs much greater investment in health informatics (ie public health information systems, the skills to use them, and networks to share them).

## Conclusions

Integration of GIS technology into routine field epidemiological surveillance offers a scientifically rigorous and quantitative method for the identification of unusual disease patterns in real time. Its potential can be synergistically maximised when linked to clinical databases collecting data at the point of care. This integration should entail the whole population and environmental data sources (including meteorological, transportation, topographical information) so as to rapidly recognise, locate and monitor disease outbreaks.

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# Viral loads in clinical specimens and SARS manifestations

**Key Messages**

1. A high viral load in nasopharyngeal aspirate (with or without a high viral load in serum) is a useful prognostic indicator of respiratory failure or mortality. The presence of viral RNA in multiple body sites is also indicative of poor prognosis.
2. Early treatment with an effective antiviral agent before day 10 may decrease the peak viral load, and thus ameliorate the clinical symptoms and mortality, and reduce viral shedding and the risk of transmission.

## Introduction

The SARS pandemic affected 8098 people with 774 fatalities in 2002-2003.[1] A novel coronavirus was isolated from SARS patients who had specific seroconversion to this virus.[2-4] Animal models using macaque monkeys, ferrets and domestic cats were established. However, no extrapulmonary lesions could be identified in these animals though virus isolation and real-time polymerase chain reaction (RT-PCR) for viral RNA were positive from their pharyngeal secretions, tracheobronchial secretions, urine, rectal swabs or stool, kidney or lung tissues. We reported the use of RT-PCR to detect SARS-CoV RNA from nasopharyngeal aspirate (NPA), throat swab, urine and stool specimens. We also developed RT-qPCR assays using the LightCycler System (Idaho Technology, Idaho Falls [ID], US) to augment the sensitivity of detection. The serial viral load in NPA was used for monitoring the clinical progress and the response to anti-viral therapy, whereas the admission viral load in serum was used as a marker of prognosis. Unlike the animal models, extrapulmonary manifestations such as haematological changes, diarrhoea, and liver derangement were common in SARS patients. In this study, we assayed and analysed the viral load of clinical specimens from different anatomic sites between days 10 to 15 after the onset of symptoms to understand the role of this virus in the pathogenesis of the clinical manifestations and abnormal laboratory tests in SARS patients.

## Aims and objectives

To correlate SARS-CoV viral load in different clinical specimens with the clinical manifestations of SARS.

## Methods

Patients who fulfilled the modified World Health Organization definition of SARS (n=154), managed in the United Christian Hospital and Caritas Medical Centre were included in this quantitative virological study. All patients were either serologically confirmed by demonstrating a four-fold rise of indirect immunofluoresent antibody titre against SARS-CoV in the serum taken on admission and within day 28 after symptoms onset, or had positive RT-PCR for SARS-CoV RNA confirmed from their clinical specimens (for those who died or failed to seroconvert before day 28). The case definition included fever of 38ºC or higher, cough or shortness of breath, and new pulmonary infiltrates on chest radiography or high-resolution computed tomography in the absence of an alternative diagnosis to explain the clinical presentation. During the first 15 days, patients were prospectively monitored for occurrence of diarrhoea, oxygen desaturation, mechanical ventilation, and laboratory evidence of lymphopaenia, renal impairment, liver dysfunction, abnormal urinalysis, and mortality. For the diagnosis of SARS-CoV infection, NPA and acute sera were taken on admission. Convalescent sera were taken between days 7 and 28 after the onset of symptoms. In all patients, RT-PCR for SARS-CoV was performed on the NPA collected on admission. RT-qPCR was performed for patients who had their NPA, sera, stool and urine specimens collected on days 10 to 15 after the onset of symptoms. All virological diagnostic laboratory tests including viral culture, RT-PCR, RT-qPCR and immunofluorescent antibody detection for IgG seroconversion against SARS-

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CoV were performed according to our protocols. From clinical samples, RNA was extracted using the QIAamp virus RNA mini kit (Qiagen) as instructed by the manufacturer. For all specimens, 140 μL of the sample were used for RNA extraction and extracted RNA was finally eluted in 30 μL of RNase-free water and stored at -20ºC. For the RT-qPCR assay, RNA and cDNA was generated as described; cDNA was amplified in a 7000 Sequence Detection System (Applied Biosystems) by using TaqMan PCR Core Reagent kit (Applied Biosystems). In a typical reaction, 2 μL of cDNA was amplified in a 25 μL reaction containing 0.625 U AmpliTaq Gold polymerase (Applied Biosystems), 2.5 μL of 10x TaqMan buffer A, 0.2 mM of dNTPs, 5.5 mM of MgCl2, 2.5 U of AmpErase UNG, and 1x primers-probe mixture (Assays by Design, Applied Biosystems). The forward primer was 5'-CAGAACGCTGTAGCTTCAAAAATCT-3' (corresponding to nt 17718 to 17742 of SARS-CoV genome) and the reverse primer was 5'-TCAGAACCCTGTGATGAATCAACAG-3' (complementary to nt 17761 to 17785). The sequence of the reporter probe was 5'-(FAM)TCTGCGTAGGCAA TCC(NFQ)-3' (FAM, 6-carboxyfluorescein; NFQ, non-fluorescent quencher; complementary to nt 17745 to 17760). Reactions were first incubated at 50ºC for 2 min, followed by 95ºC for 10 min. Reactions were then thermal-cycled for 40 cycles (95ºC for 15 sec, 60ºC for 1 min). Plasmids containing the target sequences were used as standard controls. To monitor the integrity of RNA extraction for each sample, a housekeeping gene, beta-actin was detected by RT-PCR using two primers: beta-actin forward, 5'-CCCAAGGCCAACCGCGAGAAGAT-3' and reverse, 5'-GTCCCGGCCAGCCAGGTCCAG-3'. All samples were found to contain detectable beta-actin RNA.

### *Statistical analysis*

All timed data were calculated from the onset of symptoms. We compared the viral load in these specimens with the presence or absence of diarrhoea, oxygen desaturation, mechanical ventilation, lymphopaenia, hepatic dysfunction, abnormal urinalysis and mortality by Chi squared or Fisher's exact test for categorical variables and Mann-Whitney *U* test for continuous variables. A two-tailed P value of less than 0.05 was taken to be significant. Correlation between the number of anatomical sites with detectable viral load by RT-qPCR and mortality was calculated by linear regression.

## Results

Viral load in NPA (n=142) between days 10 and 15 after the onset of symptoms was associated with oxygen desaturation (odds ratio [OR]=3.1; 95% confidence interval [CI], 1.6-6.2), mechanical ventilation (OR=11.3; 95% CI, 3.6-35.1), diarrhoea (OR=2.5; 95% CI, 1.3-5), hepatic dysfunction (OR=2.5; 95% CI, 1.2-5.2) and mortality (OR=54; 95% CI, 7-415). Serum viral load (n=53) was associated with oxygen desaturation (OR=5; 95% CI, 1.5-16.4), mechanical ventilation (OR=1.5; 95% CI, 1.1-2) and mortality (OR=17.1; 95% CI, 2.0-151). Stool viral load (n=94) was associated with diarrhoea (OR=14.1; 95% CI, 1.7-114), as was urine viral load (n=111) with abnormal urinalysis (OR=7.2; 95% CI, 1.6-32.9).

## Discussion

The viral load reflects the dynamic interaction between viral replication and viral clearance by body defence mechanisms. Viral load study in SARS has been used for virological diagnosis and monitoring of progress or response to anti-viral therapy. In our study, the viral load in the NPA peaked around day 10 and was immediately followed by a decrease with a concomitant normalisation of the lymphocyte count and a corresponding rise of serum antibodies specific for the SARS-CoV. The presence of the virus and the viral load in different body fluids may have a bearing on the possible modes of transmission. The infectivity at day 10 as reflected by a mean peak viral load of 5.8 and 7.0 $\log_{10}$ copies/mL in positive specimens of NPA and stool respectively suggested that respiratory droplets and indirect contact with faeces might be an important mechanism of transmission. Previous viral load study centred on NPA and serum at the time of admission as a diagnostic tool and a prognostic indicator. Viral load study in various body fluids in addition to NPA and serum has not been performed to determine the transmission and pathogenesis of the pulmonary and extra-pulmonary manifestations of SARS.

The SARS is predominantly a viral pneumonia with a rapid tempo of deterioration. The importance of SARS-CoV as a respiratory pathogen is supported by the strong association of the viral load in the NPA with oxygen desaturation, mechanical ventilation and mortality as evident by odds ratios of 3.1, 11.3 and 54 respectively. Unexpectedly it was also associated with diarrhoea (OR=2.5) and hepatic dysfunction (OR=2.5). Anecdotal reports of the usefulness of steroids in the treatment of SARS suggest these extra-pulmonary manifestations could just be part of an inflammatory spill-over from a process of virus induced immuno-dysregulation or excessive cytokine activation in the lungs. However, our findings suggest that viral replication in these extra-pulmonary sites may be as important since the viral load in the stool correlated strongly with diarrhoea. Moreover, electron microscopy of the ileal and colonic biopsy from SARS patients showed numerous viral particles intra- and extra-cellularly.

The serum viral load also correlated with oxygen desaturation, mechanical ventilation, and mortality. This was not surprising, as viraemia has also been reported in adenovirus, respiratory syncytial virus and rotavirus infections.[5-7] However, viraemia even if present is very short lasting in these mucosal infections. In one study, five out of 41 neonates with positive respiratory syncytial virus **(RSV)** antigen in nasal washes were positive for RSV-RNA in blood. High levels of adenovirus DNA in serum was also associated with fatal outcome in children who developed adenovirus

infection after allogeneic stem-cell transplantation. Six (86%) of seven children who died of adenovirus infection, compared with only two (7%) of 29 other patients, had high serum levels of adenoviral DNA (P<0.0001). The absence of an association between viral load in any specimens with lymphopaenia at day 10 could be explained by the routine use of steroids which induces apoptosis of lymphocytes. The apparent inferior performance of serum viral load as a prognostic indicator could be related to a lower number of available serum samples in this cohort. However, the proportion who had oxygen desaturation in these 53 (38%) patients was not significantly different from the 142 (46%) patients who had submitted nasopharyngeal samples between days 10 and 15.

Compared with other common viral respiratory diseases, the onset of peak viral load in the nasopharynx appeared to be delayed. In a prospective study of viral shedding in nasopharyngeal secretions in experimental adult infections as enumerated by $TCID_{50}$ (median tissue culture infective dose) viral titre or RT-qPCR, RSV was detected between days 2 and 12, with a plateau phase between days 3 and 8 at a peak viral load of 5 $\log_{10}$ copies/mL. In the case of experimental adult influenza, viral replication in NPA peaked at about 48 hours after the onset of symptoms and declined sharply thereafter, with an insignificant degree of viral shedding after days 6 to 8. The peak virus titres in symptomatic volunteers inoculated with influenza A H3N2 ranged from $10^{2.5}$ to $10^{7.0}$ $TCID_{50}$/mL of nasopharyngeal wash. The viral load correlated positively with the clinical symptoms of fever and malaise, as well as the degree of viral shedding. However, the reported low incidence of viraemia and the early peak nasopharyngeal viral load in these two conditions could be accounted by the inherent behaviour of viral replication, background IgG and IgA antibodies with cross-reactivity against homologous antigens (due to previous infections or innate immunity of the host). In many of these experimental infections where the profile of the viral load in NPA was documented, the volunteers were adults and had a low level of background antibodies and therefore concomitant cell mediated immunity against influenza or RSV.

One limitation of the present study was its retrospective nature. Only those who had sent the specimen at around day 10 could be tested and analysed. Changes of lymphocyte subset were also not analysed due to the retrospective nature of this study. Nonetheless, lymphocytes changes in SARS patients were well reported by two other groups who showed a consistent decrease in the peripheral blood level of dendritic cell subsets, natural killer cells, CD4+ and CD8+ T lymphocytes and B lymphocytes in SARS patients.[8,9]

## Conclusions

SARS is predominantly a respiratory infection with spread through viraemia to extrapulmonary sites where viral replication leads to non-respiratory manifestations. There could be concomitant immuno-dysregulation and associated inflammatory damage that accentuates its morbidity and mortality. A high viral load in NPA with or without a high viral load in serum is a useful prognostic indicator of respiratory failure or mortality. The presence of viral RNA in multiple body sites is also indicative of a poor prognosis. Early treatment with an effective antiviral agent before day 10 may decrease the peak viral load, and thus ameliorate clinical symptoms and mortality, and reduce viral shedding and the risk of transmission.

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# Comparative host gene transcription by microarray analysis early after infection of the Huh7 cell line by SARS coronavirus and human coronavirus 229E

**Key Message**

During the early stages of infection, SARS-CoV produces more severe perturbation of host cell gene expression in a human epithelial cell line of liver origin than the HCoV-229E.

## Introduction

SARS-CoV is the aetiological agent of SARS, which is associated with a high mortality and morbidity. Such an unfavourable clinical effect is different from that of other known human coronaviruses, including the group 1 coronavirus 229E and NL63 and the group 2 coronavirus OC43.

SARS-CoV is an enveloped positive-sense single-stranded RNA virus that can grow in embryonic monkey cell lines including the Vero E6 and foetal rhesus monkey kidney (FRhk-4) cells. It can be sub-cultured onto other Vero cells and colonic carcinoma cell lines such as Caco-2 or LoVo. Unlike other human coronaviruses, SARS-CoV proliferates rapidly and causes obvious cytopathic effects in Vero E6 within 48 h of inoculation. There are no other human cell lines known to be susceptible to infection to both SARS-CoV and other human coronaviruses. It has been reported that a human hepatoma cell line (Huh7) can be infected by the pseudotyped lentiviral particles carrying the Spike protein of the SARS-CoV and the wild type replicative SARS-CoV.[1-3]

## Aims and objectives

To report the susceptibility of the cell line Huh7 to infection by both the SARS-CoV and HCoV-229E and perform a comparative gene transcriptional profile at an early stage of such infection by these two viruses to elucidate differences in pathogenesis.

## Methods

### *Cell lines and virus*

Huh7 cells (courtesy of Prof David Ho, Aaron Diamond AIDS Research Center) were used throughout this study. The cells were incubated at 37ºC in Minimal Essential Medium (MEM) supplemented with 10% foetal calf serum, 100 IU/mL penicillin and 100 μg/mL streptomycin. Our prototype virus (SARS-CoV, HKU-39849) was isolated from the lung-tissue biopsy of the brother-in-law of the index SARS patient who travelled to Hong Kong from Guangzhou and started a superspreading event leading to the pandemic.[4] The HCoV-229E strain (American Type Culture Collection Number: VR-740) was used in this study. The SARS-CoV and HCoV-229E used in our experiments had undergone 3 passages in FRhk-4 cells and MRC-5 cells, respectively, and were stored at -70ºC. Viral titres were determined as the median tissue culture infective dose ($TCID_{50}$) per mL in confluent Huh7 cells in 96-well microtitre plates. The plates were used to standardise the viral inoculum and measure the relative susceptibility of the Huh7 cell line to these two viruses. The relative susceptibilities of Vero 1008, Vero 76, Vero, and Huh7 cell lines to SARS-CoV and HCoV-229E were also tested by $TCID_{50}$. One hundred $TCID_{50}$ was confirmed by plaque assays to be equivalent to 85 plaque-forming units. All work with infectious viruses was performed inside a type II Biosafety Cabinet, in a Biosafety Containment level III facility, and the

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personnel wore powered air-purifying respirators.

### *Monitoring of virus-induced cytopathic effect, antigen detection, semi-quantitative and quantitative PCR*

Huh7 cells and culture supernatants infected with either SARS-CoV or HCoV-229E at a multiplicity of infection of 100 $TCID_{50}$ per cell were collected at 2, 4, 12 and 24 h post-infection. A washing step was performed 1 h post inoculation. The percentages of cells developing cytopathic effects (CPE) were counted by inverted light microscopy at 24 and 48 h. The rate of viral replication was measured semi-quantitatively by RT-qPCR on the culture filtrate. The amount of coronavirus antigen expression in infected cells was measured by indirect immunofluorescence tests, using convalescent serum of patients suffering from SARS-CoV and HCoV-229E infection.[5,6] RT-PCR for SARS-CoV and HCoV-229E was performed directly on culture filtrate according to our previous protocol.[5]

### *Microarray analysis*

Human genome-wide gene expression was examined with the Affymetrix GeneChip system HG-U133A microarray, which is composed of more than 22 000 oligonucleotide probe sets interrogating approximately 18 400 unique transcripts, including 14 500 well-characterised human genes. Quality control, GeneChip hybridisation, data acquisition and analysis were performed at the Genome Research Centre, The University of Hong Kong, according to the standard protocols available from Affymetrix. Data analysis was performed using the Microarray Suite Expression Analysis software (Version 5.1; Affymetrix). For comparison across different arrays, the data for each array were normalised by a global scaling strategy, using a scaling target intensity of 500.

### *Gene expression analysis by semi-quantitative PCR, quantitative RT-PCR and immunoassays*

Genes with significant transcriptional changes known to be associated with biological significance were selected for further analysis by semi-quantitative PCR, RT-qPCR and immunoassays. RT-qPCR was performed according to our previous protocol.[7] The extracted RNA was pre-treated with DNase. Primers that specifically amplified the nine genes related to apoptosis, inflammation and coagulation were designed. The housekeeping gene porphobilinogen deaminase (PBGD) was used to standardise the initial RNA content of a sample. Experiments were performed in duplicate and the results for individual samples were expressed as mean expression level of a specific gene/PBGD relative to the reference cDNA. The relative expression of each infected sample versus the uninfected controls were then calculated and expressed as fold changes. Three sets of immunoassays (human IL8, PAI1 and TFPI2) were performed according to our previous protocols and the manufacturers' instruction.[8,9]

### *Statistical analysis*

The fold changes in the target gene expression and the differences in the concentration of protein expression between SARS-CoV and HCoV-229E at different post-inoculation time points were compared by Student's *t* test. A P value of $<0.05$ was considered significant. A statistical package (SPSS 10.0) was used for all analyses.

## Results

### *Susceptibility of Huh7 cell line to SARS CoV and HCoV-229E*

Using a multiplicity of infection of 100, CPE was visible in Huh7 cells at 24 h and progressed to about 50% cell death at 48 h in both viruses. Both viruses produced a comparable $TCID_{50}$ of around $10^7$ per mL in the culture supernatant of Huh7 cells at 48 h. In terms of viral load, one log increase of viral genome copy was noted at 12 h post-infection in both viruses, which was followed by a peak at 24 h. For both viruses, antigen expression could be observed by indirect immunofluorescence in over 50% of the cells at 24 h post-infection.

### *Effects on gene expression of host cells by microarray*

Based on the gene expression analysis, 224 genes were significantly altered within 4 h post-infection. Only 21 genes were disturbed by HCoV-229E per se, whereas 164 genes were altered by SARS-CoV infection only, and the remaining 39 by both coronaviruses. Out of the 164 genes with altered expression in SARS-CoV, 38 were up-regulated and only one was down-regulated at both 2 and 4 h post inoculation. At 2 h post inoculation, 43 were up-regulated and 16 down-regulated. At 4 h post inoculation, 49 were up-regulated and 17 down regulated. In contrast, for HCoV-229E infection, only one gene was up-regulated and no genes were down regulated at both 2 and 4 h post inoculation. At 2 h post inoculation, no genes were up-regulated and only two genes were down-regulated. At 4 h post-inoculation, 14 genes were up-regulated and four were down-regulated. When multiple transcripts of the same gene were eliminated and analysed, genes related to apoptosis (n=23), inflammatory or immune response (n=34) and coagulation (n=5) were identified in addition to the expected genes of stress response, metabolism and other unknown genes. Of the 23 apoptotic genes affected, 13 were pro-apoptotic and 11 were up-regulated in SARS-CoV infection compared to only three in HCoV-229E infection. As for inflammation and immune response, 32 genes were up-regulated in SARS-CoV compared to only three in HCoV-229E. These included NFKB1A, NFKB2, IL8, TGFβ2, chemokines CXCL1, 2, 3, 5, 6 and 10, ICAM1, and TNFα induced proteins. Surprisingly, genes of the pro-coagulation pathway were also affected by SARS-CoV infection with up-regulation of PLSCR1 (phospholipid scramblase 1), EGR1 (early growth response 1 gene), PAI1/SERPINE1 (plasminogen activator inhibitor 1) and THBS1 (thrombospondin 1). In terms of stress response, seven genes were up-regulated in SARS-CoV infection compared to only one in HCoV-229E infection. Overall

there were far more changes in gene expression related to cell cycle, transcription, metabolism, and miscellaneous and unknown functions in SARS-CoV infection. When the Pathway Assist software (Ariadne Genomics Inc.) was used for linking altered genes in cellular pathways for SARS-CoV, there was clear clustering of altered genes related to apoptosis, inflammation and coagulation.

### *Confirmation of cellular gene and protein expression by semi-quantitative PCR, RT-qPCR and immunoassay*

A similar trend to up-regulation of gene expression with SARS-CoV showed a 1.4-10.8 fold increase, compared to HCoV-229E infection for coagulation (TFPI2, PAI1 and THBS1), inflammation (IL8 and NFKB2), transcription (JUNB) and apoptotic (PHLDA1, CARD10 and BAX). Enzyme immunoassay showed SARS-CoV induced higher concentrations of PAI1 and IL8 compared to HCoV-229E at 2, 4 12 and 24 h post-inoculation. Both SARS-CoV and HCoV-229E induced similar TFPI2 expression 4, 12 and 24 h post-inoculation, but at 2 h post-inoculation SARS-CoV induced a lower concentration of this protein.

## Discussion

SARS-CoV causes respiratory failure in over 60% of those infected and has a mortality rate of around 15%.[4,10] Apart from pneumonia, occasionally SARS also manifests clinically as pulmonary vasculitis and thrombosis in the lungs among those who died.[11,12] Much has been studied including the virology, genomics, diagnostics, clinical features and progression in relation to viral load, treatment, infection control and immunisation.

No pneumocyte cell line has yet been found to support lytic or non-lytic infection by SARS-CoV. In this study, Huh7 cells were found to be susceptible to SARS-CoV.[13] HCoV-229E produced lytic infection within 48 h post-infection. A high multiplicity of infection of 100 $TCID_{50}$ per cell was used to ensure reproducibility of the gene expression study. Since the expression of a large number of genes was expected to change significantly when virus-induced cytopathology followed a rapidly lytic viral infection, we studied the difference in gene and protein expression profiles at a relatively early stage of infection (ie 2 and 4 h post-infection). This time frame is biologically relevant as proliferation of the Golgi complex and related vesicles and swelling of trans-Golgi sacs were observed in infected cells within the 1st hour of infection. Extracellular virus particles were present in 5% and 30% of the cell populations at 5 and 6 h post-infection, respectively.[14] This also facilitated the analysis as a lower number of altered genes were involved.

Comparative transcriptomic analysis indicated that far more genes (n=136) were up-regulated by SARS-CoV than HCoV-229E. Contrary to the reported findings of increased anti-apoptotic/inflammatory gene expression and decreased pro-apoptotic/inflammatory gene expression in the enterocyte cell lines,[15] far more pro-apoptotic and pro-inflammatory genes were expressed in Huh7 cells infected by SARS-CoV but not HCoV-229E. For instance, expression of BCL2 was induced by SARS-CoV in enterocytes, yet we observed up-regulation of its antagonists, including BAX and BCL2L11, in Huh7 cells. Moreover, much higher expression of other pro-apoptotic proteins, including CASP7, CARD10, PMAIP1, and GADD45B were also induced by SARS-CoV in contrast to HCoV-229E. Furthermore, there was marked perturbation of genes involved in cell cycle regulation, including induction of the CDKN2B gene, which can mediate growth arrest at the G1-phase.

The induction of pro-inflammatory cytokines by SARS-CoV was even more prominent compared to HCoV-229E. The induction of IL8 may be of pathogenic importance as its concentration was positively correlated with disease severity in pulmonary infection with RSV. Thus, the observed significantly higher level of IL8 induced by SARS-CoV in Huh7 cells, compared to HCoV-229E, may recapitulate the host response to these viruses by pneumocytes. The induction of various chemokines of the CXC or CCL families may mediate the chemotaxis of lymphocytes and neutrophils.

These alterations in gene expression are in keeping with the histological changes of SARS hepatitis in which cellular apoptosis, marked accumulation of cells in mitosis with ballooning degeneration of hepatocytes, and moderate lymphocytic infiltration were found in biopsied liver tissues.

The up-regulation of genes involved in pro-coagulation and platelet activation is interesting. TFPI2 inhibits thrombin generation by binding and inactivation of the TF: FVIIa (tissue factor: factor VIIa) complex. Up-regulation of the gene probably represents an inhibitory response to restrain the activation of the coagulation pathway during acute inflammation. In contrast, TFPI2 also inhibits both free and matrix/cell-associated plasmin, thus favouring fibrin deposition and may have a positive role in matrix turnover. Up-regulation of the gene of PAI1 accompanied by a dramatic increase in protein level results in an anti-fibrinolytic response. This may favour fibrin deposition during the acute inflammatory phase of the disease. It is important to note that the mouse hepatitis virus can activate the immune coagulation system by fgl2 gene encoding a prothrombinase. This enzyme can induce macrophage pro-coagulation activity resulting in fibrin deposition on the endothelium of intrahepatic veins and hepatic sinusoids. The result could be confluent hepatocellular necrosis. The low number of liver biopsies performed in these patients may account for the lack of reports on these large-scale changes related to vascular damage. However, systemic vasculitis including oedema, localised fibrinoid necrosis and infiltration by monocytes, lymphocytes, and plasma cells into vessel walls of various tissues has been reported.[12] Thrombosis was found in small veins. Marked up-regulation of a pro-apoptotic gene, PHLDA1, was observed in SARS-

CoV infection of Huh7. Over-expression of this gene in vascular endothelial cells leads to decreased cell adhesion and induces detachment-mediated apoptosis.[16] If similarly induced in vascular endothelial cells infected by SARS-CoV, this gene may contribute to the vascular damage induced SARS-CoV infection.[16]

## Conclusions

SARS-CoV produces more severe disturbance of host cell gene expression in a human epithelial cell line of liver origin than the HCoV-229E during the early stage of infection. There are marked alterations in gene expression related to apoptosis, inflammation and pro-coagulation. These findings are consistent with the histological changes of SARS, especially in the liver and blood vessels.

Besides antivirals against SARS-CoV, other modalities of treatment such as anti-apoptotic agents, immunomodulators against inflammation and modifiers of coagulation should be considered in future research on the treatment of SARS. It is important to note that many patients continued to deteriorate 2 to 3 weeks after the onset of SARS, despite a decreasing viral load.

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# Prevalence of SARS-CoV antibody in all Hong Kong patient contacts

**Key Message**

The near absence of transmission (seroprevalence=0.19%) resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease.

## Introduction

Since the SARS outbreak, considerable progress has been made in understanding the biology, pathogenesis, and epidemiological features of both the coronavirus and the disease. Epidemiological studies of hospitalised patients suggest that the overall transmissibility of SARS (as indicated by the basic reproductive number $R_0$=2.7; 95% confidence interval [CI], 2.2-3.7) is relatively low compared with other pathogens.[1] However, such studies could not take into account possible episodes of mild or moderate illness that did not resort to inpatient care and could not address whether asymptomatic community spread played a role in the 2003 epidemic. If this type of spread occurred, sufficient herd immunity against SARS-CoV to protect against another large-scale outbreak might have developed in the population. The full spectrum of disease associated with SARS-CoV infection should be examined to define more precisely what constitutes a case requiring quarantine and isolation to minimise potential human-to-human spread. Understanding these issues requires the systematic study of the seroprevalence of SARS-CoV antibody in a large sample stratified by age and other baseline characteristics, especially since children were disproportionately less affected by SARS, both in terms of reduced incidence and severity of infection. Serological surveys can be based on a random sample from the total population with appropriate stratification, on serum collected for other reasons (eg blood donors, all hospital admissions), or on surveys of persons who resided in sites of superspreading events or who have had close contact with a confirmed SARS patient.

We report a serological survey for immunoglobulin G (IgG) against SARS-CoV in a representative sample of close contacts of all SARS patients in Hong Kong (>76% had laboratory confirmation of SARS by either paired serology or repeat reverse transcription–polymerase chain reaction [RT-PCR] according to World Health Organization [WHO] criteria).[2]

## Aims/objectives

To estimate the seroprevalence and associated predictors of SARS-CoV IgG antibody among all close contacts of the case cohort during the Hong Kong 2003 outbreak.

## Methods

During the epidemic (from 15 February to 22 June 2003), close contacts were prospectively identified by the Department of Health through standardised telephone interviews with all 1755 confirmed SARS patients within 1 week of hospital admission. A close contact was defined as a person who had cared for, lived with (in the same household), or came into direct contact with body fluids of the SARS patients within 10 days before hospital admission. A total of 3612 close contacts were recorded; 505 were diagnosed as having SARS. Of the remaining 3107 contacts, 2805 (90%) had a telephone number available, as provided by the primary patient. We successfully contacted 2337 (83%) of the contacts from 23 October to 30 November 2003, and 1776 (57% of those eligible) consented to a telephone interview after the purpose of the study was explained to them by trained public health nurses. The interview consisted of questions that assessed the relationship between the patients and contacts; the timing, intensity

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and frequency of contact; precautionary measures adopted during contact with the patient; known contact with other SARS patients; clinical symptoms of febrile, respiratory, gastrointestinal, or constitutional illness since February 2003; medical and travel history; and sociodemographics. Participants were then invited to provide blood samples for serological testing. Shopping coupons (worth US $25) were given to participants after blood was collected as compensation for time and travel costs.

Samples were screened by the Government Virus Unit of the Department of Health by using viral lysate enzyme-linked immunosorbent assay (ELISA; GBI Biotech, Beijing). Positive results were confirmed with immunofluorescence assay (IFA) and neutralisation tests. For the IFA, microscope slides coated with SARS-CoV–infected FRhK4 cells were incubated with serum samples at serial two-fold dilutions starting from 1:25. A positive test was indicated by cytoplasmic fluorescence under ultraviolet microscopy. Using IFA as the standard, the ELISA detects antibody with IFA titre of >25 (ie sensitivity of 100%) and has a specificity of 95%. Neutralisation tests were performed by standard virological methods with Vero E6 cells and SARS-CoV isolate 6109. A titre of >10 was considered positive. The reported sensitivity of 100% was for convalescent-phase serum samples taken a few weeks after the onset of infection in SARS patients, which should apply to our study. During the early phase of infection, IgM predominates; the ELISA kit we used detects IgG only. Therefore, the sensitivity was 80 to 90% (depending on the number of days after illness onset when the serum samples were taken). However, this sensitivity should not have affected our findings, which were based on tests carried out at least 6 months after the last reported case of SARS in Hong Kong.

## Results

Of the 1068 samples analysed, two (0.19%; 95% CI, 0.02-0.67%) contacts had a positive titre (1:25 to 1:50 on IFA compared with at least 1:100 in most recovered SARS cases) for SARS-CoV IgG antibody. None of the two contacts with a positive sample reported a chronic medical condition or being sick with febrile or respiratory illness from February to August 2003. Both seropositive contacts arose from two superspreading events in Hong Kong, ie Prince of Wales Hospital nosocomial outbreak and Amoy Gardens community outbreak.[1,3] The former reported one other close contact, who was interviewed but declined to be tested. The latter was separately identified by three intrafamilial index patients, all of whom lived in the same household and reported only each other as close contacts. The participants who consented to testing were broadly similar to those who declined, except that the former group had relatively fewer children and comprised fewer men. However, those who consented to testing were more likely to report more frequent contact and closer relationships with SARS patients, more febrile or respiratory illness episodes since February 2003, and a travel history to SARS-affected regions, which may have biased our seroprevalence estimate upwards.

## Discussion

The extent of seropositivity in close contacts of confirmed patients should provide the upper limit of SARS-CoV antibody seroprevalence in the general population, given the relatively intense exposure of these persons to SARS patients. Our finding of the near absence of transmission resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease. Whereas some SARS patients (especially health care workers) might have been promptly admitted to hospitals, so that transmission to family members was reduced. Almost all SARS patients (perhaps with very few exceptions in children) had severe disease resorting to inpatient treatment; thus, infection with SARS-CoV almost always caused severe disease requiring hospitalisation.[4]

Although our results suggested that SARS-CoV was a new virus in humans without a close precursor or an antigenically related virus that would have induced at least a small degree of cross-reactivity on serological testing, a recent study on a select group of 938 healthy Hong Kong adults (whose serum had been stored as part of a hepatitis B serosurvey in 2001) indicated that 1.8% of the sample had acquired a SARS-CoV–related virus infection at least 2 years before the 2003 SARS outbreak.[5] The investigators speculated that the virus that affected these healthy, seropositive persons was antigenically closer to the recently isolated animal SARS-CoV–like virus than human SARS-CoV, but interspecies transmission from animals to humans was likely to be inefficient, as the virus might not have adapted in the new host.[3] This hypothesis may explain why only a few persons became infected but were asymptomatic. This hypothesis would be compatible with the presumed asymptomatic infection observed in Guangdong animal traders, especially in those who handled masked palm civets, who had a seropositivity rate of 72.7% (95% CI, 49.8-89.3%) in the absence of prior overt clinical disease.[6]

The limitations of the study included incomplete contact tracing (especially in the earlier parts of the epidemic) and potential recall bias (under-reporting of contacts by some patients who were too sick to answer questions). Another possible shortcoming was the lack of a survey of close contacts whose telephone numbers were not provided, although there was no reason to suspect they had a systematically different serological profile. In fact, these were mostly non-household contacts who would have had less intense exposure to SARS patients. In addition, because peak infectivity, as indicated by viral load, usually occurred during week 2 of illness, when most of the patients would

have been isolated in hospital (the mean symptom onset-to-admission interval decreased from a maximum of 9.3 days in late February to 1.0 day by mid-May). Transmission to close contacts in the later stages of the epidemic was therefore less likely.[7,8] Finally, contacts who refused to participate (n=561) or undergo serological testing (n=708) might have been due to their concerns about having SARS (possibly because of having SARS-like symptoms) and did not want to be identified and stigmatised as having been infected with SARS-CoV. Surveys in other countries with large-scale outbreaks such as Canada, China, Singapore, and Taiwan should be undertaken to confirm our findings.

## Conclusions

The near absence of transmission resulting in asymptomatic infection in this representative high-risk group of close contacts indicates that the prevailing SARS-CoV strains in Hong Kong almost always led to clinically apparent disease. It is inferred that infection with SARS-CoV almost always caused severe enough disease requiring hospitalisation.

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# Community psycho-behavioural surveillance and related impact on outbreak control in Hong Kong and Singapore during the SARS epidemic

## Key Messages

1. The promotion of personal protective health practices must take into account background perceptions of risk and psychological responses in the community-at-large.
2. Population psycho-behavioural factors in Hong Kong and Singapore are shown to be an important potential vector for the transmission of an infectious agent.
3. Comparative psycho-behavioural surveillance and analysis can yield important insights into generic versus population-specific issues that could be used to inform, design and benchmark public health infection control measures.

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## Introduction

During a new epidemic such as the SARS outbreak, medical and public health communities focused on identification of the responsible agent as well as pathophysiology, clinical presentation, diagnosis, and treatment of the condition.[1-4] Interest was less in the epidemiology of the disease and the effectiveness of infection control measures in various hospitals; population psycho-behavioural surveillance received almost no research coverage.[5,6] However, formulation and implementation of public health infection control measures deserves equal attention and such recommendations should be based on public perceptions, beliefs and attitudes. Standard data collection and analysis in outbreak control strategies rarely include information about population perceptions about the disease and their relevance to the agent-vector-host epidemiological triangle.

As there may be a possible return of SARS, it is useful to compare the public responses in different cities that were similarly affected. Such comparative analyses enable policy makers to disentangle generic issues from culture-specific concerns and to share practices that successfully controlled the outbreak.

We report a cross-sectional, population-based survey on psycho-behavioural responses to SARS in two centres of the epidemic, Hong Kong and Singapore.

## Aims and objectives

To compare public knowledge and perceptions about SARS and the extent to which precautionary measures were adopted in Hong Kong and Singapore.

## Methods

Respondents were recruited using random-digit dialling of all land-based telephone lines in Hong Kong and Singapore. A total of 705 Hong Kong (aged ≥18 years) and 1201 Singaporean (aged ≥21 years) residents completed the survey conducted from 15 May to 10 June 2003 in Hong Kong and 5 to 10 May 2003 in Singapore. The respective response rates were 54.7% (705/1288) and 62.3% (1201/1928).

The survey consisted of 60 questions, five of which had multiple parts. It was translated and back-translated from Cantonese to English and vice versa in Hong Kong, and from Cantonese to Mandarin, Malay and English in Singapore. It was pre-tested for face and content validity, length and comprehensibility. The questionnaire was administered in Cantonese in Hong Kong, and in Mandarin, Malay or English in Singapore (at the respondents' choosing).

The respondents were asked: (1) their self-perceived general health status, febrile and respiratory symptoms in the previous 2 weeks, and general anxiety levels using the State-Anxiety Scale of the State Trait Anxiety Inventory (STAI)[7];

(2) their use of health services in the previous 2 weeks; (3) the presence, intensity and setting of direct and indirect contacts with SARS patients; (4) their risk perception in terms of their self-perceived likelihood of contracting SARS and survival if diagnosed with the disease; (5) their beliefs about routes of transmission and confidence in physicians' ability to diagnose the disease; (6) the extent to which various precautionary measures were being adopted and possible changes in lifestyle practices to prevent transmission of the virus; and (7) sociodemographics.

We determined differences in proportions between baseline demographics in this survey and corresponding population statistics in the two cities by calculating the effect size. A value of 0.1 indicates a small effect, 0.3 a medium effect, and 0.5 a large effect. To adjust for possible sampling biases due to sociodemographic differences between respondents and non-respondents and to ensure that the sample was representative of the general populations, we weighted the responses based on the latest figures from the Hong Kong Census and Statistics Department and Singapore Department of Statistics for age, gender and level of educational attainment. All 95% confidence intervals (CIs) were generated using logistic and multinomial regression for dichotomous and multi-categorical variables, respectively.

Using multivariable logistic regression, we sought to identify predictors for greater adoption of a predefined set of precautionary measures (ie at least five of the seven specified strategies) and health services use (defined as presentation to western, Chinese or other complementary and alternative medical practitioners in any setting during the previous 2 weeks). Potential explanatory variables were anxiety level (STAI mean score), level of confidence in physicians' ability to diagnose SARS, self-perceived likelihood of contracting SARS and surviving the illness if infected, presence of physical symptoms, contact history, and sociodemographics. All analyses were conducted using Stata version 8.0.

## Results

Comparing the sample demographics with those from the respective population census data, most of the baseline parameters were similar to the benchmark statistics as confirmed by the small effect sizes. To improve generalisability, age, gender and education were used to weight the samples in all subsequent analyses.

### *Health and emotional status*

The anxiety level of Hong Kong respondents was significantly higher than their Singaporean counterparts (mean=2.06 vs 1.77, P<0.001), using the STAI 10-item scale (scores ranging from one [not anxious at all] to four [very anxious]).

Only 0.5% of the Hong Kong and 0.9% of the Singaporean respondents (P=0.36) reported persistent fever of 38ºC for at least 1 day within the previous 2 weeks; about half of whom (0.2% and 0.4%, P=0.47) also had cough or dyspnoea. Respondents with this combination of symptoms was eligible for a SARS diagnosis during an acute outbreak. Hong Kong respondents reported significantly higher prevalences for headaches, difficulty breathing, dizziness, running nose, and sore throat, but none of these (except for difficulty breathing) were cardinal symptoms of SARS. In fact, their presence may have suggested other diagnoses.

When the prevalences of these five symptoms were adjusted for the anxiety level (ie STAI score), they decreased by 7% to 23% in the Hong Kong sample, but remained almost unchanged for the Singaporeans. Given the higher anxiety levels in Hong Kong respondents, psychosomatic presentation may have played a role in the larger proportion of respondents giving a positive response to these symptoms.

### *Extent of direct and indirect contacts with diagnosed cases and willingness to be quarantined*

The majority (92.3% in Hong Kong and 96.7% in Singapore) of respondents reported no contact history, whereas 0.2% of Hong Kong and 0.3% of Singaporean respondents had direct, non-close contact, and 4.1% of the Hong Kong and 1.5% of the Singaporean samples had indirect contact (contact of a direct contact) with a confirmed case. The remaining 3.4% (Hong Kong) and 1.5% (Singapore) of the sample believed they might have been exposed to a possible SARS patient or infected materials (eg fomites).

There appeared to have been a high degree of willingness to comply with quarantine procedures, in the event the respondents were to be exposed to SARS patients. More than 90% of the samples in both cities were willing to be quarantined if there was close (eg household or intimate relationships) contact and at least 70% would be compliant for non-close or social contact.

### *Knowledge and beliefs about SARS*

The majority of respondents in both cities (86.7% in Hong Kong vs 71.4% in Singapore, P<0.001) knew that SARS could be transmitted by person-to-person droplets, although fewer (75.8% in Hong Kong vs 62.1% in Singapore, P<0.001) identified fomites or contact through contaminated objects as a possible transmission mode. These are the two main routes of transmission confirmed by the Centers for Disease Control and Prevention and the World Health Organization. However, 40.9% of the Hong Kong and 50.9% of the Singaporean samples thought that the infection could be transmitted via the airborne route (P<0.001), which does not appear to be the case according to the epidemiological evidence. Overall, Hong Kong respondents were more knowledgeable about the routes of transmission, in terms of the total number of correct responses (P<0.001).

A total of 23% of Hong Kong and 11.9% of Singaporean

respondents believed that they were 'very likely' or 'somewhat likely' to contract SARS during the outbreak (P<0.001). This proportion remained the same even after excluding those who reported any contact (direct or indirect) with a SARS patient. Singaporean respondents were more confident about the ability of physicians to diagnose SARS (29.5% vs 16.1% were 'very confident', P<0.001). However, the corresponding proportions for feeling 'not very confident' or 'not at all confident' were similar in the two cities. Regarding the likelihood of surviving SARS if they contracted the disease, 9.9% of Hong Kong and 11.2% of Singaporean respondents believed their survival was 'not very likely', and 1.9% and 2.2% was 'not at all likely'. Up to the time of the survey, the case fatality rates were 17.1% in Hong Kong and 13.9% in Singapore.

### *Precautionary measures*

The respective proportions of respondents who reported practising each of seven specified precautionary measures (to prevent the transmission and contracting of SARS) directed against the two main modes of transmission (person-to-person droplet spread and fomites) were analysed. There were large differences between Hong Kong and Singapore for six of the seven measures, except for washing hands with soap. Compared with Singaporean respondents, more Hong Kong respondents would cover their mouths when sneezing or coughing (83.6% vs 94.4%) and wash their hands afterwards (72.6% vs 85.6%) as well as after touching possible contaminated objects (48.3% vs 81.2%). About 47.7% of Hong Kong and 27.3% of Singaporean respondents used serving utensils during meals; this is important in Chinese culture, in which dishes are commonly shared with everyone at the table. The difference in proportion of facemask wearing was most striking (79.0% in Hong Kong vs 4.1% in Singapore). At least two thirds of the Hong Kong sample but only 12.6% of the Singaporeans practised at least five of the seven specified preventive strategies.

### *Predictors for the adoption of precautionary measures and health services use*

The level of anxiety (as measured on the STAI scale) demonstrated a positive dose-response relationship with adoption of personal protective measures, especially in Hong Kong (P<0.01). Recent physical health (as inferred from acute respiratory or febrile symptoms) or a contact history with SARS patients was not associated with adoption of precautionary measures. Higher self-perceived likelihood of contracting SARS was a positive predictor in Hong Kong (odds ratio [OR]=1.53; 95% CI, 0.99-2.38), although the results were equivocal for Singapore (OR=1.24; 95% CI, 0.83-1.87). Other variables such as the level of confidence in the ability of physicians to diagnose SARS and the likelihood of surviving SARS did not appear to be predictive. Greater knowledge about the transmission routes of SARS predicted the adoption of more precautionary measures in Hong Kong (OR=2.09; 95% CI, 1.39-3.13). The lack of significant association in Singapore may reflect the much lower adoption of personal protective measures. In terms of sociodemographics, males were much less likely to adopt comprehensive precautionary measures against SARS. There were positive dose-response relationships with increasing age and the level of educational attainment in both cities, where the former relationship was stronger in Singapore and the latter in Hong Kong. To assess whether anxiety level was an intermediary between risk perception and uptake of precautionary measures, we re-analysed the model while omitting the STAI score as an independent variable. This revealed that the OR estimates for the two self-perceived likelihood factors did not change appreciably, thus confirming that anxiety was not a significant intermediary causal factor.

The presence of symptoms was the only robust predictor for higher health services use. Respondents' health-seeking behaviour did not appear to have been influenced by extraneous factors such as risk perception, anxiety level or contact history. However, younger, male respondents were less likely to seek health care services.

## Discussion

This population-based, cross-sectional survey revealed substantial differences in the knowledge, beliefs, emotional status, and extent of adopting personal protective measures between Hong Kong and Singapore at the end of the SARS epidemic. Areas of commonalities between two cities included levels of civic compliance with public health control and quarantine directives, as well as predictors of greater adoption of precautionary steps and health services use. Public health action to curb the transmission of SARS coronavirus was mainly effected through enhanced personal hygiene and health protective measures. This was dependent on the public knowledge, psychological responses (viz anxiety level) and the perceptions of the community-at-large. There were sociodemographic subgroups that were less likely to take personal protective steps or to seek care. The strength of this study was that respondents were interviewed during an actual outbreak, compared with other studies of infectious disease epidemics or bioterrorism attacks in which hypothetical questions were usually posed.

As the survey was conducted during the epidemic (close to the end), knowledge indices were expected to be at their highest given the cumulative effects of sustained promotion of health practices through mass media. Nonetheless, there were still significant knowledge gaps in terms of the routes of SARS coronavirus transmission (more so in Singapore than Hong Kong). In addition, respondents' risk perception as indicated by their perceived likelihoods of contracting and surviving SARS were exaggerated and overly pessimistic when benchmarked against the overall probabilities based on the numbers of patients infected and died. This could be explained by a combination of knowledge deficits and excessive anxiety generated by the outbreak, although the

present analyses preclude drawing of definite conclusions.

The stage of the epidemic at which we conducted the survey could have affected our observations regarding public behavioural responses. Singapore's lower adoption prevalence of precautionary measures might arguably have been due to the low daily new case counts at the time of the survey, although it would have been difficult for the population to foretell this given that in Singapore similarly low new daily counts were observed towards the end of March and early April, only to peak again 2 weeks later. Toronto also experienced a similar bimodal distribution of cases. The Hong Kong survey was also carried out during the end of the outbreak, but a much larger proportion of respondents reported continued vigilance for personal protective precautions and more comprehensively. Assuming this cross-sectional pattern was representative of the entire epidemic in both cities and that there was no ecologic fallacy, the very different extent of the respective outbreaks in Hong Kong and Singapore must be due to other factors. For instance, the impact of the two superspreading events at the Prince of Wales Hospital (n=239) and Amoy Gardens (n=329) in Hong Kong (where the former 'seeded' the latter) might have dominated over the much smaller effects of community transmission (where one infected individual typically spread the disease to three others in the absence of any preventive measures), which was dependent on public collective adoption of personal preventive measures. This hypothesis, if substantiated, underlines the often stochastic or random nature of such epidemics.

Our findings have important implications for public health and infection control. Public health messages in providing appropriate advice and education during this epidemic were highlighted. There were significant gaps in the public knowledge about SARS such as the route of transmission and risk perception, which were associated with inadequate adoption of precautionary measures. Therefore, health education and promotion efforts should be stepped up to prepare for a possible return of SARS.

Anxiety can be either a facilitator or barrier for promoting adoption of precautionary measures. This study confirmed that the population attitudes and perception of events were important indices. They should be closely monitored during an outbreak like SARS, as they can be highly predictive of key behaviours.

Younger, less-educated males (ie traditional risk-takers) were least likely to adopt appropriate preventive measures. Targeting health promotion messages through intermediaries such as female significant others (eg mothers, wives or girlfriends) who are more health conscious and risk averse may raise the level of protective precautions undertaken by this vulnerable subgroup.

Only those with symptoms were more likely to seek medical attention. Other factors, such as risk perception and anxiety level, did not significantly influence health care use, suggesting that there was little detectable panic or irrational use of health services in both cities. This could have been due to avoidance of health care facilities by the public to minimise exposure to high-risk areas (hospitals) and health care personnel. Nonetheless, panic and irrational use of health services during large outbreaks could in theory overwhelm any health care system.

The limitation of this survey was that it was administered at a single time point such that the stability of the responses is unknown, although in Hong Kong repeated cross-sectional and time series data as well as prospective panel data at various points of the epidemic were collected. The analysis of this longitudinal data set can track possible psycho-behavioural changes as epidemics evolve and evaluate the macro impact of policy decisions. In addition, the use of structural equation modelling linking different psycho-behavioural variables to better delineate the causal chain of events deserves further examination. Further exploration of public beliefs and their interplay with traditional health beliefs and practices would be a useful adjunct to understand population psycho-behavioural responses. Such qualitative research should be a high priority to prepare for future large-scale epidemics.

## Conclusions

Promotion of personal protective health practices must take into account background perceptions of risk and psychological responses in the community-at-large. Population psycho-behavioural factors in Hong Kong and Singapore were a potential vector for the transmission of an infectious agent. Comparative psycho-behavioural surveillance and analysis can yield important insights into generic versus population-specific issues. Such issues could be used to inform, design and benchmark public health infection control policy measures.

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# Influenza-associated hospitalisation

## Key Message

The disease burden associated with influenza includes not only acute respiratory diseases but also cerebrovascular disease, ischaemic heart disease and diabetes mellitus.

## Introduction

Influenza may result in hospitalisation, especially in young children and the elderly or in those with chronic diseases. Hospitalisation for influenza is under-reported owing to the non-specific symptoms of influenza and lack of laboratory tests. Excess hospitalisation and mortality associated with influenza have been used to measure the health impacts of influenza.[1-3] Excess hospitalisation was calculated as the difference in hospitalisation numbers between the epidemic and non-epidemic periods within a year, or between the epidemic years with apparent influenza peaks and the baseline years without any apparent increase of influenza activity. It is difficult to apply the same approach to tropical and subtropical regions such as Hong Kong, where there are no well-defined epidemic periods or even clear seasonal patterns of influenza activity. We used Poisson regression to estimate the disease burden related to influenza in the circumstance without a clear influenza seasonality.[4]

## Aims and objectives

To evaluate influenza-associated hospitalisation for different age-groups in Hong Kong.

## Methods

The weekly numbers of hospital discharge diagnoses from 14 acute hospitals in Hong Kong during the period 1996-2000 were obtained from the Hospital Authority. The disease categories included acute respiratory disease (ARD) [International Classification of Diseases version 9 (ICD9) codes 460-466, 480-487] and its sub-category pneumonia and influenza (P&I) [ICD9 480-487], cerebrovascular disease (CVD) [ICD9 430-438], ischaemic heart disease (IHD) [ICD9 410-414] and diabetes mellitus [ICD9 250]. The weekly proportion of specimens positive for influenza A and B (influenza A+B), and for respiratory syncytial virus (RSV) were obtained from the microbiology laboratory of Queen Mary Hospital and were adopted to represent influenza and RSV activity.

We used Poisson regression to model the weekly counts of hospitalisation for each disease category.[5] We built a core model to control for confounding factors including temperature, relative humidity, long-term trends and seasonality. This core model was accepted once the partial autocorrelation plots of its residuals did not have any discernible patterns. The variables influenza A+B and RSV were entered into the core model to assess the impacts of influenza activity with adjustment for RSV. The lag effects of influenza were assessed by entering the variable influenza A+B measured at 0-3 weeks before the admission week and selecting the one with the most significant effect (ie the smallest P value for the coefficient).

The percentage of excess hospitalisation attributable to influenza was calculated as the ratio of the difference between the total observed and expected hospitalisation under the assumption that influenza A+B was zero, to the total number of observed hospitalisation. This percentage of excess hospitalisation was then multiplied by the total hospitalisation per year to get the excess number, which was further divided by the population to obtain a rate of excess hospitalisation per 100 000 inhabitants.

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**Table. Influenza-associated excess hospitalisation in Hong Kong from 1996 to 2000, after adjusting for co-variates including respiratory syncytial virus**

| Age-group (years) | Lag (weeks) | Excess hospitalisation per year | | P value |
|---|---|---|---|---|
| | | % (95% CI) | No. (95% CI) per 100 000 inhabitants | |
| Acute respiratory disease | | | | |
| 0-14 | 0 | 9.3 (7.7, 10.8) | 163.3 (135.2, 189.7) | <0.001 |
| 15-39 | 0 | 7.2 (3.3, 10.8) | 6.0 (2.7, 8.9) | 0.001 |
| 40-64 | 1 | 11.0 (7.9, 13.9) | 14.9 (10.7, 18.8 ) | <0.001 |
| 65-74 | 1 | 11.5 (8.4, 14.3) | 83.8 (61.2, 104.2) | <0.001 |
| 75+ | 0 | 8.7 (6.5, 10.8) | 266.0 (198.7, 330.2) | <0.001 |
| All | 0 | 10.9 (9.5, 12.1) | 60.6 (52.8, 67.2) | <0.001 |
| Pneumonia and influenza | | | | |
| 0-14 | 1 | 14.7 (11.8, 17.4) | 70.4 (56.5, 83.3) | <0.001 |
| 15-39 | 1 | 10.5 (6.1, 14.4) | 2.9 (1.7, 4.0) | <0.001 |
| 40-64 | 1 | 8.7 (5.0, 12.1) | 6.8 (3.9, 9.4) | <0.001 |
| 65-74 | 1 | 11.0 (8.1, 13.8) | 58.7 (43.3, 73.7) | <0.001 |
| 75+ | 0 | 7.1 (4.8, 9.3) | 176.3 (119.2, 231.0) | <0.001 |
| All | 1 | 11.6 (10.2, 12.9) | 29.3 (25.8, 32.6) | <0.001 |

## Results

In all age-groups, influenza was significantly associated with 10.9% of total hospitalisation for ARD, 11.6% for P&I, 1.5% for CVD, 1.8% for IHD and 3.5% for diabetes (P<0.01) [Table]. In addition, ARD and P&I were significantly associated with influenza (P<0.001). Influenza accounted for 9.3% and 11.5% of hospitalisation for ARD in the 0-14 and 65-74 years age-groups, respectively. For both disease categories, the influenza-associated excess hospitalisation rates per 100 000 inhabitants were estimated to be lowest (6.0 for ARD and 2.9 for P&I) in the 15-39 years age-group, and highest (266.0 for ARD and 176.3 for P&I) in the 75+ years age-group (Table).

Influenza was significantly associated with hospitalisation for CVD (P=0.001) and IHD (P<0.01) only in the 75+ years age-group, with rates of 55.4 and 56.4 per 100 000 inhabitants. Influenza was also significantly associated with 2.0% of all hospitalisation for IHD in the 40-64 years age-group (P<0.05). In addition, influenza was significantly associated with hospitalisation for diabetes (P<0.01), with rates being 6.6, 23.9 and 53.3 per 100 000 inhabitants in the 40-64, 65-74 and 75+ years age-groups, respectively. For all these chronic diseases CVD, IHD and diabetes, the rates of influenza-associated excess hospitalisation were highest in the 75+ years age-group (Fig).

## Discussion

In this study, influenza was significantly associated with cardiorespiratory hospitalisation in all age-groups in Hong Kong. According to our estimates, influenza accounted for 29.3 excess P&I hospitalisations per 100 000 inhabitants in all age-groups and 11.6% of total hospitalisations. These estimates were comparable with those reported by a study in the US using a similar Poisson regression approach. Annually there was a 36.8 influenza-associated excess P&I hospitalisations per 100 000 inhabitants for all ages, accounting for 8.6% of all hospitalisations for this diagnosis during the period 1979 to 2001.[6] Those aged 75+ years had the highest hospitalisation rates for all disease categories investigated. The influenza-associated hospitalisation for ARD was lowest in the 15-39 years age-group, a pattern echoed in the US study.[6]

Influenza was associated with excess hospitalisation for chronic diseases such as CVD and IHD, especially in those older than 65 years. Numerous studies have suggested that influenza infection may cause damage not only to the respiratory system, but also to the circulatory organs. Influenza might trigger alterations in circulating clotting factors, platelet aggregation and lysis, concentrations of inflammatory-response proteins and cytokine concentrations. These may enhance thrombotic tendencies, impair vasodilation, or even lead to endothelial injury. In addition, acute respiratory tract infection is associated with an increased risk of myocardial infarction and stroke.[7]

The association between influenza and hospitalisation for diabetes was significant. Our study was the first

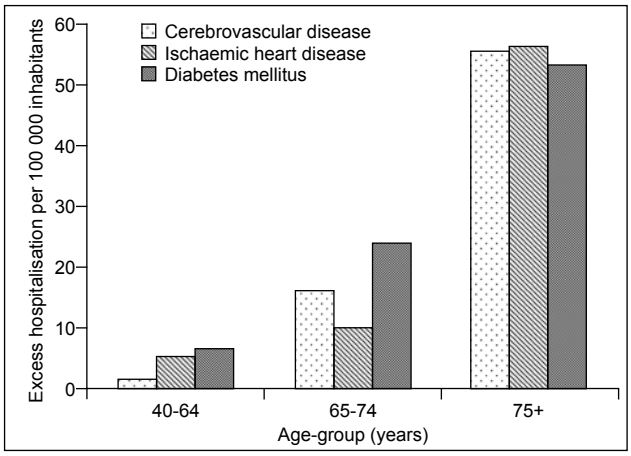


**Fig. Excess hospitalisation per 100 000 inhabitants associated with influenza at different age-groups**

showing that influenza was associated with hospitalisation for diabetes mellitus in persons older than 40 years. Although increased morbidity and mortality of diabetes during influenza epidemics has long been recognised, we were able to assess the excess hospitalisation rates due to year-round influenza virus activity rather than in epidemic periods alone. The increased hospitalisation of diabetic patients may be explained by their impaired immune responses, which probably leads to secondary bacterial infections and further increases morbidity and mortality. Moreover, influenza and its complications may increase the chance of diabetic patients suffering a range of diabetes-related complications that may or may not be directly related to influenza.

Young children and the elderly are high-risk groups in tropical and subtropical settings; they would most benefit from influenza vaccination. Influenza also significantly increased the risk of hospitalisation in patients with underlying chronic conditions such as CVD, IHD and diabetes. These results provide evidence to support the promotion of influenza vaccination in patients with these chronic diseases.

Poisson regression modelling is a valid approach to assess disease impacts of influenza in tropical and subtropical regions with uncertain seasonal patterns of virus activity. The estimates based on Poisson regression modelling have been proved to be robust to unobserved confounding factors such as smoking status and chronic co-morbidity.[8] However, this methodology has the limitation of over-controlling underlying seasonal variations of disease morbidity in the core model. It is possible that some of this seasonal variation may also be attributable to the seasonal activity of influenza, but this portion of the variation cannot be separated from others in this type of analysis. Therefore, our estimates are very likely to underestimate the true impact of influenza on hospitalisation. An approach that can also capture the seasonal variation of disease morbidity associated with influenza virus circulation is needed.

## Conclusions

Our study demonstrated an association of influenza with hospitalisation, not only for ARD (including P&I), but also for chronic diseases such as CVD, IHD, and diabetes mellitus. The excess hospitalisation rates associated with influenza in Hong Kong were estimated to be close to the rates in the US.

Influenza vaccination in the tropics should be promoted to achieve a direct benefit of reducing influenza-associated hospitalisation. The Poisson regression approach is applicable in estimating disease burden associated with influenza in Hong Kong, and can be adapted to other subtropical/tropical regions.

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# Reducing the impact of the next influenza pandemic using household-based public health interventions

**Key Message**

Household-based public health interventions can effectively mitigate the impact of influenza pandemic, and the resources and compliance requirement are realistic and feasible.

## Introduction

Wherever a pandemic influenza strain evolves, there is a period of time during which the disease has not yet reached some populations. This allows these populations to implement interventions to reduce local transmissibility (measured by the basic reproductive number $R_0$) prior to the introduction of the strain. This may reduce the infection attack rate (IAR) once the pandemic arrives. We estimated the reduction in IAR after different household-based interventions using a mathematical model of influenza transmission within and between households. Household-based interventions, such as voluntary quarantine and antiviral prophylaxis, may reduce the IAR substantially, without consuming resources at the same rate as non-targeted population-level interventions. To estimate the impact of household-based interventions on IAR, we used an individual-based stochastic model of influenza transmission with explicit household, peer-group, and community settings.

## Aims and objectives

To estimate the effectiveness of preventive measures that communities might implement to reduce the impact of pandemic influenza.

## Methods

In this simulation, the distribution of household sizes and the average numbers of children in households of different sizes were made to be consistent with Hong Kong.[2] All interventions were active prior to the arrival of the infected individuals, and the population had a constant introduction of 1.5 infected individuals per day per 100 000 inhabitants for 365 days. Susceptible individuals reported with influenza-like-illness, caused by something other than the pandemic influenza strain, at a constant rate of 74 per day per 100 000 inhabitants (according to Hong Kong Centre for Health Protection, www.chp.gov.hk, Data and Statistics, Sentinel Surveillance).

Household-based interventions were simulated as an integrated process of voluntary household quarantine, voluntary individual isolation, and contact tracing. Quarantine referred to segregation of household contacts of a suspected patient from other members of the community within their own homes. Isolation referred to relocation of symptomatic individuals from their household to a separate facility. If an individual complied with household quarantine, his infectivity to other household members increased by a factor of $\varepsilon_0$ ($\varepsilon_0$=2 at baseline). Also, the level of transmission in isolation may be higher than elsewhere. The degree of transmission in isolation was assumed to be a factor of $\varepsilon_1$ greater ($\varepsilon_1$=1 at baseline). Individuals with symptoms severe enough to warrant hospitalisation were assumed to be isolated and to receive antiviral therapy. Compliance was modelled at the individual level (ie each member of the household made independent decision). We defined $p_c$ to be the probability of compliance. These interventions were implemented using the following algorithm:

(1) An individual from households not in voluntary quarantine had the opportunity to enter the programme via one of the following three routes: developing symptoms, being contacted through contact tracing, being hospitalised.

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We assumed that the subject actually reported with a probability $p_c$ for symptoms and contact tracing, and with probability 1 for hospitalisation, and complied with the programme until released. After release, the subject was not bound by previous decisions to join or not join, ie being able to choose once again.

(2) Each of the other members of the household complied with intervention instructions with a probability $p_c$.

(3) After a delay of 1 day, all compliant non-symptomatic household members took one dose of prophylactic antivirals per day, when antiviral policies were in effect. Symptomatic household members took two doses of anitvirals per day.

(4) If contact tracing was in effect, each compliant adult member of the household would name, on average, five subjects (in their peer-group).

(5) If isolation was in effect, new symptomatic individuals who were compliant would enter voluntary isolation with a probability $p_c$ after a delay of 1 day. If the isolated individuals no longer showed symptoms after 3 days, they would be released from isolation and rejoin their household, which might be quarantined. Otherwise, they would be isolated for a further 3 days. This cycle would be repeated until the subjects no longer showed symptoms or died.

(6) Isolated individuals were given two doses of antivirals per day, without a delay, in all simulations, regardless of the policy for the use of antivirals in households.

(7) If contact tracing was in effect, contacts (whether known or not already in the programme) of all new symptomatic or hospitalised household members would be traced with a mean delay of 1 day.

(8) In the absence of new symptoms in compliant or hospitalised household members for 7 days, the quarantined household would be released from the programme at that point. Otherwise, they would return to step 5.

## Results

With compliance rates of 50%, all intervention policies substantially reduced IAR. The baseline IAR of 74% was reduced to 49% when voluntary household quarantine was in effect. However, the peak proportion of households that were quarantined, even with compliance rates of only 50%, was 9.6%. The addition of voluntary individual isolation further reduced the IAR to 43% and the peak proportion of households that were quarantined decreased to 7.1%. Voluntary individual isolation provided an incentive for households to participate: presumed infectious individuals may have been prioritised for health care services and would have protected household members. However, this approach required isolation facilities for up to 0.9% of the population at the peak of the epidemic.

We also considered the use of antivirals with voluntary household quarantine. This policy had a similar efficacy to voluntary individual isolation (IAR, 44%) at a cost of 3.9 doses of antiviral per member but with a much smaller peak level of isolation of 0.5%. The use of antivirals in addition to quarantine and isolation further reduced the IAR to 40% and the peak proportion of households that were quarantined reduced to 6.2%. The addition of contact tracing reduced the IAR to 34% but increased the proportion of the population in quarantine considerably. The additional requirements of contact tracing are unlikely to be justified unless the reproductive number is reduced to near one by other interventions. The prevalence of quarantine and isolation specifies the resources required by these programmes over time, eg the total prevalence of quarantine and isolation on a given day indicates the number of antiviral doses that needs to be distributed, when the use of antivirals in addition to quarantine and isolation is in effect.

As the influenza strain that may cause the next pandemic has not yet been observed, it is not possible to estimate its level of transmissibility (other than by using historical data from other strains) or the balance of transmission in different settings.[1] We used extensive Latin hypercube sampling to conduct sensitivity analyses. This suggests that variations in the efficacy of policies in reducing the IAR is dominated by the basic reproductive number $R_0$.[3] All interventions are considerably more cost-effective for lower values of $R_0$.

The efficacy of quarantine plus antivirals was not substantially less than that of quarantine, isolation plus antivirals for most parameter combinations. The potential for increased transmission in isolation did not seem to substantially decrease the efficacy of the voluntary individual isolation. Even with isolation transmissibility levels 10 times greater than those outside isolation, the voluntary individual isolation was still effective (IAR, 45%), compared to voluntary household quarantine alone (IAR, 49%), because the overall proportion of susceptible individuals entering isolation was low. Although this proportion may have been high during the initial stages, it would likely be small when averaged over the entire course of the epidemic.

All estimated reductions in IAR were sensitive to the population compliance rate, $p_c$ and to the proportion of transmission, $\theta$, which was either asymptomatic or pre-symptomatic. Values of $p_c$=50% and $\theta$=30% were assumed for baseline intervention scenarios. Our estimated changes in IAR were also sensitive to the average delay in the provision of antivirals and in voluntary isolation, although less so than to $p_c$ and $\theta$. In deciding whether to implement any or all of the policies described, local public health officials may wish to consider available epidemiological data (to assess $R_0$ and $\theta$) and also estimate the levels of compliance that could be achieved for the different options in their populations. As compliance may be higher for policies that provide immediate benefits to the individual, compliance will be low for voluntary household quarantine alone, higher for voluntary individual isolation alone, and the use of antivirals with voluntary household quarantine, and highest

for the use of antivirals with voluntary household quarantine and individual isolation. It is likely that the provision of antiviral prophylaxis and treatment increases compliance substantially. Our baseline assumption of 50% is intended to be conservative. It seems that household-based interventions work when levels of compliance are high. Even moderate levels of compliance render household-based public health interventions effective. Also, the marginal benefits from the use of antivirals and isolation may not be justified if the average times for provision of these services exceed 3 to 4 days, given that the quarantine period is set at 7 days.

Levels of compliance with quarantine and isolation would likely improve in the early and late stages of the epidemic, when a viable diagnostic method is available. We considered the impact of virological testing as a diagnostic support for these policies. However, current low throughput (limited by both laboratory infrastructure and supplies of reagents) and low test sensitivity (due to difficulties in obtaining adequate specimens outside of specialised care settings) meant that it was not a worthwhile addition. If an inexpensive, easy-to-perform, rapid and accurate test was available, it would have a significant impact on transmission and on peak levels of quarantine, when used as part of a wider household-based programme.

## Discussion

For lower transmissibility strains of pandemic influenza, the combination of voluntary household quarantine, individual isolation, and the prophylactic use of antivirals was highly effective and feasible across a range of transmission scenarios, even with only moderate levels of compliance. We have quantified the resources consumed by this and similar policies in terms of numbers of people quarantined, numbers of people isolated, and doses of antivirals required.

We assume that the natural history of the next pandemic strain will be similar to that of the 1918 strain, a reduction in IAR from 74% to 40% would avert 16 000 deaths during the period of the initial pandemic wave in a city about the size of Hong Kong (6.8 million people). Our results suggest that such a reduction can be achieved using the combination of voluntary quarantine, individual isolation, and antiviral therapy. Isolation on such a large scale may be somewhat controversial, given the infrastructure requirements of such a policy. Therefore, when large stockpiles of antivirals are available, the marginal benefit of the additional use of isolation may not be justified. However, when stockpiling of antivirals is not feasible, individual isolation is the best possible addition to household quarantine.

Our results build on previous modelling studies of pandemic influenza which focus on the possibility of containment using geographically targeted antiviral therapy.[1,4] Effective strategies have been identified for mitigation rather than containment.[5,6] The key outcome of mitigation is the reduction in IAR, rather than the likelihood of complete control. Given that many epidemiological parameters associated with the next influenza pandemic are unknown, comparison of results from different modelling studies is not straightforward. Our results are consistent with the reduction in IAR from 34% to 20%.[5] However, they are not consistent with other studies, in which a 10 fold reduction in the numbers of ill people is reported for the use of targeted anti-viral prophylaxis.[6] This large discrepancy is likely due to the optimistic nature of their policy: they assume that households, household clusters, schools and workplaces can be targeted very efficiently for prophylactic antiviral therapy. We suggest that a highly efficient contact tracing process be required to achieve high levels of coverage between socially connected households, which is particularly true in modern urban populations. Such a process requires large numbers of households to be recruited during short periods of time, which is not feasible.

Reducing the first-wave IAR should be the primary goal of influenza preparedness planning. When complete transmission control is not achieved, this necessarily implies a longer epidemic. If the mortality rate of the pandemic strain is considered to be low, it is likely that some governments will place priority on reducing the duration of the outbreak than on reducing the number of infections. For a longer period of societal disruption, policy should be designed to reduce mortality and peak stresses on the society as a whole. For example, for the baseline case, a combination of voluntary household quarantine, individual isolation, and use of antivirals could reduce the peak incidence of infection from 3.7 to 0.8%. Although such analyses are beyond the scope of this work, the likelihood of maintaining uninterrupted key societal services (such as law enforcement, food distribution and utility provision) may improve substantially across this range. Therefore, the potential massive adverse economic implications of a temporary breakdown may justify extending the expected period of disruption.

## Conclusions

Household quarantine was not successfully implemented on any significant scale during the 1918 city-level epidemics upon which estimates of transmissibility are based.[1,7] Therefore, the likely impact of the interventions we described is real and not already incorporated into estimates of transmissibility. Modern transport and communication infrastructures are much more advanced than those available in 1918, so it is reasonable to expect that such interventions can now succeed. Many countries have put in place formal pandemic preparedness plans following a World Health Organization framework. These national plans mention the interventions included here, but they do not specify the implementation of intervention processes in even the broadest terms, nor do they attempt to predict the levels of resources required. Our findings and future studies, which match detailed descriptions of

interventions with realistic transmission models, can help to inform pandemic preparedness plans by quantifying both the benefits of, and resources required by, household-based interventions against pandemic influenza.

Our measures to increase social distance consume substantial resources and therefore detailed planning is required. To allow quarantined individuals to remain at home, provision of food, water and medicines must be made for. This may be achieved through a central system or a neighbourhood assistance scheme. For isolation, careful planning and investment is required so that large facilities can be made operational in time to reduce transmission in the early stages of the epidemic. For antivirals to be provided efficiently, a dedicated distribution system is required.

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# Knowledge of risk and self-protection practices and the degree of influenza hazard from live poultry sales

**Key Messages**

1. Perceptions of risk from buying live chickens were moderate, but sickness anxieties did not predict buying or touching habits.
2. Buying was strongly predicted by the erroneous belief that cooking is the best means of protection from avian influenza. Health education groups seeking to increase preventive practices to control possible avian influenza outbreaks need to learn from this.

## Introduction

Pandemic human influenza strains emerging from co-infection of a human influenza carrier by avian influenza H5N1 virus is a small risk, but the public health impact could be catastrophic. Low probability, highly prevalent events have considerable public health importance.

Domestic waterfowl, chickens, and pigs act as aberrant hosts for both avian influenza (from migratory waterfowl and shorebirds) and human influenza viruses. Genetic reassortment of influenza viruses is likely to be more rapid in aberrant hosts.[1] Domestic animal and human avian influenza infection may therefore increase the chance of a potentially pandemic strain emerging.

Most human-animal contact is domestic or commercial. Most human avian influenza infections occur among persons working or living with domesticated birds.[2] Wet markets provide opportunities for people and live animal mixing, making them potential sources of viral amplification and infection. Severe acute respiratory syndrome coronavirus probably emerged in wet markets. Direct hand-to-face contact is the most likely path for infection. Highly dense urban populations increase opportunities for infection and transmission in any outbreak.

Minimising unnecessary mixing between people and domestic poultry by replacing live animal sales in wet markets with hygienic central slaughtering and chilling is therefore valuable.

## Aims and objectives

To determine population knowledge of risk self-protection practices and to estimate the degree of influenza hazard from live poultry sales at the height of the 2004 Asia avian influenza epidemic.

## Methods

A telephone survey of the general population was performed from 10 am to 10 pm from mid-February to mid-March 2004. Households were selected by using random digit dialling. Within households, respondents were selected by using random number tables based on varying household sizes. Inclusion criteria were Cantonese speakers, age of 16 to 95 years, and residing in Hong Kong for >12 months.

### *Instrumentation*

Of the six-section questionnaire, three sections are addressed here. Section 1 consisted of Likert scale items assessing self-rated health (excellent to very poor) and influenza-like symptoms (fever, chills, cough, headache, myalgia, breathing difficulties, coryza, sore throat, diarrhoea and low back pain ['yes', 'no', 'don't know']).[3] Section 2 consisted of 13 questions on household practices when buying live birds, and three of them assessed risk perceptions: worries about catching avian influenza from buying live chickens, likelihood of self/family members getting sick from buying live chickens (all using five- or seven-point

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categorical ordinal response formats) and a decile anchored 0% to 100% probability assessment of getting sick from buying live chickens.[4] To help identify attitudinal and knowledge predictors of risk perceptions and behaviour change, respondents expressed agreement or disagreement using five-point Likert scales (strongly agree to strongly disagree) with 32 statements addressing attitudes, avian influenza protection practices, and perceptions of live chicken sales. Section 3 consisted of nine items concerning demographic information.

### *Data analysis*

Categorical data were analysed with Chi squared tests and continuous data with *t* tests. Average annual live chicken purchase rates were calculated by using a conservatively estimated number of live chicken purchases per response category. To households reporting one live chicken purchase per year, one live chicken purchase was attributed; to households reporting 'a few times a year', four were attributed; to households reporting 'monthly', 12 were attributed; to households reporting 'a few times per month', 24 were attributed; to households reporting 'weekly', 52 were attributed; and to those reporting 'a few times a week', 100 were attributed. Perceived risk moderates behaviour. To identify predictors of greater risk perception and behaviour, purchase (yes/no) [model 1] and touching during purchase (yes/no) [model 2] of live chickens, and perceived likelihood of getting sick from buying live chickens (dependent variable 50th percentile dichotomised 0% to 100% probability assessment responses to the question, "How likely is it that you will get sick from buying live chickens?") [model 3] were regressed in forward-stepped multivariate logistic equations on five attitudinal factors, adjusted for demographics. Attitudinal factors were derived by reducing the 32 attitudinal statements with varimax-rotated principal components factor analysis by using scree-plot and Eigen vector-driven factor extraction. Dichotomisation and logistic regression were required for binary dependent variables in models 1 and 2, and to overcome multimodal distribution difficulties on the response scale used in model 3. All proportions were rounded to the nearest whole number. Analyses were performed using SPSS 11.0. (SPSS, Cary [NC], US).

## Results

Seven interviewers called 6603 telephone numbers in 4 weeks. Of these, 2596 were invalid (fax or answering machines), and persons reached by 1765 numbers were ineligible (non-Cantonese speakers, residing in Hong Kong for <12 months). Of 2240 eligible respondents, 1256 declined to participate or complete the survey (556 were 'too busy', 688 refused for other reasons), leaving 986 eligible respondents who completed the survey, giving a response rate of 44% (986/2240).

The sample comprised 589 women and 397 men closely matching the most recent population census data. Men had a wider age distribution than did women (P=0.006), were more likely to be single (P<0.001), born in Hong Kong (P<0.001), and better educated (P=0.015).

### *Purchase of live chickens*

Of female respondents, 20% (116/589; 95% confidence interval [CI], 17-23%) reported that their household never bought live chickens, compared to 24% (96/396; 95% CI, 20-28%) of male respondents. In households (78%) that reported buying live chickens, 76% (95% CI, 72-78%) of female and 31% (95% CI, 26-36%) of male respondents did so personally; other family members or domestic helpers did the rest of the purchasing. Of male respondents, 18% (95% CI, 14-22%) reported that all family members bought live chickens, 14% (95% CI, 10-18%) claimed to be the sole purchasers, whereas 69% (95% CI, 64-74%) reported that other household members did the purchase. The corresponding rates among females were 11% (95% CI, 8-14%), 65% (95% CI, 61-69%), and 24% (95% CI, 20-28%).

Because 65% of women but only 14% of men personally bought live chickens, we adjusted for sex differences in purchasing rates by applying the female rate to the remaining proportion of purchases in male-respondent households (86%), and all but 14% in female respondent households, the remainder being attributed at the male rate.

### *Contact with live chickens during purchase*

Of the 78% of respondents who reported their household bought live chickens, 13% (95% CI, 10-16%) of female and 19% (95% CI, 14-23%) of male purchasers touched the chickens when buying. Overall, 14% (95% CI, 9-13%) of purchases involved physical contact with a live chicken. Extrapolating these exposures (14% of 78%=11%) by the average number of chickens purchased annually (18.7), multiplied by the number of Hong Kong households (2 051 890), gives 4 220 738 person-chicken exposures annually. Of those reporting that they touched live chickens when buying, only about 30% said they 'always' or 'usually' washed hands afterwards. Anxiety scores did not differ between those who bought live chickens and those who did not.

### *Risk perception*

Among all respondents, four separate items tapped perception of risk from buying live chickens. The first assessed perceived objective risk. Overall, 36% (95% CI, 33-39%) of respondents agreed with the statement 'buying live chickens is risky to health'. The next two items considered perceived consequences of risk (odds of getting sick). Statement-based probability estimates for 'getting sick from buying live chickens' indicated that 34% (95% CI, 31-37%) of respondents considered that they would 'never' or were 'very unlikely' to get sick from buying live chickens, whereas 27% (95% CI, 24-30%) thought it was 'unlikely', 24% (95% CI, 21-27%) 'chances are even' and 15% (95% CI, 13-17%) 'likely' or 'very likely'. The

third item (0-100% probability estimates of sickness risk) produced lower risk estimates than the second item, with 53% (95% CI, 50-56%) perceiving the likelihood of getting sick at below 26%, 38% (95% CI, 35-41%) in the range 26-50%, and 9% (95% CI, 7-11%), exceeding a 51% likelihood. Item 4 assessed the risk expressed by others. Overall, 46% (95% CI, 43-49%) of respondents reported that their friends had expressed worries about catching avian influenza. Risk perceptions did not differ by age, sex, education, income, or occupation.

### *Factor analysis*

The 32 attitude statements produced a five-factor best-fit solution, which accounted for 38.5% of the score variance. These five factors were labelled according to their item content. Factor 1, 'animal husbandry risk' (10% of variance), included items attributing avian influenza to market practices, live animal sales, and poor home and market hygiene. Factor 2, 'traditional market practices' (9% of variance), items supported traditional markets, their low health risks, live chicken sales, and trivialised health 'scares'. Factor 3, 'protective practice' (8% of variance), items reflected unwillingness to continue live chicken purchases despite risks, unwillingness to take risks for enjoyment, risks from zoonotic infections, and responsibility for own health. Factor 4, 'avian influenza anxieties' (6% of variance), items reflected avian influenza worries, effect of media reports, and sense of vulnerability. Factor 5, 'feel protected' (6% of variance), items reflected reassurance from media reports, trust in government, and confidence in existing avian influenza control measures.

Models 1 to 3 were adjusted for sex, age, marital status, education, occupation, income, place of birth, years of residence in Hong Kong, and recent travel in mainland China. All models also included factors 1 to 5 plus attitudinal items not included in the factor scores.

Model 1 produced six independent predictors of buying live chickens: (1) travel: respondents reporting recent travel in mainland China were less likely to buy (adjusted odds ratio [AOR]=0.35; 95% CI, 0.1-0.9); (2) employment status: unemployed people were less likely to buy (AOR=0.18; 95% CI, 0.05-0.6); (3) traditional market practices (factor 2 score): persons supporting traditional markets were more likely to buy (AOR=1.2; 95% CI, 1.06-1.1); (4) protective practice (factor 3 score): persons reporting high protective practices were more likely to buy (AOR=1.2; 95% CI, 1.06-1.5); (5) willingness to change buying habits if other persons do the same (AOR=0.3; 95% CI, 0.1-0.8); and (6) belief that cooking food thoroughly is the best protection against bird flu (AOR=8.7; 95% CI, 1.6-46.7).

Model 2 estimated independent predictors of touching chickens when buying, using only respondents who reported buying live chickens themselves (n=451). Two variables independently predicted higher risk of touching: place of birth—persons born outside of Hong Kong—(AOR [China]=2.8; 95% CI, 1.4-5.4; AOR [elsewhere]=4.2; 95% CI, 1.4-12.5), and employment status—unemployment—(AOR=3.9; 95% CI, 1.2-12.1).

Model 3 identified adjusted independent predictors of risk perceptions for getting sick from buying live chickens. Older age lowered perceived risk (AOR [54 years of age]=0.3; 95% CI, 0.2-0.6; AOR [35-54 years of age]=0.5; 95% CI, 0.3-0.8 [reference, 18-34 years]), whereas worries about catching bird flu (AOR=2.9; 95% CI, 1.9-4.5), animal husbandry risk (Factor 1) [AOR=1.1; 95% CI, 1.04-1.14], protective practices (Factor 3) [AOR=1.1; 95% CI, 1.04-1.2], and avian influenza anxiety (Factor 4) [AOR=1.1; 95% CI, 1.0-1.2], all increased risk perception.

## Discussion

Women are usually responsible for food shopping; shopping practices differ by gender, and reporting differences by gender have been found elsewhere.[5] The observed purchase (and therefore exposure) rate of 18.7 live chickens/household/year (38 370 343 purchases annually) matches government figures of about 38 325 000 live chickens purchased in 2004 in Hong Kong. This provides important independent validation of our data accuracy.

How much risk this exposure represents is difficult to accurately quantify. A highly conservative estimation assumes that genetic reassortment of human and avian influenza viruses can occur only on day 1 of a 5-day infectious period in a person with human influenza.[6] During the two 10-week human influenza seasons that occur annually in Hong Kong, sentinel data for influenza-like symptoms 1998 to 2004 indicate that peak population infection rates (pi) average 10% (±50% lower and upper bound estimates, ie 5-15%), giving $0.2 \times (4\ 220\ 738/52) \times 20 \times \text{pi} = 32\ 467$ (16 233-48 700) episodes when persons on day 1 of a human influenza infection face exposure to live chickens. Wet markets amplify viral loads. Before the enactment in 2003 of wet market 'rest days', H5N1 isolates occurred in about 10% of chickens for sale in Hong Kong.[7] As all live chickens available in Hong Kong are vaccinated against avian influenza and the vaccine is presumed 90% effective, then only 1% (10% of 10% carrier rate) are potentially avian influenza infected, giving 325 (162-487) day 1 potential co-infection exposures when reassortment could occur, a rate of 0.0077% (0.0038-0.0115%). Influenza produces no symptoms for 24-48 hours after infection so shopping rates would be unaffected—assuming that 50% of persons shop on day 1 of infection reduces the figure by half to 162 (81-243) co-infection exposures annually. Among the 11% who touch the chickens, risk for avian influenza infection is likely to be greater. These estimates, though highly uncertain, quantify the potential risk involved.

Although one third of respondents perceived some risks from live chicken sales, risk magnitude seldom exceeded 60%, and peaks at 25% and 50% are partially artifactual.

Almost 50% indicated that their friends had expressed anxieties about avian influenza. Attributing greater concerns to others than to themselves reflects optimistic attribution bias, a protective response enabling expression of concern while preserving 'face'. Sickness anxieties reflected the fact that the markets and live chicken sales were perceived as health threats. Older persons, possibly due to past experience of buying live chickens, or past 'chicken plagues', viewed the present avian influenza outbreak as low risk. Hazard familiarity and experience can reduce associated risk perceptions. Respondents who reported higher anxiety and greater risk were no less likely to buy live chickens.

Raising population anxiety levels by warnings about disease produces only transient, inconsistent changes, and therefore appears to be ineffective as a means of reducing long-term high-risk behaviour. This is because (1) persons perceiving control over dubious 'hazards' seem to underestimate the associated risk, which reduces the likelihood of behaviour change; (2) persons who perceive little or no control over a threat adopt fatalistic responses continue with established behaviour, and direct coping efforts towards controlling emotions rather than risks; and (3) hazard exposure causes familiarity, thus reducing perceptions of risk. For these reasons, persons may dismiss the warnings as exaggerated or unrealistic. Once confidence in food safety is lost, recovery time may be protracted.

## Conclusions

Perceptions of risk from buying live chickens were moderate, but sickness anxieties did not predict buying or touching habits. Buying was, importantly, strongly predicted by the belief that cooking is the best way to protect from avian influenza. This perception is an important message for health education groups seeking to increase preventive practices to control possible avian influenza outbreaks.

When planning for education programmes that aim to increase preventive practices to control possible avian influenza outbreaks, health education groups should remember that buying habits are strongly based on the erroneous belief that cooking is the best way to protect purchasers from avian influenza. Cooking protects from infection by eating, but not from infection through contact prior to eating.

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Fielding R, Lam WW, Ho EY, Lam TH, Hedley AJ, Leung GM. Avian influenza risk perception, Hong Kong. Emerg Infect Dis 2005;11:677-82.

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# Clinical features and molecular epidemiology of coronavirus-HKU1–associated community-acquired pneumonia

**Key Messages**

1. Coronavirus (CoV)-HKU1 accounts for 2.4% of community-acquired pneumonia.
2. Clinical features alone cannot differentiate this entity from other community-acquired pneumonia.
3. Further studies are needed to understand the significance of CoV-HKU1 in upper respiratory tract infection and its potential to cause outbreaks of acute viral respiratory illnesses.

## Introduction

No microbiological cause can be identified in a large proportion of patients with respiratory tract infections. Recently, we discovered a novel group 2 coronavirus—coronavirus HKU1 (CoV-HKU1)—from a patient with pneumonia.[1] We examined the prevalence of CoV-HKU1 in nasopharyngeal aspirate (NPA) samples from patients with community-acquired pneumonia during a 12-month period.

## Aims and objectives

This study aimed to (1) define the clinical features of CoV-HKU1 infection, (2) understand the epidemiology of CoV-HKU1–associated pneumonia, (3) determine the molecular epidemiology and genotypes of the virus, and (4) assess the usefulness of diagnostic tests in identifying such infections.

## Methods

Prospectively collected NPAs from patients with community-acquired pneumonia were sent to the clinical microbiology laboratories of four hospitals in Hong Kong during a 12-month period. Community-acquired pneumonia was defined as symptoms and signs consistent with an acute lower respiratory tract infection, together with new radiographic findings that develop before or within 48 hours of presentation. Once CoV-HKU1 was detected in NPAs, hospital records, laboratory results, and chest radiographs of the corresponding patients were analysed.

Possible risk factors associated with CoV-HKU1–associated pneumonia were determined using two age- and sex-matched controls per patient with CoV-HKU1–associated pneumonia that were randomly selected from patients with community-acquired pneumonia whose NPAs were negative for CoV-HKU1. Each set of controls was within 5 years in age (older or younger) and was admitted within 15 days before or after admission of the corresponding patient with CoV-HKU1–associated pneumonia. The hospital records, laboratory results, and chest radiographs of the controls were analysed.

Viral RNA was extracted from NPAs using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany).

RT-PCR of the pol gene of CoV-HKU1 was performed using CoV-HKU1–specific primers followed by DNA sequencing. A 453-bp fragment of the pol gene of CoV-HKU1 was amplified by RT-PCR using CoV-HKU1-specific primers (LPW1926 [5'-AAAGGATGTTGACAACCCTGTT-3'] and LPW1927 [5'-ATCATCATACTAAAATGCTTACA-3']) designed by multiple alignment of the nucleotide sequences of the pol genes of CoV-HKU1. Both strands of the PCR products were sequenced twice by use of an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City [CA], US), using the two PCR primers. The sequences of the PCR products were compared with the sequences of the pol genes of CoV-HKU1 and those of other coronaviruses in the GenBank

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The ELISA-based immunoglobulin (Ig) G and IgM antibody tests were performed in accordance with our protocol. Each sample was tested in duplicate, and the mean absorbance for each serum sample was calculated.

The complete pol, S, and N genes of CoV-HKU1 from NPAs from nine of the 10 patients (from whom adequate amounts of RNA were available) were amplified and sequenced using the strategy described in our previous study. The nucleotide and deduced amino acid sequences of the pol, S, and N genes were compared with those of CoV-HKU1 and other group 2 coronaviruses. Phylogenetic tree construction was performed using the PileUp method with GrowTree (Accelrys Inc, San Diego [CA], US). Patient characteristics were compared between those with CoV-HKU1–associated pneumonia and those with non–CoV-HKU1–associated pneumonia, and between those who died of and those who survived CoV-HKU1–associated pneumonia. Fisher's exact test was used for categorical variables, and the Mann-Whitney *U* test was used for continuous variables. A P value of $<0.05$ was regarded as statistically significant.

## Results

The NPAs from 10 (2.4%) of 418 patients with community-acquired pneumonia were positive for CoV-HKU1. All 10 cases occurred in winter and spring; nine of them were adults; and four had underlying diseases of the respiratory tract. In the six patients from whom serum samples were available, all had a four-fold change in IgG titre and/or presence of IgM against CoV-HKU1. The two patients who died had significantly lower haemoglobin levels, monocyte counts, albumin levels, and oxygen saturation levels on admission and had more extensive involvement visible on chest radiographs. Sequence analysis of the pol, S, and N genes revealed two genotypes of CoV-HKU1.

## Discussion

Similar to HCoV-229E, HCoV-OC43, and HCoV-NL63, CoV-HKU1 was a human coronavirus that was endemic in humans. Similar to other human coronavirus infections, cases of CoV-HKU1–associated pneumonia occurred during winter and spring. Most patients with CoV-HKU1–associated pneumonia were old (80% were >65 years old) and had major underlying diseases, especially of the respiratory and cardiovascular systems.

Compared with SARS-CoV pneumonia, CoV-HKU1–associated pneumonia was a monophasic disease, and most patients had relatively mild symptoms localised to the respiratory tract and were therefore hospitalised only briefly. Although dyspnoea was present in 25% of patients with this pneumonia at presentation (compared to 20% in patients with SARS-CoV pneumonia), they often recovered quickly in contrast to those with SARS-CoV pneumonia who tended to deteriorate after 7-10 days.

Despite a relatively mild disease course in most patients, CoV-HKU1–associated pneumonia may be fatal in patients with low haemoglobin concentrations, monocyte counts, serum albumin levels, and oxygen saturation levels on admission and more extensive involvement on chest radiographs.

## Conclusion

CoV-HKU1 is a cause of acute community-acquired pneumonia with winter seasonality. More studies should be conducted on this emerging cause of acute viral respiratory illness.

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Woo PC, Lau SK, Tsoi HW, et al. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. J Infect Dis 2005;192:1898-907.

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1. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 2005;79:884-95.

## AUTHOR INDEX






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