SUPPLEMENT 6

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Research Fund for the Control of Infectious Diseases

Research Dissemination Reports

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控制傳染病研究基金

研究成果報告

Respiratory Infectious Diseases
呼吸道感染疾病

Vaccination
疫苗接種

Infection Control
感染控制

Supplement 6

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Health and Medical Research Fund

Research Fund for the Control of Infectious Diseases

Research Dissemination Reports

RESPIRATORY INFECTIOUS DISEASES

VACCINATION

INFECTION CONTROL

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund (and its predecessor funds) administered by the Food and Health Bureau. In this edition, we present 13 dissemination reports of projects related to respiratory infectious diseases, vaccination, and infection control. In particular, three projects are highlighted for their potentially significant findings, impact on healthcare delivery and practice, and/or contribution to health policy formulation in Hong Kong.

The nasopharynx of children is a natural microbial reservoir where pneumococcal colonisation can give rise to invasive pneumococcal disease. Introduction of pneumococcal conjugate vaccines (PCV7, PCV10, and PCV13) in Hong Kong children was expected to substantially reduce invasive pneumococcal disease in them. Serotype replacement, where increasing proportions of invasive pneumococcal disease are caused by non-vaccine serotypes, was also expected. Chan et al[1] conducted a study to assess nasopharyngeal pneumococcal carriage rates, serotypes, and antimicrobial resistance patterns in over 1500 Hong Kong children younger than 2 years. They found that serotype replacement by non-vaccine serotypes in circulating pneumococci among healthy young children in Hong Kong was evident after introduction of pneumococcal conjugate vaccine into the childhood immunisation programme, with the predominant carriage serotypes being serogroup/type 15 and 6C.

Decision to vaccinate children against human papillomavirus (HPV) can be difficult. Fielding et al[2] conducted a study to identify the underlying barriers and facilitators about HPV vaccination of adolescent daughters in 35 local Chinese families. They found that social influences significantly affect the decision-making process of parents and adolescent girls about HPV vaccination. Important facilitators favouring decisions to vaccinate included governmental involvement and recommendations from trusted healthcare professionals. Doubts about the necessity, safety, efficacy, and particularly the high cost of vaccination are major barriers to HPV vaccination.

The increasing occurrence of multidrug-resistant organisms (MDROs) in hospitals is of great concern. MDROs can survive for prolonged periods on hospital furnishings and medical items, and are associated with an increased risk of transmission and infection. Regular cleaning and disinfection is important for breaking the chain of infection. Leung et al[3] developed a multilevel antimicrobial disinfectant coating that synergistically combines 'release-killing', 'contact-killing', and 'anti-adhesion' properties to enable long-lasting disinfection of surfaces. They found that in a hospital ward setting the coating achieved consistently low bacterial load in the ward environment independent of the cleaning regimen and could be effective in reducing environmental occurrence of MDROs.

We hope you will enjoy this selection of research dissemination reports. Electronic copies of these dissemination reports and the corresponding full reports can be downloaded individually from the Research Fund Secretariat website (https://rfs2.fhb.gov.hk/). Researchers interested in the funds administered by the Food and Health Bureau also may visit the website for detailed information about application procedures.

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Nasopharyngeal colonisation and antimicrobial resistance of *Streptococcus pneumoniae* in Hong Kong children younger than 2 years

KCC Chan *, M Ip, PSK Chong, AM Li, HSHS Lam, EAS Nelson

KEY MESSAGES

1. Serotype replacement by non-vaccine serotypes in circulating pneumococci among healthy young children in Hong Kong was evident after introduction of pneumococcal conjugate vaccine into the childhood immunisation programme.
2. The predominant carriage serotypes were serogroup/type 15 and 6C.
3. Further monitoring and evaluation of these and other emerging serotypes among invasive disease and carriage isolates are warranted.

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Introduction

Introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) substantially reduced invasive pneumococcal disease (IPD) in children.[1] However, serotype replacement has been observed, with increasing proportions of IPD caused by non-vaccine serotypes.[1,2] The nasopharynx of children is a natural reservoir where pneumococcal colonisation can give rise to IPD. Therefore, surveillance of nasopharyngeal pneumococcal carriage is important in the monitoring of PCV impact.[3] In Hong Kong, PCV7 was first incorporated into the universal childhood immunisation programme in September 2009, replaced by PCV10 in October 2010 and by 13-valent PCV (PCV13) in December 2011. With the use of PCV13, it was predicted that the prevalence of both carriage and IPD would be further reduced.[4] However, it may also pose an additional selective pressure that may lead to a non-PCV13 serotype shift. A community-based epidemiological study is needed to investigate the nasopharyngeal carriage serotype distribution and antimicrobial susceptibilities of pneumococcal isolates in children. This study aimed to assess nasopharyngeal pneumococcal carriage rates, serotypes, and antimicrobial resistance patterns in children younger than 2 years.

Methods

Healthy children aged 2 months, 12 months, and 18 months were recruited by convenience sampling from June 2013 to June 2014 when they attended Maternal and Child Health Centres for routine vaccination. Informed consent was obtained from parents or primary caregivers of participants. Parents or caregivers of participants were asked to complete a questionnaire about demographics and possible predictors of pneumococcal carriage.

Deep nasopharyngeal samples were taken transnasally by a trained nurse according to World Health Organization standard procedures.[5] The nasopharyngeal swab processing and identification of pneumococcal serotypes were based on the Centers for Disease Control and Prevention study protocol.[6] PCR-based serotyping of the broth-enriched culture was performed in parallel with the isolation-based study, using the primers and conditions based on the latest updates.[6] In brief, sequential multiplex PCRs would be able to detect a total of 40 serotypes. Other untypable strains by conventional multiplex-PCRs were further resolved by a combination of traditional Quellung reaction-based testing method, as described previously and by additional primers as described.[7] Antimicrobial susceptibilities were performed by microbroth dilution using cation-adjusted Mueller-Hinton broth with lysed horse blood (5% v/v), according to Clinical and Laboratory Standards Institute 2014.[8] Interpretation of results was based on the published breakpoints.[8] Isolates identified as intermediate or resistant were grouped together as non-susceptible. Non-susceptible breakpoints for penicillin, cefotaxime, and erythromycin were minimum inhibitory concentration (MIC) of ≥4 μg/mL, ≥2 μg/mL, ≥0.5 μg/mL, respectively.

Pneumococcal carriage rate, proportion of non-vaccine serotypes carriage, and proportion of carriage that was antibiotic non-susceptible were calculated. Potential risk factors for pneumococcal carriage were determined using univariate analysis by Chi-squared test for categorical variables and Student's *t* test or Mann-Whitney *U* test for continuous variables. Variables that were significant in the univariate analysis and those that increase

the risk of carriage were further tested by logistic regression using the forward-conditional models. A P value of <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics (Windows version 21.0; IBM Corp., Armonk [NY], US).

Results

A total of 2435 eligible children were approached and 1541 of them (782 were male) with a mean age of 0.93 (standard deviation, 0.53) years were included after consent was obtained. All included children had completed the recommended vaccination schedule for their age. The overall pneumococcal carriage rate was 5.5% (84/1541). Children of older age-groups had higher colonisation rate (P<0.001) and prevalence of recent respiratory symptoms and recent antibiotic use (P<0.001) [Table 1].

In multivariate logistic regression, age-groups of 12 months and 18 months, presence of siblings younger than 6 years, and recent respiratory symptoms were significantly associated with pneumococcal carriage (Table 2).

Of 84 pneumococcal isolates obtained from 84 children, 16 different serotypes were identified. The most common serotypes were 15B/C (16.67%), 6C (15.5%), 23A (13.1%), and 15A/F (9.5%) [Table 3].

TABLE 1. Characteristics of participants

Characteristic	No. (%) of participants				P value
	Age 2 months (n=477)	Age 12 months (n=522)	Age 18 months (n=542)	Total (n=1541)	
Male sex	241 (50.5)	267 (51.1)	274 (50.6)	782 (50.7)	0.975
Vaginal delivery	311 (65.2)	352 (67.4)	367 (67.7)	1030 (66.8)	0.65
Ever breastfed	386 (80.9)	417 (79.9)	417 (76.9)	1220 (79.2)	0.26
Child-care attendance	2 (0.4)	57 (10.9)	138 (25.5)	197 (12.8)	<0.001
Presence of young siblings aged <6 years	168 (35.2)	171 (32.8)	159 (29.3)	498 (32.3)	0.13
Household tobacco exposure	130 (27.3)	172 (33.0)	183 (33.8)	485 (31.5)	0.06
Overcrowding (a living space of <5.5 m^2/person)	36 (7.5)	27 (5.2)	33 (6.1)	96 (6.2)	0.296
Household income ≤HK$20 000 per month	115 (24.1)	157 (30.1)	175 (32.3)	447 (29.0)	0.01
Recent respiratory symptoms (3 days)	44 (9.2)	110 (21.1)	122 (22.5)	276 (17.9)	<0.001
Recent respiratory symptoms (1 month)	50 (10.5)	231 (44.3)	234 (43.2)	515 (33.4)	<0.001
Recent use of antibiotics (3 months)	44 (9.2)	117 (22.4)	137 (25.3)	298 (19.3)	<0.001
Pneumococcal carriage	11 (2.3)	41 (7.9)	32 (5.9)	84 (5.5)	<0.001

TABLE 2. Univariate and multivariate analyses of risk factors for pneumococcal carriage

Variable	No. (%) of participants		Univariate analysis		Multivariate analysis	
	Total (n=1541)	Pneumococcal carriage (n=84)	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Age-group						
2 months	477 (31.0)	11 (13.1)	1		1	
12 months	522 (33.9)	41 (48.8)	3.61 (1.83-7.11)	<0.001	2.88 (1.41-5.87)	0.004
18 months	542 (35.2)	32 (38.1)	2.67 (1.32-5.33)	0.01	2.19 (1.05-4.57)	0.04
Ever breastfed	1220 (79.2)	71 (46.1)	1.46 (0.80-2.68)	0.22	-	-
Child-care attendance	197 (12.8)	13 (15.5)	1.27 (0.69-2.33)	0.45	-	-
Young siblings aged <6 years	498 (32.3)	53 (63.1)	3.88 (2.46-6.14)	<0.001	3.90 (2.44-6.23)	<0.001
Respiratory symptoms in recent 3 days	276 (17.9)	29 (34.5)	2.58 (1.61-4.13)	<0.001	2.13 (1.31-3.47)	0.002
Respiratory symptoms in recent 1 month	515 (33.4)	44 (52.4)	2.30 (1.48-3.58)	<0.001	1.71 (1.07-2.73)	0.03
Doctor visit in recent 3 months	950 (61.6)	64 (76.2)	2.06 (1.24-3.45)	0.01	1.17 (0.65-2.10)	0.60
Hospitalisation for all causes in recent 3 months	94 (6.10)	7 (8.3)	1.43 (0.64-3.20)	0.38	-	-
Use of antibiotics in recent 3 months	298 (19.3)	21 (25)	1.42 (0.85-2.37)	0.18	-	-
Respiratory symptoms in household members in recent 1 month	806 (52.3)	54 (64.3)	1.69 (1.07-2.67)	0.03	1.00 (0.60-1.65)	0.98
Antibiotic use by household members in recent 3 months	352 (22.8)	24 (28.6)	1.38 (0.84-2.25)	0.20	-	-

Overall, 2.4% of the isolates were PCV7 serotypes, 10.7% were PCV13 serotypes, and 89.3% were non-PCV13 serotypes. Multiple serotypes were detected in two samples by PCR-based serotyping of the broth-enriched culture, which were co-colonisation of 22F/A and 10A in one sample, and 15B/C and 10F/C/33C in another one. Discrepancy was noted in one sample, in which PCR-based serotyping of the broth-enriched culture was 23A but the isolation-based PCR serotyping was 15A/F.

Antibiotic susceptibility patterns for 82 out of 84 isolates were available. The proportions of penicillin (MIC ≥4μg/mL), cefotaxime (MIC ≥2μg/mL), and erythromycin (MIC ≥0.5μg/mL) non-susceptible isolates were 7.3%, 13.4%, and 79.3%, respectively. Non-PCV13 serotypes accounted for 33.3%, 45.5%, and 86.2% of the penicillin, cefotaxime, and erythromycin non-susceptible isolates, respectively (Table 4).

TABLE 3. Serotype distribution of the pneumococcal isolates

***Streptococcus pneumoniae* serotype**	**No. (%)**
Pneumococcal conjugate vaccine 7/10/13	
19F	2 (2.38)
Pneumococcal conjugate vaccine 13	7 (8.33)
6A	2 (2.38)
3	1 (1.19)
19A	4 (4.76)
Non-vaccine serotypes	75 (89.29)
15B/C	14 (16.67)
6C	13 (15.48)
23A	11 (13.10)
15A/F	8 (9.52)
10A	1 (1.19)
13	1 (1.19)
16F	1 (1.19)
35A/35C/42	1 (1.19)
35B	1 (1.19)
35F/47	1 (1.19)
34	1 (1.19)
CpsA negative/ LytA negative	14 (16.67)
CpsA negative/ LytA positive	5 (5.95)
Non serotypable	3 (3.57)
Total	**84 (100)**

Discussion

The overall pneumococcal carriage in children younger than 2 years was 5.5%; 89.3% of the isolates were non-PCV13 serotypes. Pneumococcal carriage was associated with older age-groups, presence of young siblings, and presence of recent respiratory symptoms. All of these have been reported to be risk factors.[3,9] The acquisition rate of *Streptococcus pneumoniae* from the nasopharynx is higher among children with respiratory tract infection. This may be due to the increase in secretions and a higher bacterial load within the nasopharynx during a respiratory tract infection.[10,11] In cohorts in Milan and Massachusetts, the incidence of recent antibiotic use has been reported to be 6.5%[3] and 15.5%,[9] respectively. In our cohort, it was 19.3%, which was relatively high. Nonetheless, we did not find an inverse association between recent antibiotic use and pneumococcal carriage, as has been reported in other studies.[3,9] Our cohort had a younger age and lower child-care attendance rate (12.8%) than other studies of older children (37.3%-54.4%).[3,9] There was no significant association between child-care attendance and pneumococcal carriage in our study.

The significant reduction in vaccine serotypes in nasopharyngeal colonisation reflected the effectiveness of the immunisation. This was consistent with the results from other post-PCV surveillance

TABLE 4. Antibiotic susceptibility patterns

Antibiotic	**Minimum inhibitory concentration (μg mL-1)**			**% Non-susceptible**	**Clinical and Laboratory Standards Institute breakpoint (μg mL-1)**		
	Range	**50%**	**90%**		**Susceptible**	**Intermediate**	**Resistant**
Ciprofloxacin	0.5 to 4	1	2	-	-	-	-
Levofloxacin	0.5 to 2	1	1	0	≤2	4	≥8
Lincomycin	≤0.25 to >32	2	>32	-	-	-	-
Vancomycin	≤0.03 to 1	0.25	0.5	0	≤1	-	-
Cefotaxime	≤0.25 to 32	0.25	2	13.41	≤1	2	≥4
Penicillin	0.015 to 16	0.25	2	7.32	≤2	4	≥8
Chloramphenicol	≤1 to 16	2	4	2.44	≤4	-	≥8
Erythromycin	≤0.015 to >64	4	>64	79.27	≤0.25	0.5	≥1
Tetracycline	0.12 to >32	32	>32	71.17	≤1	2	≥4
Linezolid	≤0.12 to 2	1	1	0	≤2	-	-

studies.[3,9,12-14] Our surveillance was conducted more than 1 year after the introduction of PCV13 into the childhood immunisation programme. The decline in vaccine serotypes in pneumococcal carriage and the emergence of non-vaccine serotypes were more evident than in previous local surveillance studies in hospitalised children.[12-14]

The strength of our study was that the surveillance was conducted in healthy children attending Maternal and Child Health Centres for routine vaccination, whereas previous local studies included hospitalised children with fever or respiratory illnesses.[12-14] Our study might better reveal the circulating serotypes among young children in our community, as there is possible change of the nasopharyngeal bacterial colonisation in young children with the presence of a respiratory tract infection at the time of swabbing.[9] Limitations of our study include the number of children with carriage, which was too small to perform subgroup analysis of the carriage serotypes. In addition, the response rate was suboptimal. Many parents declined to consent for nasopharyngeal swab because they considered the procedure invasive and unnecessary. This might explain why local data about the pneumococcal carriage rate in young children are lacking. Despite these limitations, our cohort is considered representative, owing to the community-based nature and territory-wide recruitment.

Conclusion

Since the introduction of PCV into the childhood immunisation programme, most circulating pneumococci in healthy young children in Hong Kong have been non-vaccine serotypes. The predominant carriage serotypes in the present study were serogroup/type 15 and 6C. Further monitoring and evaluation of these emerging serotypes is warranted.

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Influenza surveillance and vaccination in Hong Kong children

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KEY MESSAGES

1. This prospective influenza surveillance study found that mild influenza infection was common among Hong Kong children aged 2 to 12 years during the influenza seasons of 2014-2015.
2. Seasonal influenza vaccination protected against influenza-like illness (ILI) but not laboratory-confirmed influenza in surveillance samples of local children. The effectiveness of influenza vaccine for ILI varied between 42.1% and 51.9%.
3. Seropositivity, defined by a haemagglutination inhibition titre of ≥1:40, was found in 92%, 91%, 68%, 49%, and 85% of participants for pandemic A/H1N1, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata, respectively. However, neither haemagglutination inhibition titre nor seropositivity was a useful surrogate of influenza immunity in children.
4. Neither ILI nor influenza infection was associated with any demographic, environmental, or clinical factors in the children.
5. Approximately half of local preschool and primary school children had received seasonal influenza vaccination within the past 3 years.

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Introduction

In healthy children, seasonal influenza vaccination reduces the rate of laboratory-confirmed influenza by 59% and the rate of influenza-like illness (ILI) by 36%. The efficacy of seasonal influenza vaccine has been reported. Nonetheless, there are limited post-licensure effectiveness data for Asian children. In European sentinel surveillance networks, seasonal influenza vaccine offered low-to-moderate effectiveness of 43% against influenza A(H3) in the early 2011/2012 season.[1] A similar level of vaccine effectiveness has been reported from 19 influenza surveillance sites in Guangzhou,[2] supporting the need to delineate the effectiveness of seasonal influenza vaccine in Hong Kong children.

The number of laboratory-confirmed cases underestimates the number of true cases, as surveillance focuses on more severe cases. Influenza surveillance in prospective cohorts is thus necessary to define the full spectrum of influenza. Because of viral antigenic drift, the usefulness of seasonal influenza vaccination programmes varies from year to year. Annual estimates of the effectiveness of influenza vaccine could monitor changes in the impact of such programmes. This study aimed to ascertain the full spectrum of influenza infections in local preschool and school-age children, identify risk factors for clinically significant influenza in these children, and delineate the effectiveness of influenza vaccine in preventing influenza and ILI among these children and their family members and classmates.

Methods

The Joint Chinese University of Hong Kong – New Territories East Clinical Research Ethics Committee approved this study. This prospective cohort study recruited children aged 2 to 12 years from kindergartens and primary schools that were randomly selected using stratified (by districts) and clustered (all subjects within a class) sampling. Written informed consent was obtained from the parents of eligible students for influenza surveillance and blood testing. There was no exclusion criterion. We conducted surveillance during three consecutive influenza seasons throughout an 18-month period (January to May 2014, August 2014, and January to February 2015).

Subject demographics, pre-existing medical illnesses, and history of influenza vaccination within the past 3 years were recorded using a questionnaire completed by the parents. Having been vaccinated was defined as (1) ≥14 days post-vaccination, (2) received two doses 28 days apart if vaccinated for the first time, or (3) received at least one dose in a previous influenza season and one dose in the season under study.[2] Serial biweekly surveillance flocked nasopharyngeal swab (NPS) samples were collected from subjects during school visits regardless of the presence of respiratory symptoms. Surveillance was started within 2 weeks upon announcement of the start of influenza seasons by the Centre for Health Protection. Subjects' parents were called biweekly to enquire about symptoms of ILI (ie, fever ≥38°C plus

two of the following: sore throat, cough, rhinorrhoea, myalgia, arthralgia). Subjects were invited to report to the outpatient clinic at Prince of Wales Hospital within 48 hours of the onset of ILI.

NPS samples were collected using flocked swabs (Copan Diagnostics, Corona, CA). Each child was swabbed twice, using one swab in each nostril. Both swabs were placed in the same specimen bottle with viral transport medium. Viral RNA was extracted with the PureLink Viral RNA/DNA Mini Kit (Life Technologies). Real-time PCR was performed using the SuperScript III Platinum One-Step Quantitative RT-PCR System. Molecular typing of influenza viruses was based on the World Health Organization guidelines with slight modifications. Primers were designed to target the haemagglutinin genes specific to formerly pandemic A (H1N1) 2009 (A/H1N1 pdm), A/H3, B/Victoria, and B/Yamagata strains. Conventional PCR was performed using the AmpliTaq Gold 360 Master Mix (Life Technologies) system, and the PCR products were resolved in 2% agarose gel. Viral load was estimated by comparing the PCR results with standard curves generated by serial dilution of plasmids containing the PCR fragments. In addition, sera obtained from 2 mL of clotted blood were tested in parallel using haemagglutination inhibition (HAI) assays against A/H1N1 pdm, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata strains.[3] An HAI antibody titre ≥1:40 was used as the cut-off for protective immunity.[4]

The association of laboratory-confirmed influenza (primary outcome) or ILI with vaccination was analysed using logistic regression adjusted for covariates, including seasonality (month of study), subject age, sex, body mass index, and comorbid medical conditions. Surveillance data from all influenza seasons were combined. The effectiveness of the influenza vaccine was estimated using a test-negative case-control design. All analyses were two-tailed. A P value of <0.05 was considered statistically significant.

TABLE 1. Distribution of numbers of nasopharyngeal swab (NPS) samples collected

No. of NPS samples collected	Frequency
1	16
2	102
3	221
4	23
5	28
6	116
7	114
8	3

Results

A total of 630 children (322 in 2014 and 308 in 2015) with a mean age of 7.3 (standard deviation, 2.4) years from five primary schools and 10 kindergartens participated. Seven subjects withdrew consent before any NPS collection. A total of 337 (53.5%) subjects had received influenza vaccination within the past 3 years. A total of 2633 NPS samples were collected; most children recruited in 2014 provided six to seven samples, and those recruited in 2015 gave two to three samples (Table 1). Two samples and three samples were obtained from 607 (97.4%) and 505 (81.1%) of 623 subjects, respectively. Of the subjects, 99 were reported to have ILI episodes. In addition, nine illness visits were arranged for five other subjects. There was no reported ILI outbreak in the schools or transmission of influenza within the same classes and household of influenza-infected children.

Influenza A and B were detected in 27 and 30 subjects, respectively, with respective median (interquartile range) viral loads of 918 (99-14 864) copies/μL and 262 (98-324 027) copies/μL. Influenza B predominated in 2014 and influenza A in 2015 (P<0.001). Overall, 36 (11.2%) of 321 subjects had influenza A or B infection in 2014, whereas all 19 (6.3%) of the 302 subjects with influenza had influenza A infection in 2015. Six influenza A and 11 influenza B isolates were not typable. For the remaining isolates, influenza A was typed into four A/H1N1 pdm and one A/H3 in 2014 and three A/H1N1 pdm and 13 A/H3 in 2015 (P=0.025). Among the influenza B isolates, all of which were detected in 2014, nine were Yamagata and 10 were Victoria strain. All such children were not reported to be sick at the time of NPS collection. All nine illness NPS samples collected from five subjects in 2014 were negative for both influenza A and B.

ILI was not associated with demographic, environmental, or clinical factors (Table 2). Seasonal influenza vaccination at all time points was protective against ILI (P=0.022-0.002). Logistic regression confirmed such association for seasonal influenza vaccination within the past 3 years (odds ratio=0.49, 95% confidence interval=0.29-0.81, P=0.005). None of the listed factors was associated with laboratory-confirmed influenza detected by surveillance visits (data not shown).

HAI assays were successfully conducted on 181 sera samples, and data for the respective influenza type (ie A or B) of subjects whose earlier surveillance NPS samples were positive for influenza were excluded from analysis. Seropositivity rates for A/H1N1 pdm, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata were 92%, 91%, 68%, 49%, and 85%, respectively. The mean reciprocal HAI titres for A/H1N1 pdm were significantly higher in children who had been vaccinated within the past 3

TABLE 2. Association of influenza-like illness with demographic, environmental, and allergic factors

Factor	Influenza-like illness*		P value
	Yes (n=99)	No (n=523)	
Male sex	55 (55.6)	272 (52.0)	0.039
Age, y	5.8±2.2	7.6±2.4	<0.001
Born in Hong Kong	87 (87.9)	423 (80.9)	0.097
Born by normal vaginal delivery	75 (75.8)	340 (65.0)	0.087
Environmental exposures			
Breastfeeding ever	60 (60.6)	282 (53.9)	0.220
Current domestic smoking exposure	39 (39.4)	210 (40.2)	0.888
Current maternal smoking	8 (8.1)	48 (9.2)	0.727
Current dog/cat cohabitation	10 (10.1)	43 (8.2)	0.539
Indoor dampness or visible mould	41 (41.1)	180 (34.4)	0.182
Presence of older brother	24 (24.2)	117 (22.4)	0.683
Presence of older sister	25 (25.3)	103 (19.7)	0.210
Allergy phenotypes			
Wheezing ever	18 (18.2)	89 (17.0)	0.778
Current wheezing	12 (12.1)	53 (10.1)	0.553
Asthma ever	5 (5.1)	34 (6.5)	0.585
Use of asthma medication in past 12 months	4 (4.0)	13 (2.5)	0.721
Rhinitis ever	31 (31.3)	188 (35.9)	0.376
Eczema ever	26 (26.3)	137 (26.2)	0.989
History of influenza vaccination			
Within the past 3 years	39 (39.4)	294 (56.2)	0.002
25-36 months prior	28 (28.3)	212 (40.5)	0.022
13-24 months prior	27 (27.3)	229 (43.8)	0.002
Within the past 12 months	29 (29.3)	237 (45.3)	0.003
Ever received human swine influenza vaccine	3 (3.0)	21 (4.0)	0.641
Ever received pneumococcal vaccine	50 (50.5)	220 (42.1)	0.120

* Data are presented as mean±standard deviation or No. (%) of participants

years (186 vs 106, P=0.022) and within the previous year (192 vs 112, P=0.032). Neither HAI titres nor seropositivity rates differed between subjects with and without ILI or influenza in surveillance samples.

In general, influenza vaccine was moderately protective against ILI, with vaccine effectiveness varying between 42.1% (10.5%-63.1%) and 51.9% (24.5%-70.1%) when subjects were vaccinated at different time points before this study (Table 3). Nonetheless, the effectiveness of seasonal influenza vaccine was poor in preventing laboratory-confirmed influenza in surveillance samples.

Discussion

During influenza seasons in 2014-2015, NPS surveillance detected 104 children with ILI and 55 clinically asymptomatic children with laboratory-confirmed influenza. A significant proportion of patients with respiratory viral infections had mild symptoms only.

Some post-marketing studies have challenged the real-life effectiveness of influenza vaccine.[1,2,5] Multiple confounding factors, such as difficulty matching influenza A subtype for the vaccine with the dominant viruses, suboptimal vaccine uptake, and poor infection control practices affected the effectiveness of influenza vaccine, which can only be defined by well-designed observational studies. This prospective cohort study enabled biweekly surveillance of influenza A and B viruses by molecular methods. Our results supported seasonal influenza vaccine as an effective public health measure to prevent ILI in local children.

Among the typable influenza virus isolates, we found four A/H1N1 pdm and one A/H3 in 2014 and three A/H1N1 pdm and 13 A/H3 in 2015. These findings are consistent with local surveillance data for influenza A, which indicate that the 2013/14 winter influenza season was dominated by A/H1N1 pdm from early January 2014 to early March 2014, whereas A/H3N2 was predominant from late December 2014 to early April 2015. All of our influenza B virus-positive samples were detected in 2014 (nine B/Yamagata and 10 B/Victoria). Our typing results are also concordant with local data showing B/Yamagata dominance between early March and late April in 2014 and low activity of influenza B in the winter 2014/15 season.

During the period of this study, approximately 40% of Hong Kong children received seasonal influenza vaccination annually. Breaking barriers to seasonal influenza vaccination should be a public health priority against influenza outbreaks. The moderate effectiveness of influenza vaccine for ILI in children aged 2 to 12 years may suggest that the Government Vaccination Programme can be expanded to older children.

Conclusion

Mild influenza was common during influenza seasons in 2014-2015 among Hong Kong children. The effectiveness of influenza vaccine for ILI varied between 42.1% and 51.9%, depending on the year of vaccination. Our findings do not support HAI titres or seropositivity as useful surrogates of influenza immunity in children.

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TABLE 3. Effectiveness of seasonal influenza vaccine in terms of laboratory-confirmed influenza (by surveillance) and influenza-like illness

Timing of vaccination	No. (%) of participants		Vaccine effectiveness (95% confidence interval)
	Vaccinated	Not vaccinated	
Laboratory-confirmed influenza by surveillance			
Within the past 3 years	n=333	n=290	
Positive	26 (7.8)	27 (9.3)	17.5 (–39.0 to 51.3)
Negative	307 (92.2)	263 (90.7)	
Within the past 12 months	n=266	n=357	
Positive	18 (6.8)	35 (9.8)	33.2 (–16.9 to 62.2)
Negative	248 (93.2)	322 (90.2)	
13-24 months prior	n=256	n=367	
Positive	21 (8.2)	32 (8.7)	6.4 (–58.8 to 44.9)
Negative	235 (91.8)	335 (91.3)	
25-36 months prior	n=240	n=383	
Positive	22 (9.2)	31 (8.1)	–14.6 (–92.2 to 31.5)
Negative	218 (90.8)	352 (91.9)	
Influenza-like illness			
Within the past 3 years	n=333	n=289	
Yes	39 (11.7)	60 (20.8)	49.4 (24.0 to 66.9)
No	294 (88.3)	229 (79.2)	
Within the past 12 months	n=266	n=356	
Yes	29 (10.9)	70 (19.7)	50.0 (22.4 to 68.4)
No	237 (89.1)	286 (80.3)	
13-24 months prior	n=256	n=366	
Yes	27 (10.5)	72 (19.7)	51.9 (24.5 to 70.1)
No	229 (89.5)	294 (80.3)	
25-36 months prior	n=240	n=382	
Yes	28 (11.7)	71 (18.6)	42.1 (10.5 to 63.1)
No	212 (88.3)	311 (81.4)	

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Influenza-like illness and viral aetiology in Hong Kong children

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KEY MESSAGES

1. Respiratory viruses are frequently detected in paediatric outpatients in Hong Kong, and non-influenza viruses appear to be associated with a much greater burden on ambulatory care than influenza A and B viruses do.
2. Increased detection of respiratory syncytial virus, parainfluenza, adenovirus, and bocavirus among inpatients suggest that these viruses may be associated with more severe illnesses than influenza and rhinovirus are, particularly in younger children.
3. Given the substantial burden of respiratory viruses other than influenza, more attention should be given to potential measures to control these diseases in Hong Kong.

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Introduction

Respiratory viruses are responsible for a large proportion of infections, hospitalisations, and deaths every year in Hong Kong.[1,2] The most severe infections are usually due to respiratory syncytial virus (RSV) in infants and influenza in all ages. The burden of other common respiratory viruses including parainfluenza, adenovirus, metapneumovirus, coronavirus, and rhinovirus are also considerable.[1-4] These viruses often result in a broad and overlapping spectrum of symptoms commonly called the 'common cold'. Only a few of the most severe infections result in hospitalisation. Previous studies have focused mainly on infections among hospitalised patients.[1-4] Nonetheless, some community-based studies of respiratory viral infections were conducted prior to the availability of molecular diagnostics and the discovery of certain respiratory pathogens such as human metapneumovirus, coronaviruses NL-63 and HKU1, and bocavirus. Limited data specific to the community viral burden in Hong Kong are available, except for those about influenza virus, for which good local surveillance data exist.

This study aimed to characterise the incidence of common respiratory viruses in children that lead to influenza-like illness and compare paediatric outpatient with inpatient data.

Methods

The study protocol was approved by the Institutional Review Board of the University of Hong Kong. Proxy written consent from parents or legal guardians was obtained for participants aged 16 years and younger, with additional written assent from those aged 8 to 16 years.

We conducted a large field study of influenza transmission within households in the years 2007-2010.[5-7] The inclusion criteria were (1) Hong Kong residents, (2) presenting two or more symptoms of acute respiratory illness, including body temperature of ≥37.8°C, headache, cough, sore throat, myalgia, runny nose, or phlegm, (3) onset of symptoms within the preceding 48 hours, (4) living in a household with at least two other people, and (5) all household members, except for the index subject, being free from acute respiratory illness in the past 2 weeks.

Patients were asked to complete a short data collection from. A combined nasal and throat swab was then collected and tested with the QuickVue Influenza A+B rapid diagnostic test (Quidel, San Diego, California). The household contacts of patients with positive results received further follow-up. In the present analysis, we focus only on the specimens collected from patients at the clinic. While waiting for the rapid test result (5-10 minutes), an additional nasal and throat swab specimen was collected and stored immediately in a viral transport medium for subsequent virological testing.

We compared our results with those of another study in which paediatric inpatients were recruited from two public hospitals (Pamela Youde Nethersole Eastern Hospital and Queen Mary Hospital) from January to September of 2008 to 2010.[8] Nasopharyngeal aspirates were obtained and tested by the same xTAG assay for respiratory viruses as the outpatient specimens were.

The primary outcome measure was respiratory

virus infection in participants, indicated by positive multiplex assay results from the combined nasal and throat swab. The secondary outcome measure was presentation of influenza-like-illness-related symptoms (body temperature ≥37.8°C, headache, sore throat, cough, runny nose, myalgia, and phlegm).

Each specimen was stored in viral transport medium (5% bovine serum albumin in Earle's balanced salt solution with antibiotic) and kept at 2-8°C immediately after collection, then cryopreserved at –70°C within 36 hours. The specimens were tested for 18 respiratory viruses (influenza A and B, RSV A and B, parainfluenza types 1-4, metapneumovirus, enterovirus/rhinovirus, adenovirus, bocavirus, coronavirus types NL63, HKU1, 229E, and OC43) by the xTAG RVP FAST multiplex assay followed by product detection and identification using a Luminex suspension microarray. Total nucleic acids were extracted from the clinical specimens using NucliSens easyMAG extraction system (bioMerieux, Netherlands) according to the manufacturer's instructions. The extracted nucleic acids were tested for respiratory viruses.

Detection frequencies in children aged 0-5 and 6-16 years were compared using Chi-squared tests, with 95% confidence intervals obtained using the exact binomial method. Associations between individual respiratory viruses and clinical symptoms were determined by cross-tabulating the proportion of influenza-like-illness-related symptoms presented with each type of virus detected. The differences between certain symptom onset rates were compared among viruses using Chi-squared tests. For virus detection frequencies, outpatient data were compared with inpatient data using Chi-squared tests, with 95% confidence intervals obtained using the exact binomial method.

Results

Of the 2700 children recruited between 2007 and 2010, 2090 (77%) provided specimens that were extracted and tested for respiratory viruses. Children recruited in each year had similar baseline characteristics (data not shown). Of the 2090 specimens, 1343 (64%) were positive for at least one respiratory virus, among which 81 (6%) were positive for more than one respiratory virus (Table 1). The most common viruses detected were entero/rhinovirus and influenza A virus. In the 81 specimens with co-detection, many were positive for entero/rhinovirus. One notable co-detection was coronavirus and influenza A. Two specimens had three viruses detected: one with entero/rhinovirus, metapneumovirus, and RSV B and another with entero/rhinovirus, parainfluenza type 4, and RSV A.

Influenza A and B viruses were significantly more frequently detected in children aged 6-16 years, whereas rhinovirus, metapneumovirus, RSV, parainfluenza, adenovirus, and bocavirus were more frequently detected in children aged 0-5 years (Table 2). Nonetheless, we could not identify consistent seasonal patterns for any of the respiratory viruses across the 4 study years (data not shown). Many of the viruses appeared in epidemics (including influenza A and B and parainfluenza), but the timing of the epidemics differed from year to year. Other viruses (including entero/rhinovirus, metapneumovirus, and coronavirus) appeared throughout most years.

Both younger and older children infected with influenza A, influenza B, or adenovirus had a significantly higher chance of presenting with fever, whereas those infected with metapneumovirus or RSV had a significantly higher chance of presenting with coughing (Table 3). Among children infected with influenza A or B, 70% to 80% presented with both fever and cough or sore throat, compared with 50% among children infected with other viruses.

Our study's outpatient data were compared

TABLE 1. Detection of respiratory viruses among 2090 paediatric outpatients

Virus type	Detection rate (95% confidence interval)	Co-infection rate among positive detection (95% confidence interval)
Entero/rhinovirus	23.4 (21.6-25.3)	12.7 (9.8-15.9)
Influenza A	19.6 (17.9-21.3)	8.8 (6.2-12.0)
Influenza B	6.3 (5.3-7.4)	6.1 (2.7-11.6)
Metapneumovirus	5.3 (4.4-6.4)	7.2 (3.2-13.7)
Respiratory syncytial virus A and B	4.9 (4.0-5.9)	19.4 (12.3-28.4)
Parainfluenza types 1-4	3.0 (2.3-3.8)	9.5 (3.6-19.6)
Coronavirus	2.8 (2.2-3.6)	22.0 (12.3-34.7)
Adenovirus	2.5 (1.9-3.3)	11.3 (4.3-23.0)
Bocavirus	0.3 (0.1-0.6)	83.3 (35.9-99.6)

TABLE 2. Comparison of virus detection between children aged 0-5 years and 6-16 years

Virus type	Detection rate (95% confidence interval)		P value
	Age 0-5 years (n=822)	Age 6-16 years (n=1268)	
Entero/rhinovirus	28.5 (25.4-31.7)	20.2 (18.0-22.5)	<0.01
Influenza A virus	14.5 (12.1-17.1)	22.9 (20.6-25.3)	<0.01
Influenza B virus	3.8 (2.6-5.3)	8.0 (6.5-9.6)	<0.01
Metapneumovirus	8.4 (6.6-10.5)	3.3 (2.4-4.5)	<0.01
Respiratory syncytial virus A and B	10.9 (8.9-13.3)	1.0 (0.5-1.7)	<0.01
Parainfluenza	5.1 (3.7-6.8)	1.7 (1.0-2.5)	<0.01
Coronavirus	2.3 (1.4-3.6)	3.2 (2.3-4.3)	0.32
Adenovirus	3.5 (2.4-5.0)	1.9 (1.2-2.8)	0.03
Bocavirus	0.7 (0.3-1.6)	0.0 (0.0-0.3)	0.01

TABLE 3. Association between individual respiratory viruses and clinical symptoms by age group

Symptoms	Proportion									P value
	Entero/ rhinovirus	Influenza A	Influenza B	Metapneu-movirus	Respiratory syncytial virus A and B	Para-influenza	Corona virus	Adeno-virus	Boca-virus	
Age 0-5 years, n	234	119	31	69	90	42	19	29	6	
Body temperature ≥37.8°C	0.44	0.83	0.81	0.52	0.48	0.62	0.47	0.83	0.50	<0.01
Headache	0.09	0.15	0.26	0.06	0.10	0.12	0.05	0.24	0.17	0.02
Sore throat	0.32	0.42	0.29	0.28	0.30	0.36	0.37	0.48	0.33	0.34
Cough	0.84	0.85	0.74	1.00	0.97	0.76	0.63	0.59	1.00	<0.01
Myalgia	0.09	0.15	0.16	0.09	0.07	0.07	0.05	0.14	0.00	0.37
Runny nose	0.90	0.81	0.84	0.74	0.86	0.74	0.84	0.69	0.83	0.01
Phlegm	0.46	0.49	0.35	0.55	0.72	0.36	0.32	0.34	0.67	<0.01
Age 6-16 years, n	256	290	101	42	13	21	40	24	0	
Body temperature ≥37.8°C	0.38	0.80	0.82	0.43	0.46	0.57	0.50	0.75	-	<0.01
Headache	0.38	0.45	0.59	0.33	0.15	0.38	0.48	0.50	-	<0.01
Sore throat	0.61	0.65	0.50	0.52	0.62	0.67	0.72	0.54	-	0.11
Cough	0.74	0.86	0.81	0.98	0.85	0.71	0.80	0.54	-	<0.01
Myalgia	0.22	0.31	0.39	0.19	0.00	0.19	0.30	0.25	-	<0.01
Runny nose	0.86	0.82	0.87	0.76	0.92	0.76	0.82	0.67	-	0.27
Phlegm	0.52	0.64	0.63	0.81	0.69	0.48	0.65	0.33	-	<0.01

with inpatient data from two public hospitals from January to September of 2008 to 2010. Among both younger and older children, inpatients had a significantly higher chance of RSV A and B detection than outpatients had. Among children aged 0-5 years, inpatients had a significantly higher chance of parainfluenza, adenovirus, and bocavirus detection than outpatients had (data not shown).

Discussion

Many studies have confirmed the substantial morbidity and mortality burden associated with influenza virus. Nonetheless, studies of respiratory viruses such as rhinovirus, parainfluenza, adenovirus, and RSV are limited. These viruses are also associated with a substantial burden perhaps exceeding that of the influenza virus in certain age groups.[8] Influenza vaccination is effective at preventing influenza virus infection and associated morbidity in most age groups. Nonetheless, no licensed vaccines are available for these other respiratory viruses.

In our study, among children aged 6-16 years, entero/rhinoviruses and influenza A were the most common causes of outpatient visits for acute respiratory illness. Among younger children, there was a broader range of common viral aetiologies. Overall, we identified at least one respiratory virus in 64% of specimens. Almost all specimens were collected within 48 hours of illness onset, so it is unlikely that the patients had ceased virus shedding. However, it is possible that the xTAG assay used had imperfect sensitivity for some respiratory viruses. It is unclear how the 6% prevalence of co-infections, which may have special epidemiological significance, should be interpreted.

In our study, we did not have an estimate of the underlying population from which the outpatients came, and therefore, the population-based incidence of various viral infections cannot be estimated. Among outpatients and inpatients aged 0-5 years, RSV, parainfluenza, adenovirus, and bocavirus were more frequently detected among inpatients than outpatients. This suggests that these viruses may lead to more severe illnesses than other respiratory viruses. Previous studies have also identified similar patterns for RSV and influenza in children in Taiwan[9] and the United States.[10]

Influenza-like illness is defined as fever plus cough or sore throat. Nonetheless, this case definition lacks sensitivity and specificity for influenza. We could not identify any differences between the various respiratory viruses. Influenza A and B and adenovirus infections were more likely to cause febrile illness (Table 3).

Influenza has a seasonal pattern with activity peaks in the winter and summer in most years, whereas RSV tends to occur in the summer months. Nonetheless, we could not identify any consistent seasonal pattern. Another study with a longer timeframe reported consistent patterns in RSV and parainfluenza activity in Hong Kong prior to the 2009 influenza pandemic and noted that the seasonal

patterns of respiratory viruses were disrupted after 2009.[11]

One study has reported detection of respiratory viruses in the respiratory tracts of children who were not ill and questioned the role of some respiratory viruses in causing disease.[12] Further studies are needed to determine the pathogenicity of viruses that are not known to be pathogenic. In our other study, we commonly detected respiratory viruses in children with acute respiratory illness, but we rarely detected respiratory viruses in children who were not ill.[13] Preliminary results provide indirect evidence that virus detection in children with medically attended acute respiratory illness is consistent with the pathogenicity of the viruses detected. A limitation of our study is that detailed clinical data were not accessed for either outpatients or inpatients.

In terms of disease control, improved control of respiratory virus transmission in schools and increased frequency of disinfection of common surfaces in public areas are suggested. Our findings on the substantial outpatient burden of RSV and data on the burden of hospitalisations and deaths associated with RSV are useful for calculating the cost-effectiveness of an RSV vaccine. Treatment for acute respiratory illnesses is largely empirical and focused on management of specific symptoms. An understanding of viral aetiology may be used in campaigns to reduce antiviral prescription rates and preserve antibiotics.

Conclusions

The RSV was frequently detected in inpatients and outpatients with acute respiratory illness. The symptoms of parainfluenza and adenovirus infections were generally more severe than those of influenza virus and rhinovirus infections. Further studies are needed to clarify the annual burden of these viruses across the full spectrum of disease, including ambulatory care, inpatient care, intensive care, and mortality.

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Surveillance of human- and swine-origin influenza in Hong Kong children

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KEY MESSAGES

1. Of 49 children aged 5 years recruited from three randomly selected kindergartens in the New Territories East Cluster, 49 provided at least one nasopharyngeal swab for influenza surveillance. Of them, 44 (89.8%) provided four bi-weekly nasopharyngeal samples between early February and late March 2012. Serial nasopharyngeal sampling is a feasible approach for respiratory virus surveillance in local preschool children.
2. Of all samples, 13 from 12 (24.5%) children were influenza-positive, including those from 10 (20.4%) of 49 children under surveillance and three of 10 children who provided illness visit samples. Asymptomatic and mildly symptomatic influenza infection is common in these young children.
3. Of the 49 children, 27 (55.1%) had received influenza vaccination within 3 years, but only two (16.7%) who were infected with influenza had been vaccinated within the past 12 months. The low level of vaccine uptake is probably the main cause of influenza infection in our preschoolers.
4. Influenza infection was not associated with any personal or environmental factors, including influenza vaccination.

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Introduction

In 2009, the emergence of a triple reassortant H1N1 virus resulted in an influenza pandemic.[1] In contrast to seasonal influenza virus, this H1N1 virus caused illness primarily in younger age groups. In the United States, 60% of patients were 18 years of age or younger. The number of laboratory-confirmed cases underestimates the number of true cases, as surveillance focused on severe cases and caused bias with respect to the disease spectrum of novel influenza infection. Patients with mild infection or who were asymptomatic could not be identified because they were unlikely to seek medical treatment. This H1N1 virus is now circulating seasonally. The pandemic H1N1 vaccine is a component of seasonal influenza vaccine, which for 2012/2013 (northern hemisphere winter) comprised A/California/7/2009 (H1N1)-like virus, A/Victoria/361/2011 (H3N2)-like virus, and B/Wisconsin/1/2010-like virus.

The Centre for Health Protection recommends influenza vaccination for children aged 6 months to 5 years. Among healthy children aged 2 to 15 years, seasonal influenza vaccination has reduced laboratory-confirmed influenza and clinical influenza-like illness by 59% and 36%, respectively. Although influenza vaccination has proven efficacy in children, the data remain inconsistent on the real-life effectiveness of seasonal influenza vaccine against transmission of influenza infection within schools and among household members. Population-based surveillance studies are needed to address this public health issue. This study aimed to investigate the feasibility of serial nasopharyngeal swab (NPS) sampling and characterise the clinical features of influenza infections in Hong Kong preschool children.

Methods

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Clinical Research Ethics Committee. This prospective cohort study targeted 50 K3 students from three randomly selected kindergartens in Shatin and Ma On Shan. The parents were contacted to obtain informed written consent for their children. There was no exclusion criterion to facilitate recruitment of an unbiased cohort. This study was timed to cover the influenza season of early 2012. Influenza infection was defined as the detection of influenza virus by multiplex PCR assay. The primary outcome was the success rate of obtaining serial NPS samples from participants. The secondary outcomes included the detection rate and clinical features of influenza infections and rates of influenza-like illness and laboratory-confirmed influenza among children who received and had not received influenza vaccination.

Parents completed a questionnaire to record the participants' demographics, early-life events, environmental exposures, health status, and history of influenza vaccination within 3 years prior to recruitment. Two sets of NPS samples were obtained. For active surveillance during the influenza season, four serial NPS samples were collected from each participant during bi-weekly school visits between early February and late March in 2012. The other set of NPS samples was obtained from children with symptoms of respiratory infections throughout the

study period. We called the parents every 2 weeks to enquire about influenza-like illness and vaccination history. The parents were encouraged to inform us as soon as their children developed symptoms and then visit our hospital within 48 hours of the onset of influenza-like illness. Alternatively, home visits were provided to collect NPS. Possible clustering of influenza infection among household members and classmates was also recorded.

The NPS samples were collected using flocked swabs (Copan Diagnostics, Corona [CA], United States). Each child was swabbed two times, using one swab in each nostril. Both swabs were placed in the same specimen bottle containing virus transport medium. Total RNA and DNA were extracted together by the PureLink Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad [CA], United States). The extracted preparation was mixed with random primers and dNTPs at 65°C for 5 minutes. The solution was equilibrated at 4°C and completed with two units (U) of RNaseOUT, 4 μL of 5× First-Strand buffer, 0.5 mM DTT, and 10 U M-MLV Superscript III reverse transcriptase (Invitrogen) to a final volume of 20 μL. Reverse transcription was performed for 50 minutes at 50°C and then stopped by heating for 15 minutes at 70°C. The resulting complementary DNA products (cDNAs) were used immediately for PCR. Two sets of primers targeting influenza A and B viruses were used during PCR for both the first and second rounds. The 73- and 516-bp PCR products from influenza A and B, respectively, were identified by electrophoresis and visualised in 1.5% agarose gel pre-stained with SYBR-Safe (Invitrogen).

TABLE 1. Clinical features of 49 participants in influenza surveillance

Feature	Value*
Age, y	5.5±0.4
Male sex	27 (55.1)
History of influenza vaccination	
Received seasonal vaccine within 3 years	27 (55.1)
Received seasonal vaccine in 2009	24 (49.0)
Received seasonal vaccine in 2010	18 (36.7)
Received seasonal vaccine in 2011	9 (18.4)
Ever received human swine influenza monovalent vaccine	3 (6.1)
Physician-diagnosed influenza in past 12 months	22 (44.9)
Anti-viral treatment for influenza in past 12 months	4 (8.2)
Asthma phenotypes	
Wheezing ever	14 (28.6)
Current wheezing	8 (16.3)
Awakening due to wheezing in past 12 months	6 (12.2)
Asthma ever	4 (8.2)
Asthma medication in past 12 months	17 (34.7)
Exercise-induced wheezing	8 (16.3)
Rhinitis ever	18 (36.7)
Eczema ever	15 (30.6)
Environmental exposures	
Breastfeeding ever	31 (63.3)
Breastfeeding for 4 months and longer	17 (34.7)
Current cat/dog cohabitation	2 (4.1)
Current maternal smoking	2 (4.1)
Maternal smoking during infancy	3 (6.1)
Maternal smoking during pregnancy	2 (4.1)
Current domestic smoking exposure	18 (36.7)
Indoor dampness or visible mould	7 (14.3)

* Data are presented as mean±standard deviation or No. (%) of participants

Results

A total of 54 children were included, but five withdrew consent before collection of the first surveillance swab (Table 1). On the second, third, and fourth visits, NPS of 48, 46, and 44 children, respectively, were collected for bi-weekly surveillance. Of the children, 27 (55.1%) had received influenza vaccination within the previous 3 years, including nine (18.4%) vaccinated in 2011. Of the 14 children reported to have respiratory symptoms, eight visited our hospital and six visited general practitioners. NPS samples were collected from 10 of these sick children.

Influenza B virus was detected in 10 children who provided at least one NPS sample. All such children were not sick at the time of NPS collection. One child had influenza A virus followed by influenza B virus detected in two consecutive NPS samples collected 2 weeks apart. She remained asymptomatic during this period. In addition, three of the 10 sick children with available NPS had detectable influenza B virus. There was no transmission of influenza within the same classrooms or households of the children infected with influenza. Only two (16.7%) of the 12 children who were infected with influenza (either under surveillance or sick) had been vaccinated within past 12 months. Influenza infection was not associated with personal, clinical, or vaccine-related factors (Table 2).

Discussion

This prospective cohort design enabled surveillance of respiratory viruses because a large proportion of the participants had mild symptoms only. They may have had mild cough and runny nose but no fever, myalgia, or arthralgia. It is unlikely that these participants would have been identified in any hospital-based study. A study that collected weekly respiratory samples from community participants reliably defined the disease severity and seasonality of picornavirus infection.[2] We applied sensitive molecular diagnostic tests to the nasopharyngeal secretions of clinically 'asymptomatic' children.

Influenza vaccination is effective against

TABLE 2. Association of influenza infection with demographic, environmental, allergic, and vaccine-related factors

Parameter	Influenza*		P value
	Yes (n=10)	No (n=39)	
Male sex	7 (70.0)	20 (51.3)	0.478
Age, y	5.3 (5.2-5.9)	5.6 (5.3-5.8)	0.524
Environmental exposures			
Breastfeeding ever	6 (60.0)	25 (64.1)	0.542
Current domestic smoking exposure	3 (30.0)	15 (38.5)	0.458
Maternal smoking during infancy	0 (0)	2 (5.1)	0.630
Current dog/cat cohabitation			
Indoor dampness or visible mould	3 (30.0)	4 (10.3)	0.140
Allergic phenotypes			
Current wheezing	1 (10.0)	7 (17.9)	1.000
Asthma ever	2 (20.0)	2 (5.1)	0.180
Asthma medication in past 12 months	4 (40.0)	13 (33.3)	0.721
Rhinitis ever	3 (30.0)	15 (38.5)	0.726
Eczema	4 (40.0)	11 (28.2)	0.470
History of seasonal influenza vaccination within 3 years	6 (60.0)	21 (53.8)	0.727
2009	5 (50.0)	19 (48.7)	
2010	5 (50.0)	13 (33.3)	
2011	2 (20.0)	7 (17.9)	
Received influenza vaccine within 12 months	2 (20.0)	7 (17.9)	0.597
Ever received human swine influenza monovalent vaccine	1 (10.0)	2 (5.1)	0.504
Received anti-viral treatments for influenza within 12 months	0 (0)	4 (10.3)	0.569
Physician-diagnosed influenza within 12 months	7 (70.0)	15 (38.5)	0.090

* Data are presented as mean (range) or No. (%) of participants

natural infection.[3] However, vaccine trials are conducted under optimal circumstances in which participants are closely monitored for outcomes and frequently reminded about personal hygiene. These findings may not be generalisable to real-life situations. There has been growing concern about the effectiveness of influenza vaccination.[4] In 2011, a systematic review showed low effectiveness (often <60%) of seasonal influenza vaccines at protection of those at the highest risk of severe disease from infection. The Influenza Monitoring Vaccine Effectiveness in Europe Project, funded by the European Centre for Disease Prevention and Control, revealed low early-season effectiveness (43%) of the 2011-12 influenza vaccine. Vaccine effectiveness can only be defined by well-designed observational studies that account for confounding factors. Nonetheless, conclusions about the real-life effectiveness of influenza vaccination in Hong Kong children cannot be made owing to the small sample size and low vaccination rate of participants. Larger population-based surveillance studies are needed to address this public health question.

The Hong Kong Government has included children aged 6 months to 5 years in the influenza vaccination subsidy scheme. Nonetheless, the vaccine uptake rate among local children has not been satisfactory over the past few years. In this study, only 56% of preschoolers had received influenza vaccination within 3 years. Only half of these young children were vaccinated following the 2009 H1N1 influenza pandemic, and the uptake rate dropped to <20% in 2011. Breaking barriers to accept seasonal influenza vaccination should be a public health priority against influenza outbreaks and an integral component of the influenza pandemic preparedness plan.

One limitation of this study was its small sample size. We originally proposed a full-scale project with over 600 preschool and school-age children. Owing to a criticism about the feasibility of serial nasopharyngeal sampling, our study was scaled down and was insufficient to evaluate the transmissibility of influenza within household and school contacts or the effectiveness of influenza vaccination. The sampling method was another limitation. Nasopharyngeal aspirate is the most accurate method for influenza detection; further, it requires suctioning and is not suitable for field studies. NPS is an acceptable alternative for the detection of respiratory viruses, including influenza.[5] Blood was not collected from participants to determine their immune status against influenza. The baseline susceptibility of participants to natural influenza infection was unknown.

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Estimation of excess mortality and hospitalisation associated with the 2009 pandemic influenza

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KEY MESSAGES

1. We estimated the excess mortality and hospitalisation associated with influenza strain A(H1N1)pdm09 using the classical Poisson model and the Poisson prediction modelling approach.
2. During the first wave of the pandemic from May to December 2009, 127 all-cause excess deaths were associated with the A(H1N1)pdm09, of which 115 were due to cardiovascular and respiratory diseases and 22 were due to pneumonia and influenza.
3. During the whole pandemic period from May 2009 to July 2010, 10 377 hospitalisations secondary to acute respiratory diseases and 7204 secondary to pneumonia and influenza were associated with A(H1N1)pdm09 infections.
4. An age shift towards children and younger people was found in A(H1N1)pdm09-associated excess hospitalisation for acute respiratory diseases and its subcategory pneumonia and influenza.
5. The age pattern of A(H1N1)pdm09-associated excess mortality was similar to that for seasonal influenza, with high mortality risk observed in people aged ≥65 years. This suggests that control measures adopted by the government were effective in reducing the mortality risk in younger age-groups.

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Introduction

In 2009, a novel A(H1N1) influenza strain, later termed as A(H1N1)pdm09, emerged to cause an influenza pandemic. To assess the severity of this pandemic, it is critical to reliably estimate the mortality and morbidity burden associated with A(H1N1)pdm09. Hospitalisations and deaths due to influenza in health records represent only the tip of the iceberg for influenza-associated disease burden, owing to its non-specific symptoms and the lack of timely laboratory tests. The Poisson regression model has been used to quantify influenza disease burden by estimating excess mortality/hospitalisation, which is defined as the difference in deaths/hospitalisation during epidemic periods compared with baseline periods when influenza viruses are not circulating.[1] Given the potential underreport of pandemic cases, it is necessary to compare excess hospitalisation associated with seasonal and pandemic influenza to accurately assess the severity of the 2009 H1N1 pandemic.

This study aimed to estimate the excess mortality or hospitalisation associated with A(H1N1)pdm09 using a valid modelling approach, and to evaluate the severity of the pandemic by comparing the disease burden of this pandemic with past seasonal influenza epidemics, and to identify the susceptible age-groups in the H1N1 pandemic by comparing laboratory-confirmed cases.

Methods

Weekly numbers of cause-specific deaths for the age-groups <20, 20-39, 40-64, 65-84, and ≥85 years were obtained from the Census and Statistics Department; three categories were considered: cardiovascular and respiratory, pneumonia and influenza, and all-cause. Hospitalisation records were obtained from the electronic health record system of the Hospital Authority. We aggregated the records into weekly hospitalisation numbers based on any-listed discharge diagnoses (up to 15 diagnoses) for the following disease categories: acute respiratory disease, pneumonia and influenza for the age-groups of 0-5, 6-17, 18-39, 40-64, 65-74, ≥75 years; and cardiovascular disease, diabetes, ischaemic heart disease, and stroke for the age-groups of 40-64, 65-74, ≥75 years.

Virology data were retrieved from: (1) the dataset of weekly numbers of laboratory-confirmed cases for influenza type A (two seasonal subtypes A(H3N2) and A(H1N1), the pandemic strain A(H1N1)pdm09) and type B during the period of 2005-2010 from the Public Health Laboratory Centre's weekly report 'Flu Express' at http://www.chp.gov.hk/en/guideline1_year/441/304.html; and (2) the Microbiology Laboratory of Queen Mary Hospital for influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza (type 1, 2 and 3) during the period of 1998-2009, as influenza

subtypes data before 2005 and other respiratory viruses other than influenza were not available to the public.

We developed a Poisson prediction method to estimate influenza-associated mortality by modifying the classical Poisson models for disease burden of influenza. The first step was to detrend the weekly mortality data of entire study period. In the second step, a Poisson regression model with the predictor variables of temperature, humidity, and an influenza proxy variable of weekly proportions of specimens positive for influenza (influenza proportions) was fitted to the detrended mortality data of 1998-2008. This 10-year Poisson model was then used to predict a mortality level for the assessment year, with the data of temperature and humidity taking the values as observed in that year and the influenza variable simultaneously set to zero. Then the time trend removed in the first step was added back to the predicted mortality to derive the baseline mortality. Excess mortality for the assessment year was then calculated by deducting the baseline mortality from the observed mortality data in that year.

We fitted classical Poisson regression models to weekly numbers of hospitalisation for each age-disease category in Hong Kong, as described in our previous study.[2] The virus proxies of weekly proportions of specimens tested positive for A(H1N1), A(H3N2), influenza B, and A(H1N1) pdm09 were simultaneously added to assess the effects of these viruses. The variables of weekly average temperature, relative humidity, weekly proportions of specimens positive for respiratory syncytial virus, adenovirus and three types of parainfluenza viruses were added into the model as confounding factors. For hospitalisation, we also added the dummy variable for the period of this containment phase, and its product term with the pandemic virus proxy into the model to allow a different hospitalisation risk during the containment phase. Baseline hospitalisation number was first calculated as the number of expected hospitalisation when the proxy variables for A(H1N1)pdm09, A(H1N1), A(H3N2), and influenza B viruses were set to zero, respectively. Excess hospitalisation number was then calculated as the difference between the baseline and predicted hospitalisation numbers with all variables set as observed.

In terms of calibration and validation of models, we compared the estimates from different models with the hospitalisation rates directly observed from a prospective cohort of paediatric patients to justify the choice of dummy variables and degrees of freedom for smoothing functions of confounding variables.[3] The model with the estimates closest to these observed rates for each age-disease category was then chosen as the best and used for subsequent analyses.

Since 1 May 2009, the Hospital Authority and Department of Health had routinely tested nearly all the suspected cases with acute respiratory symptoms for A(H1N1)pdm09 by RT-PCR and established an electronic reporting system 'eFlu' in response to the H1N1 pandemic. Because laboratory tests were intensively conducted in suspected cases during the first wave of pandemic from 1 May 2009 to 2 January 2010, the total numbers of hospitalised or fatal cases with laboratory-confirmed A(H1N1)pdm09 infections could be regarded as the lower bound of the true burden.

Results

We estimated that there were 127 excess deaths attributable to A(H1N1)pdm09, with underlying cause of cardiovascular and respiratory and its subcategory pneumonia and influenza being estimated to be 115 and 22, respectively, corresponding to the excess rates of 1.6 and 0.3 per 100 000 population, respectively. The higher mortality rate attributable to the pandemic was observed in the age-groups of 65-84 and ≥85 years for all-cause, cardiovascular and respiratory, and pneumonia and influenza (Table 1). In 2009, the age-standardised annual crude mortality rate was 9.4 per 100 000 population, which was within the range of annual rates in the preceding years (5.1-13.9).

After age standardisation, annual excess hospitalisations were higher in 2009 than in other years for acute respiratory disease, pneumonia and influenza, and ischaemic heart disease, but were slightly lower than in 2010 for cardiovascular disease, stroke, and diabetes (Table 2). A total of 10 377 acute respiratory disease hospitalisation and 7204 pneumonia and influenza were attributable to A(H1N1)pdm09 during the whole pandemic period from May 2009 to July 2010. 60% of these hospitalisations occurred in children and 13% in the elderly. For chronic diseases, the total numbers of A(H1N1)pdm09-associated hospitalisation were 1676, 848, 359, and 1550, for cardiovascular disease, ischaemic heart disease, stroke, and diabetes, respectively. More than 80% of these hospitalisations occurred in people aged ≥65 years. Overall, 80% of A(H1N1)pdm09-associated hospitalisation occurred during the first wave of the pandemic from May 2009 to January 2010.

Among all the virus types/subtypes, A(H1N1)pdm09 was associated with the highest annual excess rate of acute respiratory disease hospitalisation, followed by A(H3N2), influenza B, and A(H1N1). Excess rates of acute respiratory disease and pneumonia and influenza were found higher in children aged <5 years for A(H1N1)pdm09, A(H1N1), or influenza B, whereas the rates were higher among persons aged >75 years for A(H3N2). For acute respiratory disease and pneumonia and

TABLE 1. Annual excess rate of all-cause mortality (per 100 000 population) associated with influenza for different age-groups, estimated by the Poisson prediction model

Year	Excess rate (95% confidence interval) of all-cause mortality associated with influenza						
	<20 y	20-39 y	40-64 y	65-84 y	≥85 y	All age-groups (crude)	All age-groups (standardised)*
1998	0.3 (–1.1 to 1.6)	0.1 (–1.4 to 1.8)	3.6 (–0.8 to 7.7)	29.4 (2.8 to 55.2)	175.2 (12.0 to 327.7)	4.4 (0.5 to 7.9)	5.1 (–1.3 to 11.2)
1999	0.7 (–0.4 to 1.8)	–0.8 (–2.2 to 0.6)	–0.7 (–4.2 to 2.9)	45.0 (21.1 to 68.3)	142.1 (–10.9 to 284.1)	7.6 (4.2 to 11.0)	9.0 (3.4 to 14.5)
2000	0.6 (–0.5 to 1.8)	–0.5 (–2.0 to 1.0)	1.6 (–2.4 to 5.2)	51.4 (24.3 to 76.3)	159.0 (–7.2 to 315.5)	7.6 (3.6 to 11.2)	8.7 (2.5 to 14.4)
2001	0.1 (–1.1 to 1.3)	0.3 (–1.3 to 1.8)	1.4 (–2.2 to 4.8)	29.4 (5.3 to 52.6)	75.4 (–63.4 to 202.1)	5.0 (1.1 to 8.7)	5.6 (–0.3 to 11.0)
2002	1.0 (0.1 to 2.1)	0.1 (–1.5 to 1.6)	3.3 (0.1 to 6.4)	44.6 (20.9 to 66.0)	204.5 (68.2 to 327.8)	8.8 (4.8 to 12.2)	9.6 (4.0 to 14.9)
2003	–0.6 (–1.7 to 0.5)	0.0 (–1.7 to 1.6)	1.5 (–2.2 to 4.9)	51.2 (26.9 to 76.1)	130.9 (–33.9 to 276.4)	7.6 (3.4 to 11.5)	8.4 (2.1 to 14.0)
2004	0.7 (–0.3 to 1.6)	0.7 (–0.7 to 2.0)	–0.4 (–3.4 to 2.5)	31.4 (9.3 to 54.1)	191.8 (47.1 to 334.1)	6.3 (2.5 to 10.1)	6.8 (1.5 to 12.0)
2005	0.4 (–0.7 to 1.6)	–0.3 (–1.9 to 1.2)	5.3 (2.2 to 8.7)	69.4 (44.5 to 94.1)	258.2 (90.8 to 429.1)	13.2 (8.5 to 17.3)	13.9 (7.7 to 20.0)
2006	0.4 (–0.8 to 1.5)	–0.2 (–1.6 to 1.2)	0.6 (–2.6 to 3.6)	28.3 (6.5 to 52.2)	183.7 (35.0 to 336.6)	6.4 (2.2 to 10.4)	6.7 (1.1 to 12.3)
2007	0.4 (–0.6 to 1.3)	0.8 (–0.5 to 1.9)	0.9 (–2.0 to 3.9)	55.5 (34.9 to 76.2)	196.8 (62.0 to 332.7)	10.0 (6.2 to 13.6)	10.3 (5.2 to 15.4)
2008	–0.2 (–1.5 to 1.0)	–0.9 (–2.3 to 0.4)	1.0 (–2.3 to 4.0)	29.5 (7.8 to 51.0)	298.7 (142.6 to 455.8)	8.1 (3.8 to 11.9)	8.4 (2.5 to 13.7)
2009	0.4 (–0.6 to 1.3)	0.0 (–1.2 to 1.1)	1.3 (–1.6 to 4.3)	34.1 (13.0 to 55.7)	276.2 (157.1 to 396.4)	9.1 (5.2 to 13.0)	9.4 (4.2 to 14.5)

* The 2009 mid-year population was used as standard population

TABLE 2. Age-standardised annual excess rate of hospitalisation (per 100 000 population) associated with influenza in 2005 to 2010

Disease	Age-standardised excess rate (95% confidence interval) of hospitalisation associated with influenza					
	2005	2006*	2007	2008	2009	2010
Acute respiratory disease	115.9 (70.2 to 157.0)	80.5 (39.8 to 120.3)	116.1 (70.3 to 158.8)	96.7 (51.7 to 138.1)	166.8 (109.5 to 221.7)	152.7 (96.3 to 204.9)
Pneumonia and influenza	76.2 (44.3 to 105.1)	56.6 (27.9 to 84.4)	75.0 (44.1 to 104.0)	64.9 (33.7 to 94.1)	130.9 (88.0 to 170.3)	109.5 (67.5 to 147.6)
Cardiovascular disease	30.4 (–24.9 to 84.2)	51.1 (10.2 to 90.3)	31.5 (–10.7 to 71.9)	48.3 (–2.3 to 99.5)	63.3 (–3.9 to 125.9)	102.3 (6.6 to 192.4)
Ischaemic heart disease	3.7 (–15.5 to 21.9)	8.8 (–6.2 to 23.4)	3.3 (–11.8 to 17.7)	6.0 (–11.1 to 21.9)	19.4 (–2.6 to 39.7)	12.6 (–21.0 to 44.4)
Stroke	8.5 (–6.0 to 21.5)	12.0 (1.6 to 22.2)	8.1 (–2.9 to 18.6)	12.3 (–1.7 to 25.4)	18.9 (0.5 to 35.5)	19.4 (–6.5 to 43.1)
Diabetes	9.2 (–9.7 to 27.9)	16.5 (1.6 to 30.6)	9.3 (–6.3 to 24.0)	16.7 (–2.9 to 36.6)	36.7 (7.9 to 63.6)	50.6 (8.2 to 91.4)

* The 2006 mid-year population was used as standard population

influenza hospitalisation, excess rates associated with A(H1N1)pdm09 were 200% to 500% of influenza rates in children aged <5 years and 500% to 1200% of those in children aged 6-17 years, but 50% and 70% of seasonal A(H3N2) rates in persons aged 65-74 years and ≥75 years, respectively.

Discussion

Combined with our estimates of excess mortality as numerators and the estimated attack rates from the previous serological studies,[4] the case fatality risk per infection case was 0.01% and 1.8% for the all-ages and ≥60 years age-groups, respectively. This suggests that the 2009 A(H1N1)pdm09 pandemic was much less severe than the previous pandemics.

Our estimates of the pandemic-associated excess mortality (particularly in children and young adults) in Hong Kong are generally lower than those for most of other countries/regions, but the rates of excess hospitalisation were markedly higher. Most pandemics in history were characterised with an age shift of mortality towards younger people. Some studies in other regions/countries also showed that most fatal cases occurred in younger age-groups. Nonetheless, there was no strong evidence of such an age shift in Hong Kong, given that the elderly had significantly higher mortality risk of A(H1N1) pdm09 than younger population.

In contrast, we observed a clear age shift of hospitalised cases towards younger age-groups in Hong Kong. Such discrepancy may simply reflect regional heterogeneity in disease severity and

population susceptibility, but could also suggest that the control measures adopted by the government were effective in reducing the mortality risk of younger age-groups.

The upper bound of our estimates for both hospitalisation and mortality all matched laboratory-confirmed cases of younger age-groups reported by eFlu. This indicates that the true mortality burden of influenza in these age-groups could be obtained through intensive virological surveillance. Surprisingly, we found a big gap between the numbers of reported fatal/hospitalised cases and our model estimates in older population, although the suspected cases were intensively screened for pandemic infection by virological tests. The underestimation of influenza-related illness in this age-group requires further investigation. We speculate that many influenza-initiated mortalities are attributed to secondary bacterial complications and exacerbation of underlying chronic respiratory and cardiovascular diseases. Although some evidence suggests that older persons were protected by pre-existing immunity against A(H1N1)pdm09,[5] those who were fragile and susceptible could have acquired infections and were more likely to have had serious complications. Our findings highlight a need to enhance the laboratory surveillance at the community level, particularly in older people.

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Development of adaptable pandemic simulation models

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KEY MESSAGES

1. A pandemic simulation programme was developed with three components: an adaptable population generator that creates synthetic populations with various structures; a disease simulator that handles combinations of reactive intervention strategies; and a user interface that offers flexible input and graphical output for analysis.
2. Simulation experiments of different reactive strategy combinations were carried out over the regional synthetic populations.
3. The effectiveness of various practical schooling strategies under a realistic demographic situation and movement dynamics were modelled. Limiting contact amongst students in more schools can reduce the overall attack rate. Increasing the duration of school closures can reduce the overall infection attack rate.

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Introduction

It is important to protect healthcare workers and patients from infection during pandemics, especially in a densely populated city such as Hong Kong. A simulation-based model mimicking realistic population structures can simulate possible scenarios in a timely manner.

This study aimed to develop an adaptable disease-spread simulation model with a user-friendly platform and interface that can accurately and reliably mimic regional community structures, including demographic dynamics and healthcare facilities. Simulation efficiency and robustness can be improved through appropriate probability and statistical approximation methods.

Methods

We developed a susceptible-exposed-infected-removed compartmental model, a stochastic agent-based heterogeneous model, and a stochastic simulation model with two major improvements to the Longini model.[1] First, the input synthetic population was based on Hong Kong demographic properties to reflect person-to-person contact patterns, especially in the educational and healthcare sectors. Second, extra refinements were made to intervention strategies to provide more reactive strategy combinations. We used C++ Qt programming to develop a visual simulation platform for the disease-spread simulation model.

Results

We calibrated the model using the 2009 H1N1 outbreak in Hong Kong in two major workable models: one based on the Hong Kong regional structure (HKmodel) and another comprising both Hong Kong's regional structure and the partial design of the high cycle fatigue (HK_HCFmodel). The simulators were calibrated to match the documented illness attack rate and basic reproductive number. We calibrated the age-group–specific cumulative infection attack rate at day 180 to match an independent study in Hong Kong. The basic reproduction number of the calibrated model was 1.5 which fell within the range of estimates between 1.1 and 2.1 of 2009 H1N1 pandemic influenza.

The effectiveness of various reactive strategies was examined using the Hong Kong regional structure model. With 10% antiviral coverage alone, the attack rate reduced to 8.56%. With school closure and social distancing, the attack rate significantly reduced to 0.96% for scenarios without antiviral coverage and to 0.24% for scenarios with antiviral coverage. The attack rate reduced to 3.19% when school closure (individual schools) was adopted and to 5.05% when social distancing was adopted. In general, for containing the spread of a disease, closing more types of schools was more effective than increased school closure length. The overall attack rate of closing all school types is lower than that for closing kindergartens and primary schools or closing kindergartens only.

Four alternative algorithms (ie, the Longini, Longini+S, Tai-S, and algorithms) were reviewed from the viewpoint of either infectious or susceptible individuals. Through theoretical analysis and simulation experiments, some standards for selecting the most efficient algorithm were identified. In addition, methods for applying the algorithms to real applications and potential directions for further studies were indicated. Algorithm efficiency was examined with respect to the changing factors of infectious individuals, transmission probabilities magnitude, and population size. The performance measure was based on the computational time of a 1-day simulation setting. A computationally efficient algorithm for a population with heterogeneous transmission rates was presented. Based on a heterogeneous subset sampling method,[2,3] our combined algorithm is more efficient than other algorithms for sampling disease transmission in a subset of a heterogeneous population.

Discussion

Current simulation models for infectious diseases often overlook regional variations when constructing detailed community structures. Simulation with more detailed community dynamics provides a better opportunity to contain potential pandemic influenza strains at the source. We developed a population-generation algorithm for Hong Kong–based simulations and a disease transmission simulation model taking into consideration the healthcare facilities.

We used C++ Qt programming to develop a visual simulation platform to facilitate graphical user interface–based displays and advanced modelling of pandemic disease transmission. It enables programming of the complicated dependencies between modules from the local community perspective. It also displays visual outputs and data analysis to facilitate effective decision-making. The platform allows users to analyse the disease transmission results. By simulating various scenarios, the impact and effectiveness of interventions can be studied prior to their implementation.

We proposed to rebuild the daily contact model from the perspective of infectious individuals to track each susceptible individual, which is different from the Longini model.[4] Such an approach has been reported by Tsai et al.[2,3] In summary, the approach from the infectiousness perspective is not as efficient as we expected. Accordingly, the Longini model from the susceptibility perspective may still have its advantages compared with the Tsai model. A reference guideline for selecting an efficient algorithm of disease transmission under various model settings is summarised.

Our simulation models allow epidemiologists, public health professionals, and policy makers to evaluate the impact of pandemic outbreaks and associated mitigation strategies, to develop effective countermeasures to minimise the impact of pandemics, to improve the performance of regional medical operation and public health systems in the face of pandemic outbreaks, and to prepare action, prophylaxis, and intervention strategies. Nonetheless, further development of the healthcare component of the model is needed to represent a more realistic situation. For instance, a fever clinic, an emergency department, and an infectious disease centre will be added in future, as well as realistic dynamics among various healthcare units. Furthermore, district-specific demographics for hospital components may be taken into account.

Bernoulli trials are time-consuming from the infectiousness perspective and susceptibility perspective. Binomial or normal approximation methods may reduce the time on Bernoulli trials and increase algorithm efficiency. Although we have not reached this stage, the idea should be applicable in developing a new and more efficient algorithms for disease transmission models. In addition, computational epidemiology approaches such as disease detection surveillance, simulation studies, and microbiological informatics[1] can play complementary roles to enable more comprehensive outbreak detection and tracking the spread of infectious disease at its origin. Exploring the interoperability among different methods for disease detection can justify optimal data-sharing for effective containment of infectious diseases. The development of a more robust model will require collaboration and understanding among statisticians, simulation modellers, epidemiologists, microbiologists, practitioners, and public health policy makers.[5]

Conclusions

A pandemic simulation programme was developed with three components: an adaptable population generator that creates synthetic populations with various structures; a disease simulator that handles combinations of reactive intervention strategies; and a user interface that offers flexible input and graphical output for analysis. Simulation experiments showed that the overall attack rate can be reduced by limiting contact amongst students in more schools and increasing the duration of school closures. However, school closure incurs high costs as parents have to stay at home to look after their children. Reference guidelines for selecting an efficient algorithm of disease transmission under various model settings were summarised.

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Real-time forecasting of infectious disease epidemics

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KEY MESSAGES

1. The validity and predictability of three epidemic models were evaluated: a hybrid-type homogeneous stochastic model, an age-structured variant of the previous model, and a power-law logistic model.
2. Reporting rates affect the interpretation of model parameters only but not the performance of parameter estimation or real-time epidemic forecasting.
3. Reliable and precise real-time epidemic forecasting is improbable during the early phase of an epidemic and unlikely to be robust until the epidemic has peaked, when using only epidemic curve data and any of the three models.
4. Robust real-time epidemic forecasting, if possible at all, requires other sources of epidemic data, such as seroprevalence, household transmission data, and phylogenetic data.
5. Epidemiologists and public health policymakers should be aware of these results when using models for real-time epidemic forecasting.

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Introduction

Mathematical modelling of infectious disease has greatly progressed. Much effort has been devoted to statistical inference of model parameters in real time with certain assumptions about the underlying transmission dynamics.[1] In contrast, real-time epidemic forecasting has been understudied. Although robust and well-established forecasting techniques for epidemics remain largely elusive, the ability to predict future incidence is regarded as one of the key functions of epidemic models (especially among non-modellers and policymakers).

Various scientific approaches have been attempted to forecast the course of epidemics of influenza and other directly transmitted diseases. These approaches can be broadly classified into two categories. One relies on a mechanistic model of transmission dynamics, and the other uses statistical extrapolation of epidemic curves. The mechanistic approach explicitly accounts for so-called 'dependent happening', which refers to the dependence of the risk of infection in one individual on the risk in other individuals. These models are built by describing the underlying dynamics of infectious disease transmission to explain the resulting epidemic curves. The other approach includes studies that use a parsimonious but flexible power-law logistic equation to directly fit the flexible parametric model to epidemic curves.[2] Although this approach necessarily disregards dependent happening, mechanistic models can be approximated by a family of logistic equations.

Forecasts of incidence are largely inaccurate and imprecise until the epidemic has peaked and depend strongly on the proportion of population that is susceptible at the beginning of the epidemic. We examined these aspects by comparing three forecasting methods in five case studies to improve the understanding of the feasibility and reliability of these models for real-time epidemic forecast. This study aimed to validate the three forecasting methods by varying precision of data and length of forecast and to assess the predictability of the three forecasting methods by timing and length of forecast.

Methods

We evaluated the following three forecasting methods.

(1) A hybrid-type homogeneous stochastic model.[3] Let R_i be the reproductive number at the beginning of an epidemic, T_g be the mean generation time, S_k be the number of susceptible individuals at the beginning of period k, r_k be the epidemic growth rate in period k, and C_k be the number of cases in period k. Given C_0, ..., C_k, the probability distribution of C_{k+1} is a Poisson distribution with mean $A_k C_k$ where

$$A_k(S_0,R_i,C_k,...,C_0) = \frac{r_k e^{r_k \Delta t}}{r_{k+1}} \frac{e^{r_{k+1}\Delta t}-1}{e^{r_k \Delta t}-1},\ r_k = \ln\left(\frac{S_{k-1}R_i}{S_0}\right)^{\frac{1}{T_g}},\ S_k = S_{k-1}-C_k$$

This model has two parameters: (S_0, R_i).

(2) An age-structured variant of the previous model that is analogous to the model developed by Katriel et al.[4] Let n be the number of age-groups. We assume that given the same level of exposure,

age-group j is a_j times as susceptible to infection as age-group 1 (ie a_1=1). We assume a contact matrix B=[$b_{i,j}$], where $b_{i,j}$ is the contact frequency between age-groups i and j normalised such that the largest eigenvalue of B is 1. We parameterise B using the UK contact matrix from the POLYMOD study.[5] Let $C_{k,j}$ be the number of cases in age-group j on day k. Given $C_{0,j}$, ..., $C_{k,j}$, j = 1, ..., n, the probability distribution of $C_{k+1,j}$ is a Poisson distribution with mean $S_{k,j}R_0a_j\sum_{i=1}^{n}b_{ij}\sum_{l=1}^{k}C_{k-l,i}g_l$, where $S_{k,j} = S_{k-1,j}-C_{k,j}$ is the number of susceptible individuals in age-group j at the beginning of day k, R_0 is the basic reproductive number, and g is the generation time distribution. This model has 2n parameters: (R_0, a_2, ..., a_n, S_1, ..., S_n).

(3) A power-law logistic model[2] in which the cumulative number of cases at time t is $I(t) = \frac{K}{[1+e^{-r(t-t_m)}]^{1/\alpha}}$. The incidence between time t and and $t+\Delta t$ is assumed to be a Poisson distribution with mean $I(t+\Delta t) - I(t)$. This model has four parameters: (K, r, t_m, α).

Parameters are estimated in a Bayesian framework with non-informative flat priors for all parameters. Model validity is assessed using mean absolute error (MAE), root mean squared error (RMSE), and mean absolute percentage error (MAPE):

$$MAE = \frac{1}{nm}\sum_{j=1}^{n}\sum_{k=1}^{m}\left|E(C_{k,j})-x_{k,j}\right|$$

$$RMSE = \sqrt{\frac{1}{nm}\sum_{j=1}^{n}\sum_{k=1}^{m}\left(E(C_{k,j})-x_{k,j}\right)^2}$$

$$MAPE = \frac{100\%}{nm}\sum_{j=1}^{n}\sum_{k=1}^{m}\left|\frac{E(C_{k,j})}{x_{k,j}}-1\right|$$

where n is the number of age-groups, m is the number of reporting periods over the course of the epidemic, and $E(C_{k,j})$ and $x_{k,j}$ are the number of cases in the jth age-group during the kth period in the model and the observed data, respectively. Model predictability is assessed for short-range and long-range forecasts (Fig 1) by examining (1) the coefficient of variation of forecasted incidence, (2) the percentage of forecasting periods for which the actual incidence lies outside the 95% prediction intervals of the forecasted incidence, and (3) f MAE, defined as the MAE of the model forecast and actual future incidence over the forecast periods.

The first step is to use simulated data to understand the behaviour and performance of the three models before applying them to real epidemic data. We generate the simulated data using a standard age-structured SIR model with a basic reproduction number R_0 of 1.3 and a mean generation time of 3 days (ie epidemiologically similar to a mild influenza pandemic). We assume that the three groups correspond to the 0-19, 20-59, and ≥60 years age-groups in Hong Kong and that group j is j times as susceptible as group 1. To assess the effect of under-reporting on forecast performance, we consider reporting rates of 100% and 5%.

The smallpox dataset contains the monthly number of smallpox cases in the Netherlands from 1870 to 1873 (with no age information). The mean generation time is assumed to be 15 days.

The polio dataset contains the daily number of polio cases in New York City in 1916 (with no age information). The mean generation time is assumed to be 10 days.

The pandemic influenza A/H1N1 dataset contains the daily number of confirmed cases of pandemic influenza A/H1N1 in five age-groups (0-12, 13-19, 20-29, 30-59, and ≥60 years) between 1 September and 15 November 2009 in Hong Kong. This period was selected because schools were closed before 1 September 2009, and the exogenous force of infection from Shenzhen contributed substantially to the transmission of pandemic influenza A/H1N1 in Hong Kong after 15 November 2009 (ie, the transmissibility of the virus was relatively constant during this period.) The mean generation time was assumed to be 3 days.

The SARS dataset contains the daily number of confirmed SARS cases in three age-groups (0-31, 32-49 and ≥50 years) between 15 February and 31 May 2003 in Hong Kong. The age partition was chosen so that the total number of cases in each age-group were similar (which facilitates model fitting). The mean generation time was assumed to be 8 days.

Results

The results of model validation and predictability for the three methods are shown in the Figure. With a reporting rate of 100%, Markov chain Monte Carlo inference did not converge until just before the epidemic peak for models 1 and 2 but converged sooner for model 3, although the model fit was poor before the peak. In terms of model fitting and real-time epidemic forecast, model 2 had the best performance, and model 3 performed better than model 1 until near the end of the epidemic. This was unsurprising, because model 2 is an age-structured variant of model 1 with the correct contact matrix, and model 3 has two more parameters than model 1, giving more flexibility. However, the predictive power of all three models was generally poor; future incidence almost always fell outside the 95% prediction intervals for both short- and long-range forecasts until near the end of the epidemic.

With a 5% reporting rate, the comparative performance of the three methods was similar to that with a 100% reporting rate. Thus, reporting rate had little effect on parameter estimation and epidemic forecasting for all three methods. Further analysis revealed that the mathematical structures of models 1 and 2 were not affected by the incorporation of

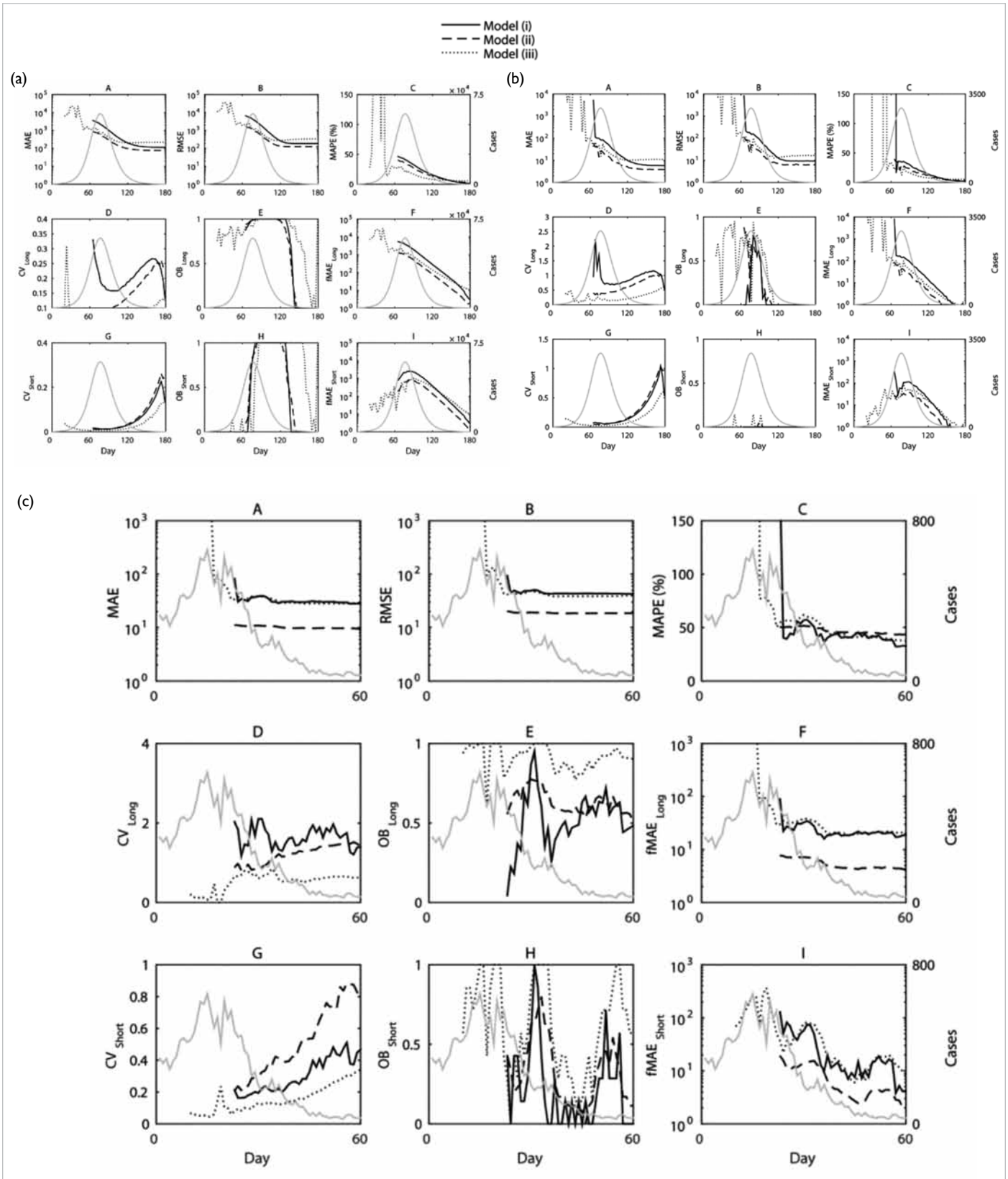

FIG. Real-time epidemic forecasting of the three models in a simulation with (a) a 100% reporting rate, (b) a 5% reporting rate, and (c) the pandemic influenza A/H1N1 case study.

Abbreviations: CV, coefficient of variation; fMAE, forecast mean absolute error; MAE, mean absolute error; MAPE, mean absolute percentage error; OB, out of bounds; RMSE, root mean square error

reporting rate (data not shown).

In the pandemic influenza A/H1N1 case study, models 1 and 2 did not converge until the epidemic peak, but model 3 converged much earlier. Furthermore, the goodness-of-fit of all three models was almost constant over time, indicating that the posterior distributions of the parameters were robust as soon as the epidemic had peaked and the parameters were identifiable. After the peak, model 2 provided the best fit to the data. Model predictability was limited for both long-range and short-range forecasts.

The results in the other three case studies were similar to those of this case study and are documented in the final report of this study.

Discussion

Our results showed that reporting rates affected only the interpretation of model parameters but not the performance of parameter estimation or real-time epidemic forecasting (aside from increased stochasticity because of lower case counts). In all five case studies, the parameter values were largely not identifiable (ie Markov chain Monte Carlo did not converge) for models 1 and 2 until or even after the epidemic peak.

Reliable and precise real-time epidemic forecasting is improbable during the early phase of the epidemic and unlikely to be robust until the epidemic has peaked when using only epidemic curve data and any of the three models. Robust real-time epidemic forecasting, if possible at all, requires other sources of epidemic data, such as seroprevalence, household transmission, and phylogenetic data. Model predictability should be evaluated not only by computing simple error measures between actual and forecasted incidence but also by interpreting these measures in the context of the forecast's level of uncertainty (the wider the prediction interval, the less useful the forecast, but the more likely that the actual incidence falls within the prediction interval). Epidemiologists and public health policymakers should be aware of these drawbacks when using models for real-time epidemic forecasting.

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Decision-making process of families about human papillomavirus vaccination of adolescent daughters: a qualitative study of Hong Kong Chinese families

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KEY MESSAGES

1. Social influences significantly affect the decision-making process of parents and adolescent girls about human papillomavirus (HPV) vaccination.
2. Governmental involvement and recommendations from trusted healthcare professionals are important facilitators of decisions about HPV vaccination.
3. Doubts about the necessity, safety, efficacy, and particularly the high cost of vaccination are major barriers to HPV vaccination. Vaccination costs contribute to inequalities in women's health, especially among lower socioeconomic groups.
4. The government should subsidise a school-based HPV vaccination programme if high coverage is desired.
5. Future HPV vaccination programmes should focus on the necessity of early vaccination and provide unbiased information about safety and efficacy.

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Introduction

Human papillomavirus (HPV) vaccination became available in the private sector in 2006 in Hong Kong. Promotion of HPV vaccination occurs mainly through advertising from the pharmaceutical industry. By 2012, only about 7% to 9% of teenage girls had received HPV vaccination.[1,2] Barriers against vaccination include worry about potential side effects, doubts about effectiveness, and high cost of HPV vaccination; facilitators of vaccination are perceived high risk of cervical cancer and recommendations from healthcare professionals. Nonetheless, two recent studies have reported that HPV vaccination uptake is more likely in girls with advantageous sociodemographic characteristics, such as being locally born and having mothers educated to a tertiary level or above,[2] whereas the principal barrier against HPV vaccination uptake was the financial cost.[1] The present study aimed to identify the underlying barriers and facilitators about HPV vaccination of adolescent daughters in Chinese families.

Methods

Hong Kong Chinese women with at least one adolescent daughter aged 10 to 18 years who were aware of HPV vaccination, together with the daughter and her father, were invited to semi-structured interviews. Theoretical sampling was used for recruitment. All interviews were audio-taped, transcribed, and analysed using a grounded theory approach.

Results

A total of 51 respondents (35 mothers, 15 adolescent girls, and one father) from 35 families were interviewed between March and September 2013. Of the 35 families, five (14%) had daughters who had been vaccinated against HPV (Table).

Most participants reported that the mother took primary responsibility for childcare. Most parents whose daughters had not been vaccinated against HPV had had no formal discussions about vaccination, as there was no perceived and immediate need. Many mothers and daughters had never discussed HPV vaccination with fathers, perceiving that men were uninterested and ignorant about 'a female topic'. Mothers often reported that fathers felt embarrassed when these issues were considered, so the decisions tended to fall to mothers and daughters, especially when fathers were neutral on the question of vaccination. However, when fathers held strong negative opinions, mothers' opinions were influenced. Social factors significantly influenced most parents' and daughters' HPV vaccination decision making, along with balancing

TABLE. Participants' characteristics and attitudes about human papillomavirus vaccination

Characteristic	No. of participants*
Mothers (n=35)	
Mean (range) age, y	43.6 (33-52)
Marital status	
Married	27
Divorced/widowed	8
Education	
Tertiary	7
Secondary	26
Primary	2
Family income, HK$	
≤10 000	6
10 001-19 999	10
20 000-50 000	11
>50 001	8
Cervical cancer diagnosis	2
Daughter vaccinated	5
Daughter unvaccinated	30
Supportive (delaying vaccination)	16 (11)
Undecided/declining	14
Father (n=1)	
Age, y	59
Education	Secondary
Daughter unvaccinated	1
Supportive	1
Adolescent girls (n=15)	
Age, y	17.5 (10-18)
Education	
Tertiary	1
Secondary	10
Primary	4
Vaccinated	2
Unvaccinated	13
Willing to receive at current age	6
Wait until adulthood	5
Refuse	1
Unknown/not answer	1

* Data are presented as No. of participants unless otherwise stated

of the risks and benefits (Fig). Most parents who currently declined HPV vaccination stated that they would re-consider when their daughters grew up, by which time they felt that the vaccine will have been evaluated more thoroughly.

The factors influencing HPV vaccination decision making are as follows:

(1) General attitude towards and experience with vaccination. All parents ensured that their children had received government-mandated vaccines. These were important to most parents, who believed (correctly) that young children are highly susceptible to pathogens but (incorrectly) that they have weaker immunity (rather than lacking exposure). Some parents expressed reluctance towards optional vaccines, either owing to negative experiences (such as unpleasant adverse effects) or a preference for 'natural immunity'. A few mothers believed that having childhood diseases (such as chickenpox) helps to strengthen children's immune systems.

(2) Knowledge and perceptions about HPV and cervical cancer. Both parents and daughters reported not knowing much about cervical cancer or HPV. Many participants knew that the HPV vaccine can reduce the risk of cervical cancer but were unaware that the vaccine protects against HPV itself.

Almost all parents and some senior girls linked sexual activity with cervical cancer. Some mothers and girls attributed cervical cancer to promiscuity. Some mothers believed that heredity, unhealthy diet, stress, and environmental pollution also contributed to cervical cancer.

Many parents and girls considered cervical cancer as fatal for women. Many parents believed that young people are more susceptible to cervical cancer because they are more likely to have early and casual sex in addition to living in a polluted environment.

(3) Reasons for accepting HPV vaccination. For many parents and girls who accepted HPV vaccination, fear/worry about cervical cancer was a key motivating factor, although some girls did not know exactly what cervical cancer was:

> "*I am very scared of it, cervical cancer. In fact, I have no idea what it is, but it sounds scary.*" (girl 9)

Many mothers who accepted HPV vaccination reported that although they taught their daughters self-protection and hoped they would lead responsible lives, they could not predict or control their daughters' behaviour and life. They felt better protecting their children with vaccination, particularly when daughters approach rites of passage, such as graduation or overseas study:

> "*Anyway, they will go abroad for study. I cannot take care of them for such a long time, cannot keep 'talking' to them, so better to vaccinate them.*" (mother 33)

Despite few participants ever having received a recommendation for HPV vaccination from healthcare professionals, concrete advice, particularly from trusted doctors, was a key cue to

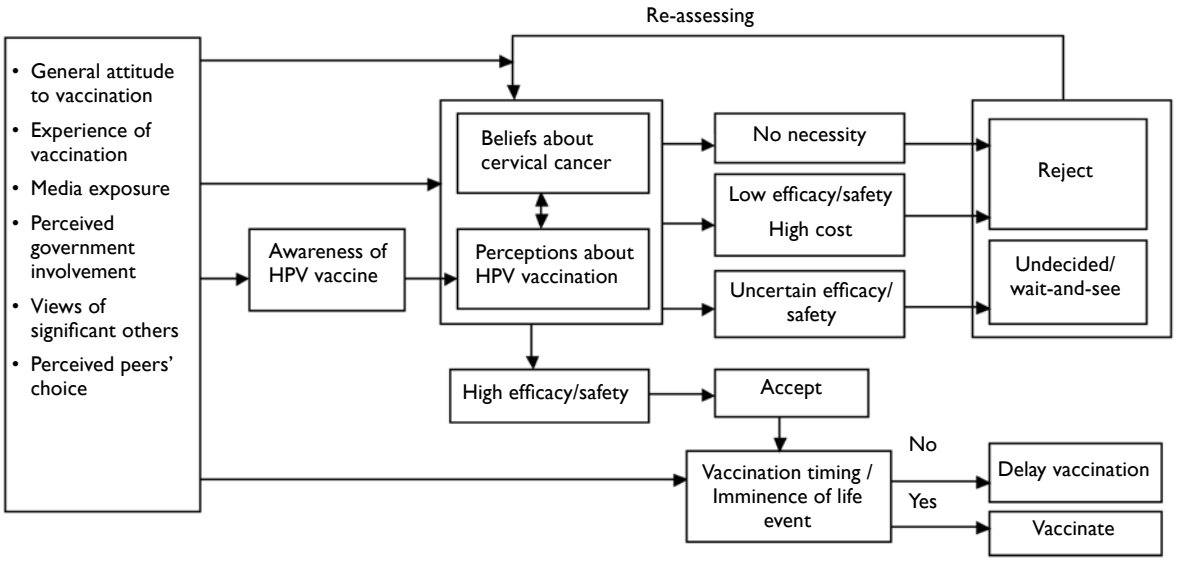

FIG. Parents' decision-making process about human papillomavirus (HPV) vaccination for adolescent girls

action for those who received it:

> "*In fact, I have no idea about this (vaccine), but I trust Dr X and her recommendation.*" (mother 2)

Some participants were confident about the safety and efficacy of HPV vaccination, as they (incorrectly) perceived that the vaccine was promoted by the government, which must have approved it before it was marketed:

> "*I never considered them (side effects), because I think it is promoted by the government… In terms of effectiveness, it must give some protection, so that's why the government vigorously promotes it.*" (mother 6)

Some participants reported that their friends had been vaccinated or had vaccinated daughters without experiencing any apparent side effects, and this reduced their concern about possible adverse effects of vaccination. If respondents perceived that a high proportion of their peers were getting vaccinated, then they felt more confident about their decision:

> "*In the beginning, (I) was slightly concerned about side effects. Recently, I heard from the radio that many people have been vaccinated. I feel nothing, feel assured.*" (mother 24)

(4) Reasons for declining/delaying HPV vaccination. Many parents perceived that the susceptibility to HPV infection was lower than that to other childhood diseases owing to the difference in transmission modes. This was particularly so in participants who attributed cervical cancer to promiscuity, who seemingly considered HPV vaccination as unnecessary. Few parents believed that using condoms could be effective as an alternative prevention approach. Religious faith was cited by some parents who declined the HPV vaccine:

> "*If she (a person) does not fear God or grow up with reverence, she will do something beyond the bottom line…So I don't see it as necessary to give her a sense of security.*" (mother 27)

Some parents felt that a new vaccine needed around 10 years of observation before people would have more confidence in it. However, despite its availability in Hong Kong for 8 years, many parents thought HPV vaccines had only been available for 2 to 3 years. Many parents who declined the HPV vaccine expressed concerns about vaccination safety, including potential unknown side effects, harm to physical development and fertility, and even impacts on subsequent generations:

> "*In the long term, do the children of people who are vaccinated have side effects from this vaccination?*" (mother 15)

Because the HPV vaccine primarily targets adult-onset cervical cancer, any immediate benefits are minimal and invisible. Some parents questioned the effectiveness of the vaccination and duration of protection:

> "*I read (a news article) that stated that currently, there is no medical evidence to support that it is definitely effective.*" (mother 12)

Most parents and some girls objected to the vaccine being recommended for girls aged ≥9 years as being unnecessarily early. Many parents saw no immediate need because their daughter was not likely to become sexually active anytime in the near future, usually based on their daughter's general

characteristics/personality:

> "*Because she is very conservative, she won't fool around, I know her personality. She is very good, well-behaved*." (mother 7)

Some parents also worried that their young, immature daughters were particularly fragile, vulnerable to any potential harms resulting from vaccination, and preferred to delay vaccination:

> "*Because she is young, it is very important, she cannot resist it (side effects)... when she is an adult, (her tolerance) will be better*." (mother 8)

Among mothers who declined the HPV vaccine, despite the lack of communication or discussion within family or with peers, any disapproval from other significant family members or friends, particularly their husbands, significantly hampered their decision making about the HPV vaccination:

> "*He (the father) said, 'Don't do something for nothing.' He feels it has risks*." (mother 23)

Most parents and girls interviewed had never received HPV vaccination recommendations from healthcare professionals. Several mothers reported that despite initiating consultations with family doctors, they failed to receive concrete advice:

> "*Actually, I have asked my doctor, he didn't give me a very concrete answer.... His response, 'Taking it doesn't matter, and I am not sure whether it will be really helpful,' is neutral*." (mother 22)

Some parents saw it as uneconomical or discretionary consumption and declined it. Some parents in favour of the vaccine ultimately decided to delay vaccinating their daughters because the present cost was unaffordable:

> "*I want to wait until my daughter makes some money and then vaccinate her, we cannot afford it now*." (mother 10)

Discussion

Financial cost was a major barrier against HPV vaccination, particularly for disadvantaged families. Fear of cervical cancer was a key factor motivating vaccination. This is consistent with the utility model, which proposes that the primary motivating factor to adopt preventive behaviour is to resolve threat-associated anxiety, rather than the threat itself.[3] Some mothers and girls attributed cervical cancer to promiscuity and believed that monogamy or condom use was the best protection. Healthcare providers should emphasise the prevalence and transmission of HPV infection, as a monogamous woman may also face the risk of HPV infection, and condoms provide limited protection from HPV prevention.

The decision-making process among families about HPV vaccination reflects social and formal professional influences along with risk-benefit assessments under conditions of uncertainty. Lay responses often revert to reliance on heuristics, primarily 'imitate the majority'.[4] Many parents adopted a wait-and-see approach, which may significantly impair the value of prophylactic vaccination. Our findings are consistent with those reported about local families[1,2] and new immigrant families.[5]

There are reasons to implement a school-based programme for vaccination against HPV for all Hong Kong girls. There are disparities in access to health resources based on socio-economic status. These disparities are unjustifiable, as they contribute to avoidable later life inequalities in women's health. Meanwhile, a programme to educate primary care doctors should be initiated to improve parents' knowledge about HPV vaccination. However, this is unlikely to be an effective strategy for new immigrant and low-income families who remain suspicious about the fiscal motives of private clinicians.[5]

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Mothers' preference and willingness to pay for human papillomavirus vaccination for their daughters: a discrete choice experiment

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KEY MESSAGES

1. Concern about adverse effects is the most important barrier to human papillomavirus (HPV) vaccination.
2. Vaccination strategies should focus on knowledge exchange and education on the safety and benefits versus risk to improve vaccination uptake.
3. Demand for HPV vaccines is high as indicated by the maximum willingness to pay. However, the willingness to pay for current vaccines is lower than the current market price.
4. Subsidy or co-payment from the government should be considered to meet demand for HPV vaccination.

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Introduction

In Hong Kong, cervical cancer is the seventh commonest cancer among females and accounts for about 3.6% of all new cancer cases. To reduce the disease burden, a cervical cancer screening programme was launched in 2004 and two vaccines were introduced in 2006. However, HPV vaccination is not included in the government immunisation schedule and is largely taken up in private clinics opportunistically. The uptake rate of HPV vaccination among adolescent girls in Hong Kong is low (2.1%-9.1%).[1,2]

The success of a HPV vaccination programme largely depends on the local stakeholders' attitude towards risks and benefits of vaccination. Our study used a discrete choice experiment to elicit Hong Kong mothers' preference and willingness to pay (WTP) for HPV vaccination for their daughters.

Methods

A cross-sectional survey of mothers with daughters aged 8 to 17 years was conducted at the paediatric specialist outpatient clinics of two public hospitals using a stratified sampling approach. A discrete choice experiment was used to elicit the preferences and WTP of mothers.

The survey was delivered through Survey Monkey (Palo Alto, California, USA) using a portable device. Sociodemographic data, health and vaccine experiences, and the discrete choice experiment responses were recorded. Trained research assistants screened the eligibility of participants, and written consent was obtained. The discrete choice experiment choices were analysed using a multinomial logistic regression model. Regression coefficients (with 95% confidence intervals) and WTP were reported. The marginal WTP for one unit change in each attribute was calculated by equation 1:

$$\text{Marginal WTP} = \frac{\text{Preference Weight}_{\text{attribute}} \times \Delta\text{Level}_{\text{attribute}}}{\text{Preference Weight}_{\text{out-of-pocket cost}}} \quad (1)$$

Total WTP for vaccines was calculated by equation 2:

$$\text{Total WTP} = \text{Marginal WTP}_{\text{protection}} + \text{Marginal WTP}_{\text{protection}} + \text{Marginal WTP}_{\text{side effects}} \quad (2)$$

All statistical analyses were conducted using Statistical Analysis System version 9.3.

Results

A total of 482 mothers (mean age, 42.9 years) were interviewed with a response rate of 79.1% (Table 1). About 58.5% of mothers were born in Hong Kong and about 97.4% had secondary education or above. About 45.5% of mothers had monthly household income more than HK$30 000. More than 75% of mothers had heard about HPV vaccines and were concerned about their daughters' risk of HPV infection and cervical cancer. However, approximately 55.4% of mothers believed vaccines

are somewhat unsafe or very unsafe and some refused vaccine for their daughter. Approximately 96.7% of mothers reported either sex education or abstinence should be taught at school, which was a proxy for conservative values.

In the validity test of scenario 1, 99.4% of mothers made a reasonable choice with higher protection effectiveness, longer protection duration, and lower probability of adverse effects. In the actual discrete choice experiment of scenarios 2 to 9, 58.3% to 86.3% of mothers chose one of the two vaccines after considering the trade-off among health benefit, risk, and cost in each scenario. Table 2 shows the mothers' preferences estimated from the statistical model. All attributes had a significant impact on the utility (P<0.001). The most important attribute was adverse effects, followed by protection against cervical cancer and protection duration.

The marginal WTP for each attribute and the overall WTP for vaccine are shown in Table 3. Vaccine effectiveness, defined as cervical cancer protection rate, was highly valued with the largest marginal WTP of HK$5431. Mothers were willing to pay similarly for lifetime protection duration (HK$3545) and the greatest adverse effect reduction from 14 to 0 in 100 mothers (HK$3550). Maximum WTP for ideal technology with the best of all features was HK$12 526. The WTP for current vaccines is approximately HK$1700, which is relatively lower than the current market price (HK$2100-4000).

TABLE 1. Characteristics of respondents

Characteristics	Total (n=482)*	Princess Margaret Hospital (n=181)*	Queen Mary Hospital (n=301)*
Mean±standard deviation mother age, y	42.9±5.5	41.4±5.6	43.8±5.2
Place of Birth			
Hong Kong	282 (58.5)	95 (52.5)	187 (62.1)
Mainland China	174 (36.1)	72 (39.8)	102 (33.9)
Others	26 (5.4)	14 (7.7%)	12 (4.0)
Education			
Primary or below	27 (5.6)	15 (8.3)	12 (4.0)
Secondary	322 (66.9)	136 (75.1)	186 (61.8)
Tertiary or above	133 (27.5)	30 (16.6)	103 (34.3)
Monthly household Income			
<HK$10 000	33 (6.85)	21 (12.5)	12 (4.0)
HK$10 000-20 000	130 (27.0)	66 (36.5)	64 (21.3)
HK$20 001-30 000	84 (17.4)	28 (15.5)	56 (18.6)
HK$30 001-50 000	103 (21.4)	29 (16.2)	74 (24.6)
HK$50 001-100 000	89 (18.5)	22 (12.2)	67 (26.3)
>HK$100 000	27 (5.6)	4 (2.2)	23 (7.6)
Retired	6 (1.2)	4 (2.2)	2 (0.7)
Unemployed	10 (2.8)	7 (3.9)	3 (1.0)
Has heard of HPV vaccines before	385 (79.9)	151 (83.4)	234 (77.7)
Familiar with HPV	107 (22.2)	42 (23.2)	65 (21.6)
Familiar with cervical cancer	312 (64.7)	113 (62.4)	199 (66.1)
Knows a child/teenager who has had HPV vaccination	94 (19.5)	28 (15.5)	66 (21.9)
Personal history of HPV vaccination	23 (4.8)	7 (3.9)	16 (5.3)
Personal history of HPV	12 (2.5)	5 (2.8)	7 (2.3)
Personal history of cervical cancer	5 (1.0)	2 (1.1)	3 (1.0)
Personal history of other cancer	15 (3.1)	4 (2.2)	11 (3.7)
Personal history of abnormal Pap smear test result	26 (5.4)	9 (5.0)	17 (5.7)
Daughter has had Pap smear test	8 (1.7)	1 (0.6)	7 (2.3)
Has concerns about daughter's risk of HPV	363 (75.3)	135 (74.6)	228 (75.8)
Has concerns about daughter's risk of cervical cancer	370 (76.8)	142 (78.5)	228 (75.8)
Believes daughter not at risk of HPV because not sexually active	466 (96.7)	175 (96.7)	291 (96.7)
Refused vaccine for daughter	32 (6.6)	7 (3.9)	25 (8.3)
Believes vaccines are somewhat unsafe or very unsafe	267 (55.4)	98 (54.0)	169 (56.2)
Believes either sex education or abstinence should be taught at school	466 (96.7)	174 (96.1)	292 (97.0)

Abbreviation: HPV= human papillomavirus

* Data are presented as No. (%) of respondents unless otherwise stated

Discussion

Cervical cancer is one of the deadliest and yet most preventable cancers. Disease burden of cervical cancer is relatively higher in Hong Kong than other developed areas, as there is no universal screening or vaccination programme in Hong Kong. In Hong Kong, HPV vaccination among teenage girls is largely opportunistic and the uptake rate is low.[1,2] Understanding the determinants of HPV vaccine uptake is crucial for designing more effective vaccine-promotion programmes and for re-evaluating immunisation policies. In our study, significant determinants of the HPV vaccine uptake were cervical cancer protection effectiveness, protection duration, adverse effects, and out-of-pocket costs.

In contrast to the commonly recognised attribute of protection effectiveness in the US,[3] Vietnam,[4] and Thailand,[5] Hong Kong mothers weighted adverse effects as a priority when making choices between vaccines. This may be explained by differences in culture, ethnicity, and education levels. For example, our sample appears to be more conservative on sexual health issues (abstinence should be taught in schools: 96.7% vs 21.6%) and less–well educated (tertiary education or above: 27.5% vs 39.7%), compared with US mothers.[3] Further, 55.4% of Hong Kong mothers believed that vaccines are somewhat unsafe or very unsafe, compared with only 9.8% of US mothers. This suggests that safety concern is the main barrier to vaccination uptake in Hong Kong. Education should

TABLE 2. Coefficients estimates for attribute main effects using multinomial logistic regression

Attribute	Preference weights	Standard error (95% confidence interval)	P value
Protection against cervical cancer	0.01633	0.0007514 (0.01486 to 0.0178)	<0.0001
Protection duration	0.01066	0.0005 (0.00968 to 0.01164)	<0.0001
Adverse effects	-0.07626	0.00487 (-0.0858 to -0.0667)	<0.0001
Out-of-pocket cost	-0.0003007	0.0000207 (-0.0003 to -0.0003)	<0.0001

TABLE 3. Willingness to pay for the attributes of human papillomavirus vaccination

Attributes	Marginal willingness to pay (HK$)
Protection against cervical cancer, %	
0-100	5430.66
0-80	4344.53
0-70	3801.46
0-50	2715.33
Protection duration, y	
0 to lifetime	3545.06
0 to 10	354.51
0 to 5	177.25
0 to 2	70.90
Adverse effects, %	
14 to 0 in 100	3550.52
10 to 0 in 100	2536.08
6 to 0 in 100	1521.65
2 to 0 in 100	507.22
Maximum willingness to pay*	12 526.24
Willingness to pay for current vaccine†	1619.89

* Calculated by incorporating 100% protection, lifetime protection (100 years), and 0% risk of adverse effects

† By incorporating 70% protection against cervical cancer, 10-year protection duration, and 10% risk of adverse effects

focus on the safety of vaccines, and communication between mothers and providers on the benefits and risks of HPV vaccination should be encouraged as part of the health education programme. About 79.9% of mothers had heard of HPV vaccines, and the demand and perceived health benefits of HPV vaccines or risks of HPV were high, as indicated by the maximum WTP. This might reflect the fear of cancer and the efforts of health education and advertisement for cervical cancer prevention. Nonetheless, the overall WTP for current vaccines was lower than the market price for current vaccines. Subsidy or co-payment from the government should be considered to meet demand for HPV vaccination.

Our study has limitations. All decisions were made on hypothetical scenarios. Our choice sets considered a limited number of attributes from the literature and a pilot study. Other attributes, especially for the protection against genital warts, may also reflect the preference. The sample was from two public hospitals; response bias from the convenience sampling method cannot be avoided, and the generalisability of the findings to entire Hong Kong population needs to be interpreted cautiously.

Conclusion

The perceived health benefits of HPV vaccines were high among Hong Kong mothers. They weighted concern about adverse effects as the most important attribute when considering HPV vaccination for their daughters. Their WTP was lower than the current market price of HPV vaccination and was diverse among different socio-economic groups.

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Multilevel antimicrobial disinfectant coating in reducing the viability of multidrug-resistant organisms in hospital environment

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KEY MESSAGES

1. Proper training of cleaning personnel and rigorous oversight and strict enforcement of cleaning routine can improve overall cleanliness and decrease bacterial load in the ward environment.
2. Multilevel antimicrobial disinfectant coating achieves consistently low bacterial load in the ward environment independent of the cleaning regimen.
3. The number of multidrug-resistant organism (MDRO)-positive environmental samples between two cleaning regimens does not differ significantly owing to rapid surface reacquisition of MDROs.
4. The occurrence of MDROs in the environmental samples is lower on surfaces coated with the multilevel antimicrobial disinfectant coating regardless of the cleaning regimen.

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Introduction

The increasing occurrence of multidrug-resistant organisms (MDROs) in hospitals is of great concern. Infections caused by MDROs can result in longer hospitalisation, increased treatment cost, and higher mortality. MDROs can survive for a prolonged period on hospital furnishings and medical items. MDROs in hospital environments have been associated with an increased risk of transmission and infection.[1] Reducing environmental contamination through improved cleaning practices reduces patient acquisition of pathogens. Regular cleaning and disinfection is important for breaking the chain of infection. Nonetheless, MDROs can still be found in frequently touched areas in hospitals. Antimicrobial coatings in these areas might reduce MDRO infection rates.

Numerous antimicrobial systems have been reported, including biocidal nanomaterials such as nanosilvers, light-activated photocatalysts based on TiO_2, surface-tethered bactericides such as quaternary ammonium compounds, and phosphonium salts. These materials impart 'contact-killing' bactericidal properties and are effective against a wide spectrum of microorganisms. Ultrahydrophobic coatings and bacteria-repelling poly(ethylene glycols) discourage bacterial adhesion and growth on surfaces. Another strategy is to store antibiotic drugs, chemical biocides, and bactericidal metal ions in bulk and coating materials for gradual release and sustained 'release-killing' bactericidal activity. Contact-killing and anti-adhesion bactericidal materials are susceptible to surface fouling that can drastically diminish their effectiveness, whereas release-killing bactericides depend on water-bridge for the transport of active compounds. Combinations of one or more of these approaches are necessary to overcome these deficiencies.

Our research team at the Hong Kong University of Science and Technology developed a multilevel antimicrobial disinfectant coating that synergistically combines 'release-killing', 'contact-killing' and 'anti-adhesion' properties to enable long-lasting disinfection of surfaces. The coating has been reported to be responsive to contamination and rapidly inactivate a panel of Gram-positive and Gram-negative bacteria (>99.99%) within 1 minute of contact with the coated surface.[2] The coating is safe and does not cause skin irritation or affect the lung function in mice. The coating can be applied by spray or wipe on variety of surfaces without altering the visual, tactile, or olfactory property of the coated surface. This study aimed to investigate the use of the multilevel antimicrobial disinfectant coating in reducing the viability of MDROs including Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and multidrug-resistant *Acinetobacter* (MDRA) in hospital ward environment.

Methods

Four orthopaedic wards at Queen Elizabeth Hospital participated during July 2013 to January 2015 in three stages: stage 1 (July 2013 to November 2013), washout stage (December 2013 to June 2014), and stage 2 (July 2014 to January 2015). In stage 1, wards A and B were planned as the treatment group and wards C and D as the control group. The study was suspended by the occurrence of a H7N9 case in Hong Kong until late May 2014. In stage 2, wards C and D were the treatment group and wards A and B the control group. A total of 2249 environmental samples were taken from stage 1 (n=966) and stage 2 (n=1283).

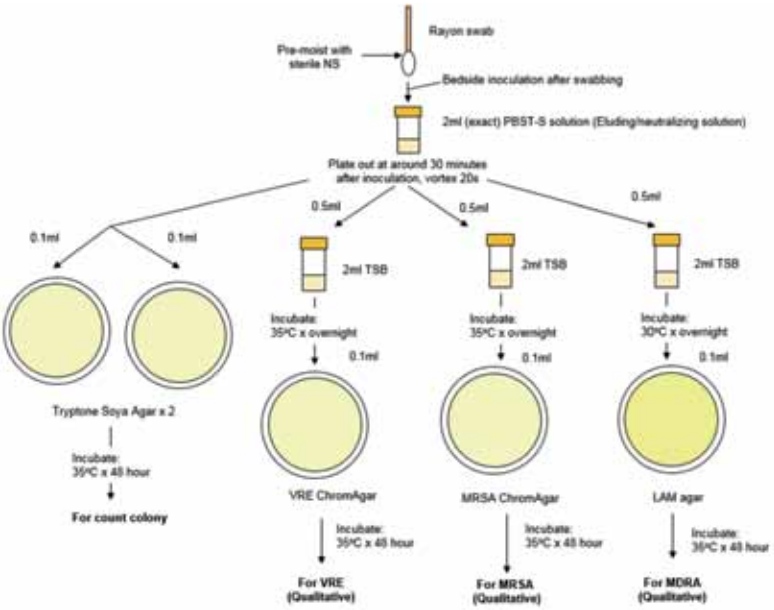

FIG 1. Workflow for assessment of bacteria and multidrug-resistant organisms in hospital environmental samples

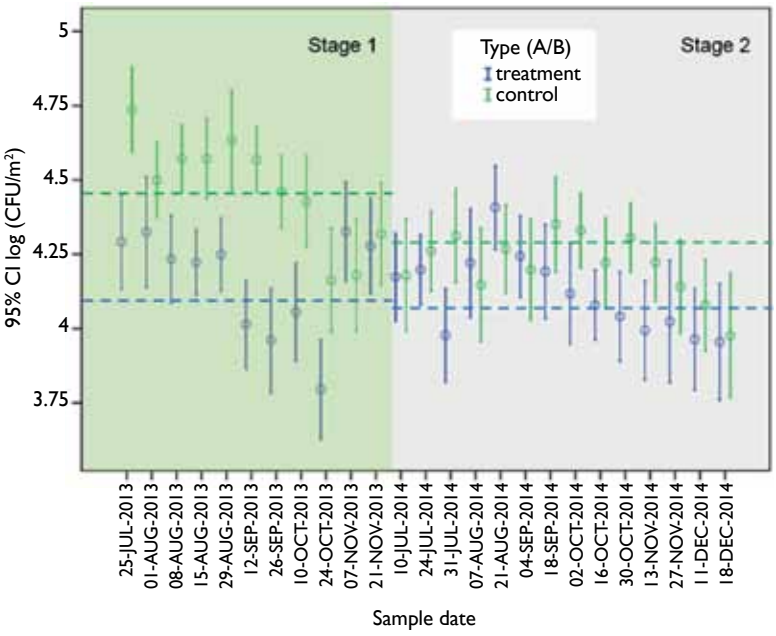

FIG 2. Viable bacteria found on select hospital surfaces in log colony forming unit (CFU)/m² over time

Daily cleaning routine in the wards was performed at 10:00 am from patients' outer surrounding to the inner zone by in-house cleaning workers using 1000 ppm sodium hypochlorite solution. Gloves and masks were worn. 10 000 ppm sodium chlorite solution was used for blood and bodily fluid spillage. Beds that were identified to contain MDRO carriers were cleaned twice a day. In stage 2, cleaning was performed by an outsource cleaning company from 9:15 am to 3:00 pm using the same protocol.

Every Tuesday, 25 beds were selected, and three items from each bed were coated, including the top surface of the hospital chest table, the top surface of the bedside cabinet, and right and left bed rails. All beds identified with MDRO carriers were included, and the remainder was selected randomly. The multilevel antimicrobial disinfectant coating was used in the treatment group, and a placebo coating that contained mainly water, dye, and dilute (0.06%) sodium hypochlorite was used in the control group.

Environmental samples were collected on Thursday every other week before the daily cleaning. Swab samples were taken from three items (225 cm^2 of the hospital chest table, 225 cm^2 of the bedside cabinet, and 225 cm^2 of the bed rails). The sterile rayon swab was moistened with 0.9 wt % sodium chloride. After sampling, the tip of the swab was severed and submerged in 2.5 mL neutralising solution for storage (0.001 M sodium thiosulphate, 0.2% Tween 80, 0.9% w/v sodium chloride) and processed within 2 hours.

To assess environmental bacteria and MDRO, the swab was vortexed to recover trapped bacteria and 100 μL of the solution was transferred to a TSA plate (Fig 1). Duplicate plates were made for consistency. The colony forming unit (CFU) on Müller-Hinton Agar represented the total aerobic bacteria count in the sample. Plates without any CFU were given a 0.5 CFU count based on the minimum detection limit of the method. Plates with considerable difference in plate counts and with evident contamination were discarded. A culture sample was prepared by transferring 0.5 mL of the remaining sample to 2-mL sterile TSB solution and incubating at 35°C. A second culture sample was prepared in similar manner but incubated at 30°C. The amplified cultures were tested for the presence of MRSA, MDRA and VRE.

Results and discussion

Environmental bacteria found during stage 1 and stage 2 are summarised in Figure 2. Blind test and use of placebo minimises bias. Comparing stage 1 and stage 2, the change in cleaning personnel and management system and stricter observance of cleaning routine had an effect on controlling microbial contamination of surfaces. The use of the multilevel antimicrobial disinfectant coating achieved consistently low viability of bacteria on treated surface. An improvement of 36.1% was observed in the treatment group, indicating that the multilevel antimicrobial disinfectant coating was more effective than cleaning alone in maintaining low bacterial load in hospital environment.

Environmental MDROs including MRSA, VRE, and MDRA were present in hospital environment. The environmental samples were sub-cultured and the amplified samples were tested for the presence of MRSA, VRE, and MDRA. This method only indicated the presence or absence of MDRO instead of the quantity of the MDROs in the sample. Of the 2249 environmental samples, 14.2% were positive for MRSA, 1.3% positive for VRE, and 2.4% positive for MDRA. Ward patients were actively screened for MRSA, VRE, and MDRA from wound, blood, and urine. The preferential inclusion of the beds of MDRO carriers and the unequal distribution of MDRO carriers among the wards mean that the ANOVA method is not strictly valid. In addition, a more vigorous cleaning routine was used for the beds of MDRO carriers. Indeed, 73.5% of samples from the beds of MRSA carriers tested negative for MRSA, and approximately 66.8% of samples containing MDROs were found from beds of non-MDRO carriers.

The MDRO dataset was categorised into 0 (negative from both environmental sample and patient bed), 1 (positive from environmental sample and negative from patient bed), 2 (negative from environmental sample and positive from patient bed), 3 (positive from both environmental sample and patient bed). Therefore, MDRO in the environment over the sampling period can be normalised as (1+*[3]*)/n, whereas MDRO carriers can be accounted by (*[2]*+*[3]*)/sampling. Thus,

$$\frac{(1+[3])/n}{([2]+[3])/\text{sampling}} = \text{MDRO (environment)} : \text{MDRO (patient)}$$

The above equation takes into account the inequality in the distribution of MDRO carriers in the wards; a decrease in the value indicates a decrease in environmental MDRO isolates in the ward. Although the bacterial load on hospital surfaces was greatly reduced during stage 2, the effect was minimal on MDRO occurrence in the environmental samples (Table). This may be due to the reacquisition of MDROs between cleaning. In contrast, the multilevel antimicrobial disinfectant coating provided sustained surface disinfection and decreased the incidence of MDROs in the environmental samples. Thus, the use of multilevel antimicrobial disinfectant coating along with strict observance of cleaning routine and hand hygiene can be effective in reducing environmental occurrence of MDROs in hospital environment.

TABLE. Ratio of environmental multidrug-resistant organisms (MDRO) to number of patients with MDRO

Parameter	Ratio of environmental MDRO to number of patients with MDRO
Change in cleaning routine	
Stage 1 (control)	0.099
Stage 2 (control)	0.095
Impact of coating	
Overall (control)	0.097
Overall (treatment)	0.088

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Development of an automated 16S rRNA gene sequence database, 16SpathDB, for identification of medically important bacteria

JLL Teng *, PCY Woo, SKP Lau

KEY MESSAGES

1. We developed 16SpathDB, a database for identification of medically important bacteria.
2. 16SpathDB offers efficient and accurate analysis of 16S rRNA gene sequences of medically important bacteria.
3. The updated 16SpathDB version 2.0 is available at http://www.microbiology.hku.hk/16SpathDB and is updated periodically for every new edition of the *Manual of Clinical Microbiology*.

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Introduction

Clinical microbiology and research laboratories regularly receive clinical isolates that require accurate bacterial identification and differentiation, particularly for medically important bacteria. Traditional identification via phenotypic characteristics is time-consuming, and bacteria isolates often cannot be fully characterised owing to poorly defined phenotypes and subjective bias. In the past 10 years, laboratories have relied on 16S rRNA gene sequencing as an identification tool. High-throughput analysis of 16S rRNA sequencing can be performed using a wide range of databases, including GenBank,[1] Ribosomal Database Project,[2] MicroSeq,[3] Ribosomal Differentiation of Medical Microorganisms,[4] and SmartGene Integrated Database Network System.[5] The database sequences of Ribosomal Database Project and SmartGene Integrated Database Network System are derived from GenBank, whereas the database sequences of Ribosomal Differentiation of Medical Microorganisms and MicroSeq are based on the sequencing of 16S rRNA genes of bacterial culture strains. Each database produces a list of top hits according to its own algorithm, and the 'first hit' or 'closest match' can be interpreted as the identity of the bacterial isolate. Nonetheless, accurate interpretation of 16S rRNA gene sequencing results is difficult for inexperienced users. For example, if the database does not contain the bacterial species of interest, the real identity of the isolate cannot be determined. If there is minimal difference between the 16S rRNA gene sequences of multiple bacterial species, it may be difficult to differentiate the identity of the isolate based on the list of top hits. Hence, we aimed to develop a database that allows determination of the most likely identity of medically important bacteria using 16S rRNA gene sequencing. An automated user-friendly platform was generated, and the accuracy of the database in identification of bacteria was evaluated.

Methods

16SpathDB is a web-based database that uses 16S rRNA gene sequences for identification of medically important bacteria. The most representative 16S rRNA gene sequence of each medically important bacterial species listed in the 9th edition of *Manual of Clinical Microbiology* was retrieved from GenBank. Strain sequences with the following criteria were preferred: good phenotypic and/or genotypic characterization, isolated from humans, few undetermined bases, and longer sequence length. More than one 16S rRNA gene sequence was included for bacterial species that had >2% intragenomic difference in their 16S rRNA gene sequences or intervening sequences in their 16S rRNA genes.

To evaluate the usefulness of 16SpathDB, 16S rRNA gene sequences of 250 medically important bacterial isolates, including those of 82 aerobic Gram-positive bacteria (staphylococci, streptococci, enterococci, mycobacteria, corynebacteria, nocardia, and members of *Bacillus*), 82 aerobic Gram-negative bacteria (*Bartonella*, *Bordetella*, *Burkholderia*, *Neisseria*, *Desulfovibrio*, *Campylobacter* and *Helicobacter* species and members of *Aeromonadaceae*, *Enterobacteriaceae*, *Legionellaceae*, *Pasteurellaceae*, *Moraxellaceae*, *Pseudomonadaceae*, and *Vibrionaceae*), 85 anaerobic bacteria (*Actinomyces*, *Clostridium*, *Bacteroides*, *Porphyromonas*, and *Prevotella* species), and one

Mycoplasma hominis that had been archived in our clinical microbiology laboratory in the past 10 years were input to the database for analysis. The exact identities of these isolates were determined by a polyphasic approach using a combination of phenotypic tests and 16S rRNA gene sequencing.

Results

16SpathDB includes 1014 16S rRNA gene sequences from 1010 unique bacterial species. The database interface is clear and simple to use. One can enter the 'query page' by clicking the 'Identify bacteria by 16S rRNA gene sequence' hyperlink. Users can then submit their query by inputting one or more queries for 16S rRNA gene sequences in the textbox or by uploading a file that contains the sequences via the 'Browse' button. Next, after clicking the 'Begin identification' button, the percentage nucleotide identity calculated from the alignment between the query sequence and each of the sequences in 16SpathDB is then displayed on the 'query results' page and can be used to determine the identity of the query sequence. The length of the input sequence affects the sequence percentage identity for species identification. In general, the 5' end of the 16S rRNA gene is more variable than the other parts of the gene and is thus preferred. An example data file is also provided to allow users to familiarise themselves with the system.

The following algorithm was used to report results generated by 16SpathDB. If there is one species in 16SpathDB with >98.0% nucleotide identity with the query sequence, this bacterial species, as well as the percentage nucleotide identity between the query sequence and the sequence of the most likely bacterial species, is reported (category 1). If there is more than one species in 16SpathDB with >98.0% nucleotide identity with the query sequence, the species that shares the highest nucleotide identity with the query sequence ('best match in 16SpathDB') as well as those with 16S rRNA gene sequences having <1% difference with the 'best match in 16SpathDB' are reported, and the user is alerted that further tests, such as biochemical tests or sequencing additional genes, may be necessary for differentiation between the most probable identities (category 2). If there are no species in 16SpathDB with >98.0% nucleotide identity with the query sequence, but there is one or more species in 16SpathDB with >96.0% nucleotide identity with the query sequence, only the genus is reported (category 3). The user is also reminded that further tests are necessary for definite species identification. If there are no species in 16SpathDB with >96.0% nucleotide identity with the query sequence, the results page would state, "No species in 16SpathDB was found to share high nucleotide identity with your query sequence" (category 4). This indicates that the query sequence may represent a bacterial species not included in the *Manual of Clinical Microbiology* or a novel bacterial species. When this occurs, users are advised to perform a BLAST search against the GenBank nr database to differentiate between the two possibilities.

Following the analysis of the submitted 16S rRNA gene sequences, users can inspect the detailed contents of the database as well as the information of individual sequences. This can be performed by clicking the 'Browse bacterial 16S rRNA gene information' hyperlink to enter the 'sequence information' page.

In 16SpathDB, among the 250 medically important bacterial isolates tested, 140 (56%) were reported as a single bacterial species having >98.0% nucleotide identity with the query sequence (category 1), 109 (43.6%) as more than one bacterial species having >98.0% nucleotide identity with the query sequence (category 2), none as genus level matches (category 3), and one (0.4%) as "No species in 16SpathDB was found to share high nucleotide identity with your query sequence" (category 4). For the 140 bacterial isolates reported as a single bacterial species, all results were identical to the true identities of the isolates as determined by the polyphasic approach. For the 109 bacterial isolates reported as more than one bacterial species, all results contained the true identities of the isolates as determined by the polyphasic approach.

Discussion

We developed 16SpathDB for identification of medically important bacteria using 16S rRNA gene sequencing. The platform has a simple user-friendly interface. One advantage of 16SpathDB is that it includes only the 16S rRNA gene sequences of medically important bacteria listed in the *Manual of Clinical Microbiology*, which contains nearly all bacterial strains ever recovered from patients. In contrast, other databases, such as the Ribosomal Database Project and SmartGene Integrated Database Network System, include 16S rRNA gene sequences from other bacterial species that have never been isolated from patients. In the clinical setting, inclusion of non-medically important bacteria could potentially hinder accurate differentiation and identification of clinical isolates. 16SpathDB is also superior to MicroSeq, which does not include an adequate number of medically important bacteria for proper isolate identification via 16S rRNA gene sequencing. Moreover, MicroSeq generates only a single 'identity' result of the query sequence while disregarding other bacterial species with similar 16S rRNA gene sequences. In contrast, 16SpathDB reports the species that shows the highest nucleotide identity to the query sequence (ie, 'best match') as well as those with <1% difference

from the 'best match' to alert the user that further tests may be required to differentiate between the probable isolate identities.

16SpathDB is accurate for identification of the 16S rRNA gene sequences of medically important bacteria. 16SpathDB successfully identified all 250 bacterial isolates archived in our clinical microbiology laboratory. Among these 250 bacterial isolates, 43.6% showed multiple possible identities, which reflected an inherent limitation of using 16S rRNA gene sequencing for bacterial identification. Phenotypic tests or sequencing of additional gene loci should be performed to differentiate among the reported bacterial species. It should be noted that this bacterial collection is from a single laboratory in Hong Kong, and thus, complete assessment of the database is not provided.

One limitation of 16SpathDB is that it includes only the sequences of bacterial species listed in the *Manual of Clinical Microbiology*, but in the clinical setting, the exclusion of bacterial species that have never been reported to cause infection is beneficial to data interpretation, as it would markedly minimise the results that have multiple possible identities. The 16S rRNA gene sequence of one isolate was reported as "No species in 16SpathDB was found to share high nucleotide identity with your query sequence". This is because *Gordonibacter pamelaeae* has never been reported to be associated with human disease and is not included in the *Manual of Clinical Microbiology*. Users should perform a BLAST search against the GenBank nr database to identify such 16S rRNA gene sequences. Users should also bear in mind that bacterial species that have never been reported to be associated with infection may still have the potential to do so.

An updated version, 16SpathDB 2.0, is available at http://www.microbiology.hku.hk/16SpathDB. It is updated periodically for every new edition of the *Manual of Clinical Microbiology*.

Acknowledgement

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Sonodynamic bactericidal activity of curcumin against foodborne bacteria

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KEY MESSAGES

1. Sonodynamic treatment with curcumin could eradicate *Bacillus cereus* and *Escherichia coli*, with *Bacillus cereus* being more sensitive to treatment.
2. The production of reactive oxygen species, including singlet oxygen and hydroxyl radical, increased significantly after sonodynamic treatment with curcumin.

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Introduction

Treatments for foodborne disease do not reduce levels of foodborne pathogens in environmentally contaminated sources, and most disinfectants have potential genotoxicity and/or carcinogenicity.[1] There is a need to develop alternative strategies to eradicate foodborne pathogens. This study aimed to evaluate the sonodynamically bactericidal efficacy of curcumin on foodborne bacteria such as *Bacillus cereus* and *Escherichia coli* and the reactive oxygen species (ROS) production of foodborne bacteria after sonodynamic treatment using flow cytometric analysis.

Methods

This study was conducted from 1 February 2013 to 31 March 2014. Non-pathogenic strains of Gram-positive *Bacillus cereus* 14579 and Gram-negative *Escherichia coli* 35218 were used. To evaluate the bactericidal effect of curcumin-mediated sonodynamic treatment, treated bacterial suspensions were spread on Mueller-Hinton agar and incubated for 24 hours. The growth of treated bacteria was indicated in terms of colony-forming units per mL ($\log_{10}$). The bactericidal effect of the treatment in anaerobic conditions was assessed in terms of production of ROS measured by flow cytometry with 2',7'-dichlorodihydrofluorescein diacetate staining. The specific detective probes and quenchers of free radicals and singlet oxygen were used to determine the types of ROS produced. The ultrasound exposure system was used as described in our previous study.[2]

Results

Sonodynamic treatment with curcumin had significant bactericidal activity, with reduction in colony-forming units for *Bacillus cereus* and *Escherichia coli* by 5.6 and 2.0 log units, respectively (Fig 1). Bacterial survival was higher without oxygen than with oxygen (Fig 2). Compared with the control condition, sonodynamic treatment with curcumin significantly increased production of ROS, singlet oxygen, and hydroxyl radicals, but bactericidal efficacy was significantly reduced by all three kinds of quenchers (Fig 3).

Conclusions

Sonodynamic treatment with curcumin significantly inactivated foodborne bacteria, especially Gram-positive *Bacillus cereus*. Significant production of ROS, including singlet oxygen and hydroxyl radicals, may result in important bactericidal activity.

Acknowledgements

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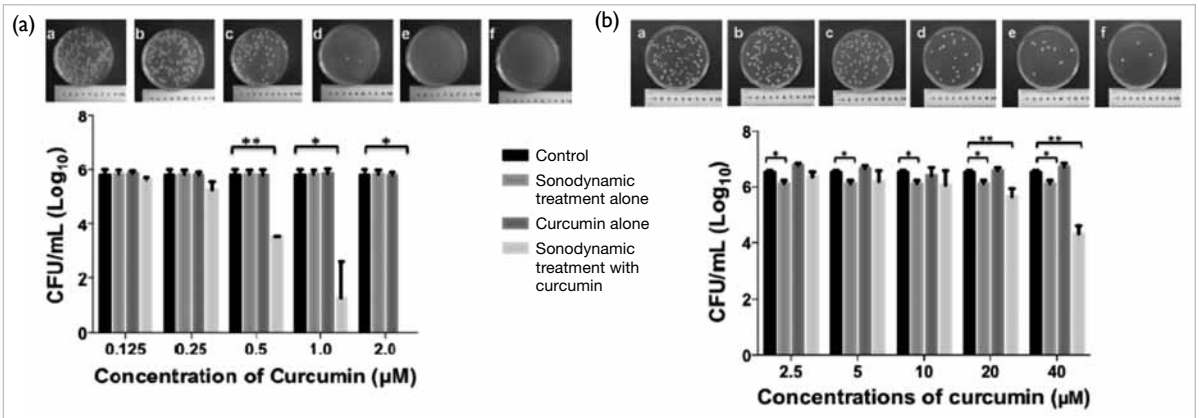

FIG 1. Colony-forming units (CFU) per mL after sonodynamic treatment with curcumin against (a) *Bacillus cereus* and (b) *Escherichia coli*

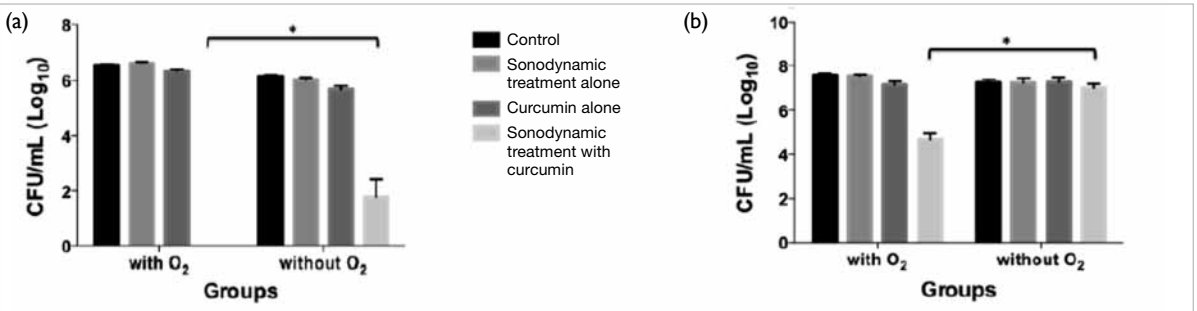

FIG 2. Bacterial survival in terms of colony-forming units (CFU) per mL for (a) *Bacillus cereus* and (b) *Escherichia coli* after curcumin-mediated sonodynamic treatment with or without O_2

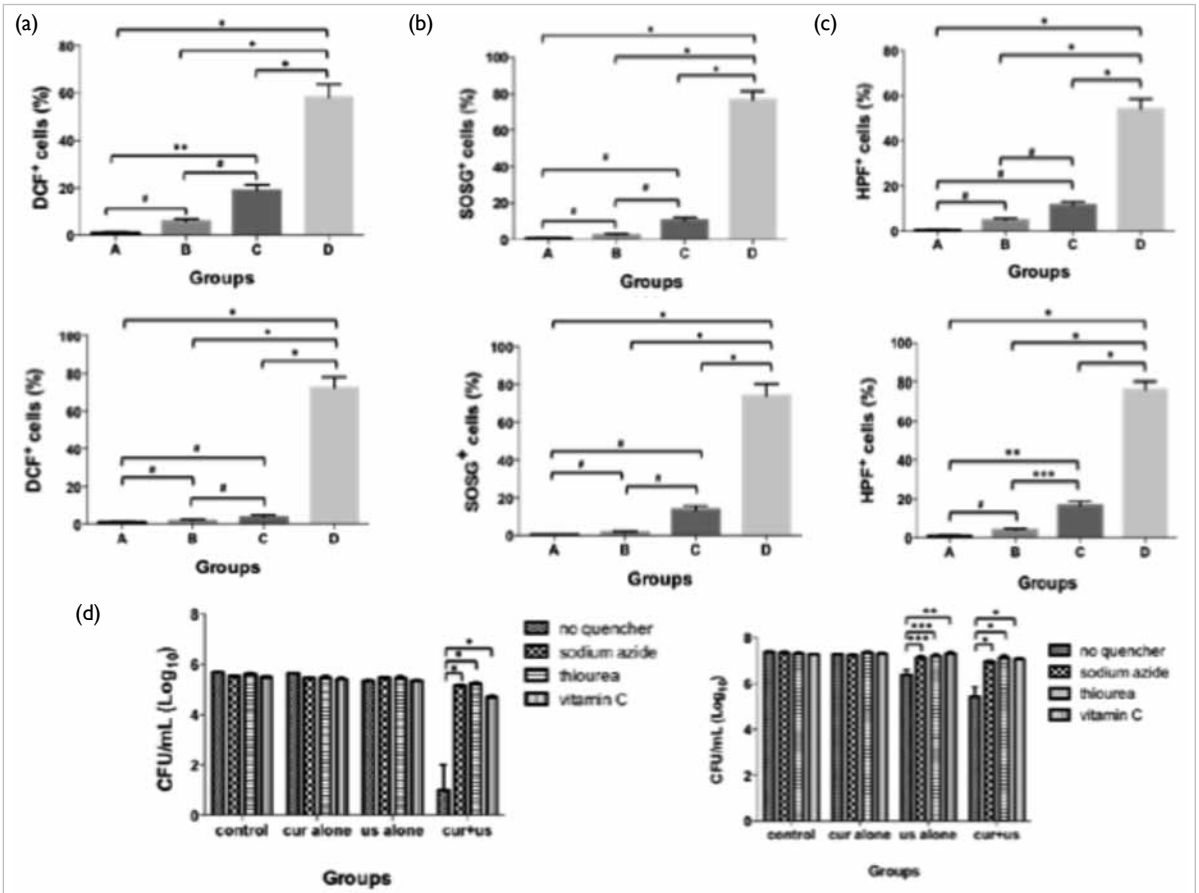

FIG 3. Production of (a) reactive oxygen species, (b) singlet oxygen, and (c) hydroxyl radical after sonodynamic treatment with curcumin against *Bacillus cereus* and *Escherichia coli*. (d) Bacterial viability after sonodynamic treatment with curcumin with or without different quenchers (sodium azide, thiourea, and vitamin C) against *Bacillus cereus* and *Escherichia coli*

Indirubin-3′-oxime as an antiviral and immunomodulatory agent in treatment of severe human influenza virus infection

MCW Chan *, RWY Chan, CKP Mok, NK Mak, RNS Wong

KEY MESSAGES

1. Two indirubin derivatives (indirubin-3′-oxime and E804) demonstrate strong antiviral and immunomodulatory effects on human macrophages and type-I alveolar epithelial cells after influenza H5N1 virus infection.
2. In mice infected with H5N1 virus, the use of E804 does not improve survival or weight loss but significantly reduces cytokine and chemokine expression and secretion, compared with controls.
3. Cyclin-dependent kinases pathway is involved in the antiviral and immunomodulatory effects of indirubin upon influenza H5N1 virus infection.

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Introduction

Patients with influenza H5N1 virus infection have a fulminant viral pneumonia with rapid progression to adult respiratory distress syndrome and multiple organ dysfunctions. Although virus replication and tissue tropism were important drivers of pathogenesis.[1] Clinical and *in vivo* studies showed that pro-inflammatory cytokines were highly induced during H5N1 infection, suggestive of pathogenesis.[2] We have shown that primary human peripheral blood–derived macrophages (macrophages) and type-I alveolar epithelial cells (ATI) were target cells of H5N1 virus and produced higher levels of cytokines.[3] These results highlighted the need to modulate cytokine responses during H5N1 infection as an adjunct to antiviral therapy.

Indirubin, a 3,2′-bisindole isomer of indigo, is an active ingredient of a traditional Chinese medicine preparation Danggui Longhui Wan for various chronic diseases.[4] This chemical compound exhibits strong anti-inflammatory and anti-leukaemic activities. One of the derivatives, indirubin-3′-oxime (IDO) has been found to have anti-inflammatory and anti-viral effects.[5] The therapeutic role of IDO and E804 in H5N1 virus infection has yet been investigated. This study aimed to demonstrate that IDO and E804 can be a beneficial adjunctive therapy for human H5N1 infection through its antiviral and immunomodulatory effects.

Methods

Antiviral activity of IDO and E804 was examined by evaluating the infectious virus titres in human macrophages and ATI using plaque assay. We then infected human macrophages and ATI with H5N1 and H1N1 viruses with or without IDO and E804 treatment, and determined the mRNA and protein expression of cytokine and chemokine by real-time qPCR and ELISA. cDNA microarray was performed to identify the gene expression profile that involved in the antiviral and immunomodulation of cytokines by E804. Mice infected with H5N1 (A/Hong Kong/486/97) and H1N1 (A/HK/54/98) viruses were used to study the effect of E804 on survival, weight loss, infectious viral titre, and cytokine and chemokine expression.

Results

Compared with cells without treatment, indirubin derivatives (IDO and E804) inhibited viral replication by about 10-fold in H5N1 virus–infected macrophages and ATIs and in H1N1 virus–infected macrophages at 24 hours post-infection, as well as in H1N1 virus–infected ATIs at 48 hours post-infection (Figs 1a and 1b). IDO effectively suppressed the viral matrix 1 protein expression in H5N1 virus–infected macrophages (Fig 1c).

In infected macrophages, E804 (5 μM) suppressed the induction of IP-10, MIG, IL-1β, and RANTES (Figs 2a to 2d), as well as IL-8, MIP-1α, MIP-1β, and MCP-1 (data not shown). In infected ATIs, E804 significantly reduced the secretion of IP-10 and RANTES (Figs 2e and 2f). E804 exhibited a stronger and more potent immunomodulatory effect than IDO.

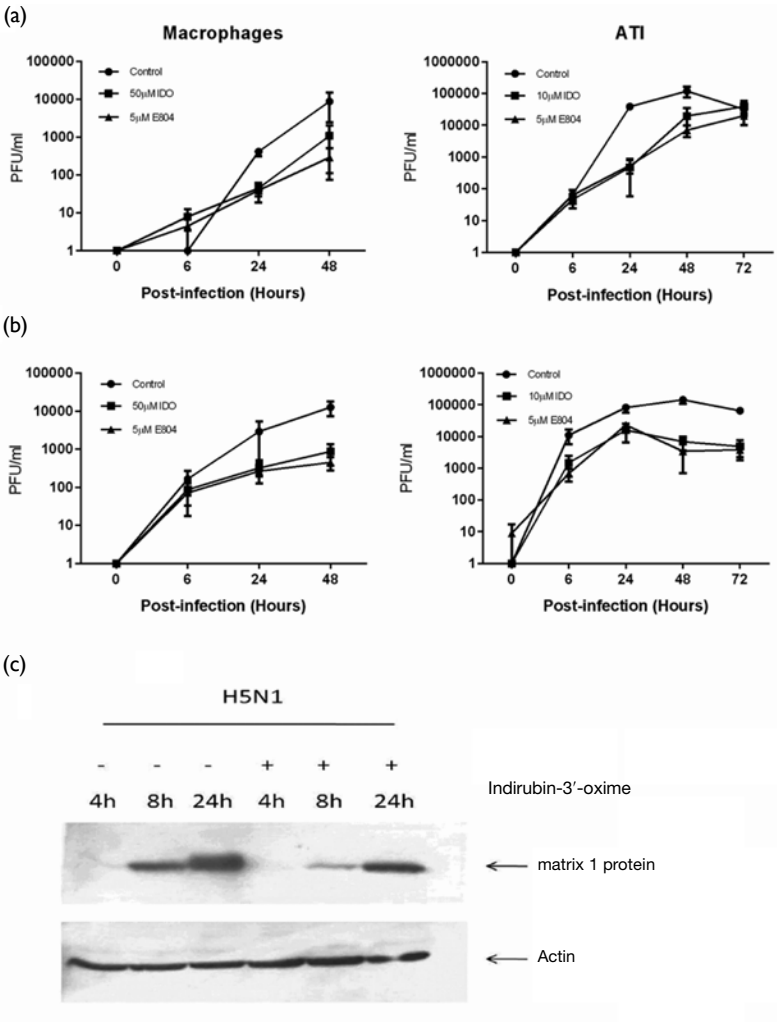

FIG 1. Viral replication in (a) H5N1- and (b) H1N1-infected macrophages and type-I alveolar epithelial cells (ATI) pre-treated with indirubin-3′-oxime (IDO) and E804. (c) IDO effectively suppressed the viral matrix 1 protein expression in H5N1 virus–infected macrophages.

Discussion

Indirubin derivatives, IDO and E804, exhibited strong cyclin-dependent kinase inhibition and suppressed induction of pro-inflammatory cytokines together with inhibition of viral replication in human macrophages and ATIs, the two major cellular targets of H5N1 virus. The antiviral and immunomodulatory effects of indirubin derivatives are partly due to cyclin-dependent kinase inhibition. The underlying mechanism needs further investigation.

Mice treated with E804 did not have any survival advantage. Mice treated with both peramivir and E804 had better lung pathology with less inflammation and infiltration of immune cells at day 6 post-infection, compared with other treatment groups. Mice treated with E804 alone or peramivir and E804 showed a significant decrease in the expression and secretion of pro-inflammatory cytokine and chemokine in the lung lysate. This suggests that direct injection of E804 can improve the immunopathology, and recovery and regeneration of injured lung may be more robust after E804 treatment. Nonetheless, mice infected with H5N1 cannot be used to evaluate the E804 treatment. A better animal model (eg, ferrets) is needed to evaluate the potential therapeutic effects. In addition, we were unable to use a higher concentration of E804 to treat the H5N1-infected mice, owing to the poor solubility of E804. Developing novel indirubin derivatives with higher solubility is needed to achieve therapeutic effect. Furthermore, the infection dosage (1-2 LD_{50}) of the H5N1 virus in the in vivo model was too high; it is the marginal infectious dosage to develop lethal outcome for the mice. In future studies, sub-lethal dosage may be used to determine the therapeutic effect of E804 on weight loss, virus titre, and cytokine level.

Pandemic influenza is a public health concern. The long vaccine production process can hardly prevent the first wave of the pandemic, and high morbidity and mortality may be unavoidable. Antivirals may lead to amelioration of disease but are not effective in preventing severe complications. Novel therapeutic strategies against severe influenza infection are needed. Additional therapeutic strategies such as modulating the immune response may be key to successful treatment. Indirubin derivatives, IDO and E804 have antiviral and immunomodulatory effects and could be used in treatment of H5N1 infection.

Indirubin has been used to treat leukaemia. This naturally occurring compound has low toxicity and may be used to treat H5N1 infection and other respiratory infections such as novel influenza H7N9, SARS-CoV, MERS-CoV, for which the 'cytokine storm' may be involved in the pathogenesis.

Conclusion

The two indirubin derivatives (IDO and E804) demonstrate strong antiviral and immunomodulatory effects on human macrophages and ATIs after influenza H5N1 virus infection. In mice infected with H5N1 virus, the use of E804 does not improve survival or weight loss but significantly reduces cytokine and chemokine expression and secretion, compared with controls.

Acknowledgement

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Results from this study have been published in: Mok CK, Kang SS, Chan RW, et al. Anti-inflammatory and antiviral effects of indirubin derivatives in

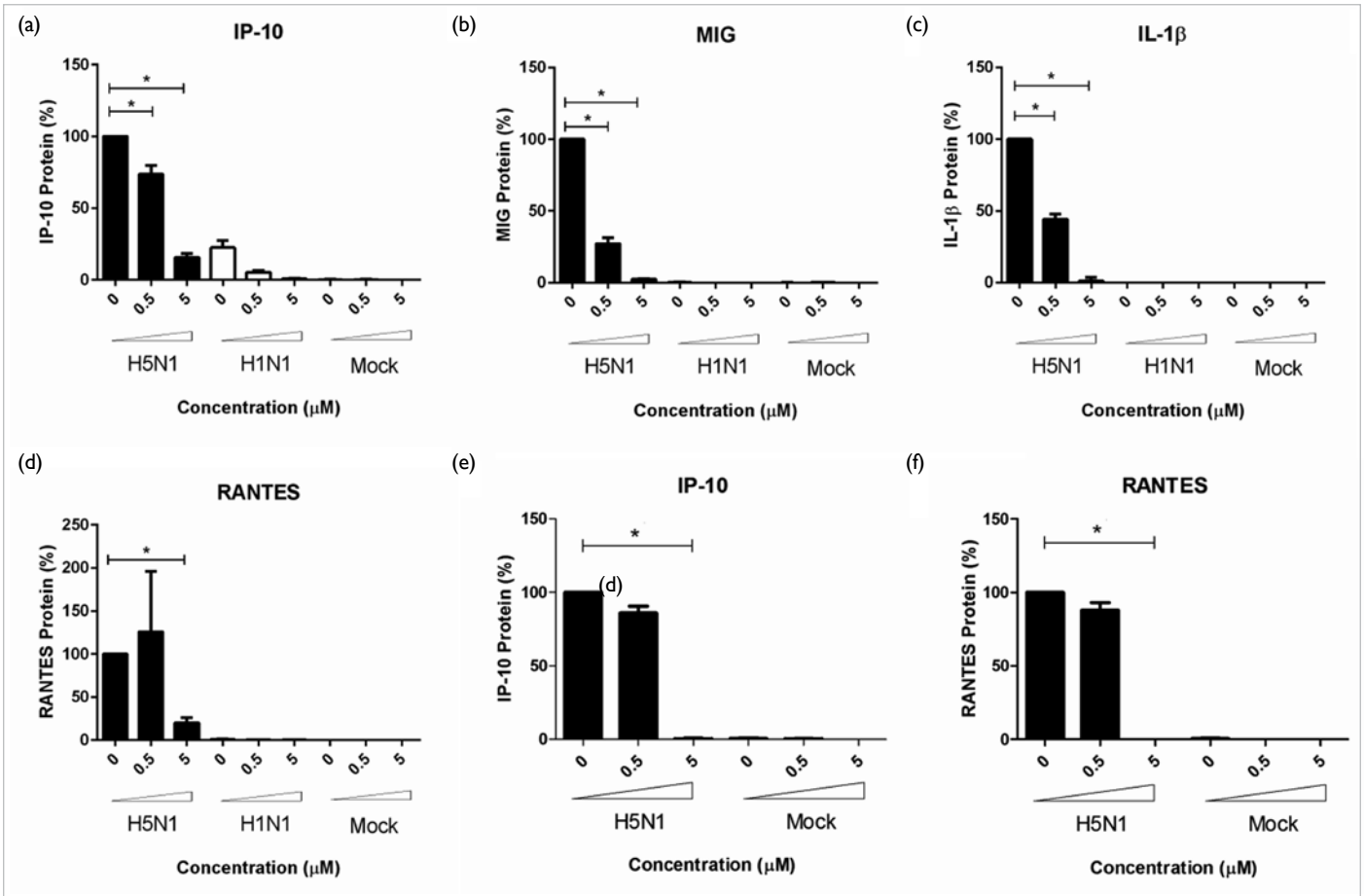

FIG 2. Immunomodulatory effect of E804 on the cytokines protein expression in terms of (a) IP-10, (b) MIG, (c) IL-1β, (d) RANTES in infected macrophages and (e) IP-10 and (f) RANTES in infected type-I alveolar epithelial cells measured by the Cytometric Bead Array assay.

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