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Determinants of late-stage human immunodeficiency virus infection at first diagnosis

The first case of human immunodeficiency virus (HIV) infection was detected in Singapore on 16 May 1985. The notification rate of HIV/acquired immune deficiency syndrome (AIDS) in Singapore has been on the rise from 3.9 per million population in 1987 to 71.9 per million population in 2003 and further increased to 117.8 per million population in 2007. More than half of the new cases already had late-stage HIV infection when they were diagnosed (*Fig. 1*). Late-stage HIV infection was defined as having a CD4 cell count of less than 200 per cu mm or developing AIDS-defining opportunistic infections at first diagnosis or within one year after HIV diagnosis.

It is known that HIV-infected patients diagnosed at a late-stage of the disease have poorer prognosis[1]. Late-stage diagnosis results in missed opportunities to start treatment early, leading to increased risk of further transmission and death. A retrospective study was conducted to identify the determinants of late-stage HIV infection at first diagnosis. The findings would help in the planning of targeted public health initiatives to facilitate early diagnosis and achieve better overall survival for people living with HIV/AIDS in Singapore.

Materials and methods

The data for the retrospective study were obtained from the national HIV registry. We included 3250 patients who were infected via the sexual route and diagnosed from 1985 to 2007.

Associations between patients with late-stage HIV infection and various epidemiological characteristics were examined using Chi-square

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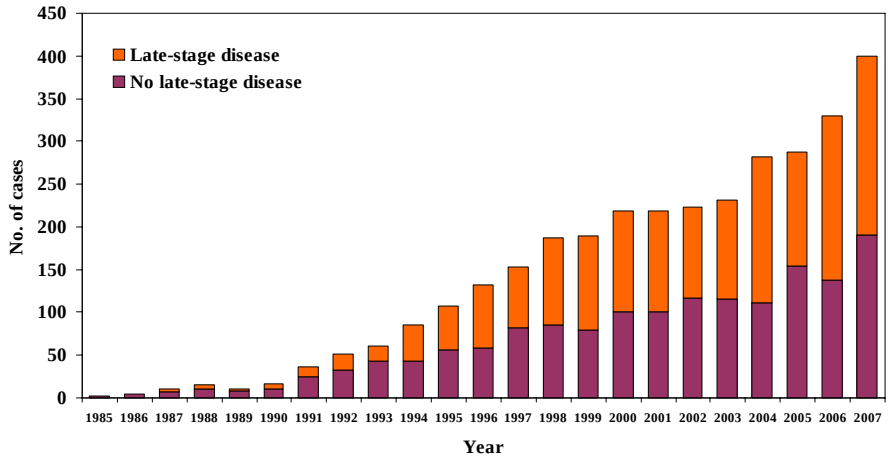
Figure 1
Number of HIV/AIDS notifications by stage of diagnosis, 1985-2007



test. To describe the survival pattern of patients with late-stage HIV infection, we used the Kaplan-Meier method to estimate the median survival time. Multivariate logistic regression was used to determine independent epidemiological risk factors for late-stage HIV infection at first diagnosis, using backward stepwise selection based on maximum partial likelihood estimates. In the final model, all remaining predictors were significant at $p < 0.05$.

Results

The median survival of patients with late-stage HIV infection was 5 years (95% CI: 3.7-6.3 years). In comparison, the cumulative proportion of patients without late-stage HIV infection surviving till the 5th year since diagnosis was 80%. Bivariate analyses showed that patients with late-stage HIV infection at first diagnosis were more likely to be 30 years and older, male, Chinese, married, lower social-economic status (based on occupation) or unemployed, detected during medical care, and having escorts and sex workers as sexual partners($p<0.005$) (*Table 1*).

Independent factors significantly associated with the late-stage HIV infection were male, 30 years and older, occupations which are administrative or service-oriented, blue-collar workers or unemployed, HIV testing done in the course of some form of medical care, and infection via the heterosexual route (*Fig. 2*).

Comments

We found that late-stage HIV infection was mainly detected through diagnostic testing as a result of illnesses, similar to findings from other studies[2,3]. Regular HIV testing can help limit the spread of infection. To encourage more to come forward for voluntary testing, since August 2007, the Ministry of Health (MOH) has allowed medical clinics to offer HIV testing using oral-fluid or blood-based rapid HIV test kits, which can produce results in approximately 20 minutes.

Since December 2007, Changi General Hospital (CGH) has started to offer HIV screening programme for adult inpatients on a voluntary opt-out basis. Following the successful pilot project of the volun-

Table 1
Epidemiological characteristics (%) of HIV-infected patients

Characteristics	With late-stage HIV infection (n=1677)	Without late-stage HIV infection (n=1573)
Age at diagnosis		
Less than 30 years	9.0	28.2
30 years and older	91.0	71.8
Gender		
Female	7.5	13.7
Male	92.5	86.3
Ethnic group		
Chinese	86.7	82.5
Malay	8.3	9.0
Indian	2.6	5.2
Others	2.4	3.3
Marital status		
Single	51.4	58.6
Married	34.0	30.4
Divorced/separated	10.8	9.2
Widowed	3.8	1.8
Occupation		
Professional / executive	11.4	19.0
Administrative / service-oriented	30.1	34.6
Blue-collar worker	32.7	22.0
Unemployed	17.2	8.7
Others	8.6	15.7
Mode of detection		
Own request	4.1	16.3
Medical care and screening	92.0	52.7
Contact tracing	1.7	11.5
Others	2.2	19.5
Mode of sexual transmission		
Homosexual/bisexual	19.4	35.3
Heterosexual	80.6	64.7
Type of sexual partners		
Regular only	10.7	17.2
Regular and casual only	17.3	32.0
Escorts and sex workers	72.0	50.8

Figure 2
Adjusted odds ratios (95% CI) for late-stage HIV infection*

* The referent category is the first row of the respective characteristic in Table 1.
CI: Confidence interval.

tary HIV opt-out screening in CGH, all acute public and private sector hospitals would also be implementing the opt-out HIV screening programme for inpatients aged 21 years and above from October 2008. The objective of encouraging more HIV testing is to increase the proportion individuals who are aware of their HIV status and hence reduce the number of HIV-infected patients who present with late-stage HIV infection.

This study identifies the high-risk groups for late-stage HIV infection at first diagnosis. The findings serve as important references for the formulation of targeted and focused public health initiatives to educate and increase awareness of the disease and to encourage HIV screening. Moreover, early detection would improve the overall survival of these high-risk groups through early treatment and management of the disease.

(Reported by Ang LW[1], Tey SH[2] and James L[1],
[1]Communicable Diseases Division, [2]Manpower Standards & Development Division, Ministry of Health)

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An outbreak of influenza-like illness in a primary school

Introduction

In Singapore, influenza viruses circulate throughout the year. Based on sentinel surveillance of acute respiratory illnesses, including influenza, the annual incidence typically displays a bimodal distribution with peaks around April-July and November-January. This seasonal increase seems to coincide with the southern and northern hemisphere winter flu seasons respectively. Virological surveillance of respiratory specimens obtained from polyclinics, medical centres and hospitals showed a marked increase in the percentage tested positive for influenza A in Jan 2008 (5.7% to 10.1% compared to 1.1%-4.4% for the same period in the previous year). No such increase was noted for influenza B.

On 17 Jan 2008, the Ministry of Health (MOH) was notified of an outbreak of influenza-like illness involving students and teachers in a primary school. As soon as the notification was received, epidemiological investigations were conducted to determine the cause and extent of the outbreak, source of infection and mode of transmission.

Epidemiological findings

The single-session school had 1,181 students and 72 teachers/staff. The classrooms were well ventilated with ceiling fans and windows. The average number of students per class was 30 for the lower primary classes and 35 for the upper primary classes.

The staff room was air-conditioned and desks were situated close to one another. The canteen was satisfactorily maintained with five food stalls. There were three staggered recess periods (each lasting 30 minutes) from 9.30 am to 11.00 am.

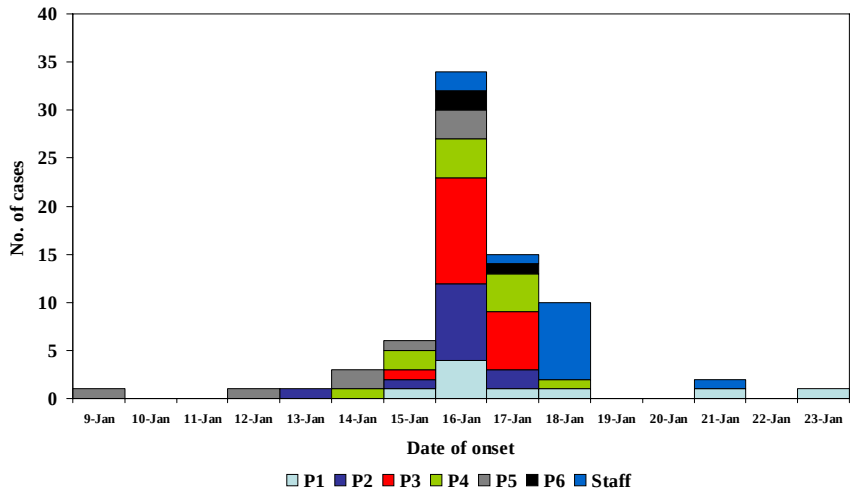
We defined a case of influenza-like illness as any person having two or more symptoms of fever and acute respiratory tract illness[1].

A total of 62 students and 12 teachers reported ill with onset of symptoms between 9 Jan and 23 Jan 2008. The illness was first reported in a primary 5 student on 9 Jan. The infection subsequently spread to other susceptible students and teachers in the school with the peak of the outbreak on 16 Jan. It then declined rapidly over the next two days and the last case was reported in a primary 1 student on 23 Jan (*Fig 3*). The duration of the outbreak was 2 weeks. None of the canteen food handlers, cleaners and other staff were affected.

The main clinical symptoms were fever (100%), rhinitis (66.2%), cough (85.1%), sore throat (62.2%), headache (18.9%), malaise (12.2%) and diarrhoea (4.1%). Of the reported cases, one (1.4%) was hospitalized while 63 cases (85.1%) sought outpatient treatment and the rest (13.5%) self-medicated. The distribution and attack rates of the reported cases among students by class and gender are shown in *Table 2*. The attack rate was highest among primary 3 students (9.0% compared with an overall rate of 5.9%). Overall, teachers had an attack rate (16.7%) which was more than 3 times higher than that of students (5.2%). Except for primary six, female students had a higher attack rate compared with males. All the teaching staff affected were females.

Seven random throat swab samples were taken from six students and one teacher and tested for influenza virus. Influenza virus type A (H1N1) was detected in two, influenza virus type A (H3N2) in another two and influenza type B in one. The four influenza A samples were also sequenced and the H1N1 virus was found to be A/Hong Kong/2652/2006-like strains and the H3N2 virus, A/Wisconsin/67/2005 (low reactor)-like strains.

Figure 3
Onset of symptoms of 74 reported cases of influenza-like illness in a primary school, 9-23 Jan 2008



Prevention and control

The principal of the school was advised on the following measures to break the chain of transmission:

- distribute circulars to inform parents not to send sick children to school;
- inform all bus drivers to ensure that children are well before they board the bus in the morning;
- reduce congregation of students by stopping morning assembly and cancel all supplementary lessons after school;
- ensure that the school premises are cleaned, disinfected and well maintained daily; identify students and staff who develop symptoms, isolate them early and refer them for medical treatment;
- observe personal hygiene etiquette, including covering of mouth when coughing/ sneezing and washing hands thereafter;
- promote frequent hand washing;
- ensure that toilets are in a sanitary condition and adequately equipped with soap and toilet papers; and
- have adequate ventilation in places of congregation and avoid overcrowding.

Comments

This outbreak coincided with the annual seasonal increase in influenza-like illness from November to January in Singapore. All the three influenza strains (H1N1, H3N2 and B) were detected in this school. This is consistent with our virological surveillance which shows these strains circulating in the general population with influenza A dominating over influenza B during the first 4 epidemiological weeks of 2008.

In temperate countries, school-age children have the highest attack rates during typical seasonal influenza outbreaks, and they play a central role in sustaining influenza transmission[2,3]. In this primary school, once the influenza virus was introduced, it spread easily among the large numbers of highly susceptible children who congregated during morning assembly, at the canteen and other crowded environment. Teachers were also affected with an attack rate significantly higher than that of students. This could be due to the staff room being air-conditioned and teachers sitting close to one another.

Table 2

Distribution of 74 reported cases of influenza-like illness by class and gender in a primary school, 9-23 Jan 2008

Class category	No. enrolled			No. affected and attack rates		
	Male	Female	Total	Male (%)	Female (%)	Total (%)
Primary 1	87	68	155	3 (3.4)	6 (8.8)	9 (5.8)
Primary 2	110	97	207	6 (5.5)	6 (6.2)	12 (5.8)
Primary 3	114	85	199	9 (7.9	9 (10.6)	18 (9.0)
Primary 4	89	81	170	6 (6.7)	6 (7.4)	12 (7.1)
Primary 5	131	100	231	4 (3.1)	4 (4.0)	8 (3.5)
Primary 6	100	119	219	3 (3.0)	0	3 (1.4)
Staff	11	61	72	0	12 (19.7)	12 (16.7)
Total	642	611	1253	31 (4.8)	43 (7.0)	74 (5.9)

It is also noteworthy that the school was quick to respond to the outbreak. Besides notifying MOH, the principal also informed parents and school bus drivers not to send unwell students to school. A high standard of personal hygiene was maintained. Thorough cleaning of the premises was carried out during the weekend when the school was closed. All these measures could have contributed to the sharp decline in the number of cases.

(Reported by Lim S, Toh HY, Lalitha K, Foong BH and Ooi PL, Communicable Diseases Division, Ministry of Health)

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A case of human simian malaria in Singapore

Introduction

Nonhuman primates are naturally infected by many malaria species. *P. knowlesi* is one of the simian malarias that cause human infection. Naturallly acquired human *P knowlesi* infection has been reported in East Malaysia, Peninsular Malaysia, Thailand, Philippines and Myanmar with the largest focus of cases reported in East Malaysia. *P. knowlesi* is commonly misidentified as *P. malariae* since the blood stages are morphologically similar on microsocopy, and molecular methods of detection are necessary for accurate diagnosis.

The clinical and epidemiological features of the first case of naturally acquired *P. knowlesi* infection in Singapore are described.

Clinical presentation

On 28 April, 2007, a previously healthy soldier, aged 20 years, from the Singapore Armed Forces sought treatment for fever of 4 day duration, myalgia, anorexia, nausea, and occasional vomiting. On initial examination, his temperature was 39.5 degrees C with a pulse rate of 106 beats/ min. He was lethargic with a tender enlarged liver, Laboratory investigations showed thrombocytopenia, hyperbilirubinaemia, and mild transaminitis.

Initial diagnosis was dengue fever. The patient experienced daily fever spikes from 39.5 degrees C to 40.4 degrees C. When fever persisted (40.4 degrees C on day 6 of his illness and day 3 of hospitalization), the clinical picture was atypical of dengue fever. Blood films for malarial parasites were ordered. Microscopy showed *Plamodium* parasitaemia of 0.2% (equivalent to 7,700 parasites/nmol/L blood) with morphological features consistent with *P. malariae*. Results of dengue reverse transcription-PCR on serum, 2 sets of blood culture, and *Rickettsia typhi* serological testing were negative. A chest radiograph and ultrasound of the abdomen were normal.

The patient was treated with oral chloroquine with an initial dose of 600 mg base, followed by 300mg 6 hours later and another 2 doses over the next 2 days. He defervesced rapidly; blood smears were negative 3 days after chloroquine therapy. At 2 weeks follow-up, he was well clinically.

Epidemiological features

For a year leading up to his illness, the patient had trained in a forested area inhabited by the long-tail macaque in Lim Chu Kang. His only travel out of Singapore was a 3-week training visit to a non-malarious endemic foreign country in Sept 2006 and another visit to Bukit Batok Nature Reserve, an area with monkeys (*M. fascicularis*) 1 month before onset of symptoms

Further laboratory investigations

As *P. malariae* infection was not consistent with the clinical findings of the initial examination, further investigation was conducted to determine the aetiology of this case. End-point nested *Plasmodium* genus- and species-specific nested PCR carried out on DNA extracted from whole blood samples were positive for *Plasmodium* sp. but negative for the 4 species that cause human malaria (*P. vivax, P. falciparum, P malariae, P. ovale*). Similarly, the sample was negative on real-time PCR for the 4 human parasites. *P. knowlesi* species-specific PCR resulted in a 153-bp fragment indicative of *P. knowlesi*. This 153-bp PCR product was directly sequenced and verified in the BLAST database to match only *P. knowlesi* small subunit ribosomal RNA (SSU rRNA).

The pathogen was confirmed by using previously described approaches to compare the sequences of the 5' and 3' ends of the circumsporozoite protein (csp) gene, as well as the gene encoding of the SSU rRNA in this case sample, to other *Plasmodium* parasites. Sequences were obtained by direct sequencing of PCR products and aligned by using the ClustalW method. Phylogenetic trees were constructed by using the MegAlign software. The case sample is clustered with other *P. knowlesi* isolates and is clearly distinct from other *Plasmodium* species.

Discussion

This is an unequivocal case of *P. knowlesi* infection supported by clinical findings and laboratory diagnostics classic for this pathogen. The classical scenario that raises the suspicion of *P.knowlesi* infection is a blood smear consistent with *P. malariae* but with parasitaemia exceeding 5,000 parasites per nmol/L blood, daily fever spikes, and pronounced symptoms, features atypical of *P. malariae* infection. The daily fever spike is due to the *P. knowlesi* 24-hour asexual life cycle, the shortest of all primate malarias. *P. malariae* has a 72-hour asexual life cycle and manifests as chronic, asymptomatic infection with low level parasitaemia.

P. knowlesi is commonly mistaken for *P. malariae* by microscopy due to similarity of the blood stages. *P. knowlesi* can be misidentified as *P. falciparum* if only ring forms are identified. The *P. knowlesi*-specific primers used by both independent laboratories have previously been shown not to detect any of the 4 *Plasmodium* species that cause human infection or the 3 agents that cause simian malaria: *P. cynomolgi*, *P. fieldi* and *P. fragile*. PCR detection using *P. knowlesi*-specific primers, followed by sequencing and phylogenetic analyses of the csp

and SSU rRNA genes confirmed *P. knowlesi* infection in this patient.

The patient likely acquired the infection in the forested area in Lim Chu Kang where he had been training for the entire year before his illness. Experimental *P. knowlesi* studies showed a prepatent period of 9-12 days in humans. *P. knowlesi* has no liver hypnozoite stage and does not cause relapse. The patient's previous overseas travel 7 months before and his visit to Bukit Batok Nature Reserve a month before onset of illness are beyond the incubation period.

P. knowlesi's natural hosts are the macaques, *M fascicularis* and *Macaca nemestrina*. Notably, the first studies on *P. knowlesi* were on a parasite isolated from a macaque imported into India from Singapore. *M fascicularis* and *Presbytis femoralis* are the 2 native monkeys in Singapore, with *M. fascicularis* being the only species in Lim Chu Kang and Bukit Batok Nature Reserve. Mosquitoes of the *Anopheles leucophyrus* group have been identified as vectors of *P. knowlesi* and are present in surrounding countries in Southeast Asia. Studies are ongoing to determine potential mosquito vectors and whether macaques are hosts of *P. knowlesi* in Singapore.

The patient's condition was diagnosed within 6 days of illness, and the infection responded rapidly to oral chloroquine. Although most patients' infections respond well to antimalarial agents, 4 fatal cases of *P. knowlesi* infection were reported recently in patients aged 39 to 69 years, whose conditions were all diagnosed within 7 days of symptom onset. Common clinical features included fever, abdominal pain, thrombocytopenia, renal impairment, and jaundice. All the patients received a misdiagnosis of *P. malariae* infection.

P. knowlesi infection should be considered as an aetiologcial agent of malaria acquired in Singapore, particularly in cases with daily fever spikes and blood smears suggestive of *P. malariae*. Epidemiological studies into the parasite's reservoir and mosquito vector will be important in the prevention of this emerging zoonotic disease.

(Based on Ng OT et al. Naturally acquired human Plasmodium knowlesi *infection, Singapore. Emerg Infect Dis 2008; 14: 814-6)*

Sequence-based typing (SBT) for investigation of *Legionella pneumophila* in environmental water samples

Introduction

Legionellosis is an infectious disease caused by the bacterium *Legionella pneumophila*. The main mode of transmission is through inhalation of aerosols contaminated with this pathogen. This disease ranges from the milder form, Pontiac fever, to the more severe form, legionnaires' disease [1]. *L. pneumophila* serogroup (SG) 1 is the major group reported to cause legionellosis. In recent years, there are also reported legionellosis traced to other SG of *L. pneumophila* [2, 3].

In Singapore, there is an average of 30 reported legionellosis cases each year over the last 19 years. So far, all cases have been sporadic and there has been no reported outbreak. The Environmental Health Institute (EHI), National Environment Agency, performs regular environmental surveillance to assess the risk of *Legionella* infection through aerosol generating water bodies. Bacteria isolates derived from these surveillance efforts are further investigated to understand the molecular diversity of the *L. pneumophila* flora.

We have adopted the sequence-based typing (SBT) system developed by the European Working Group for *Legionella* Infections (EWGLI) for this study. This method allows excellent resolution, reproducibility and epidemiological concordance of *L. pneumophila* serogroups 1-14.

Methods

Sample collection

Water and swab samples were collected from potable water sources such as spa facilities, mist fans and hot water storage tanks via showerheads while non-potable water sources include cooling towers. A subset of 17 isolates from the potable sources and 15 isolates from the non-potable sources were used for the analysis. All samples collected were cultured for *L. pnuemophila* in accordance with the British Standards Institute method (6068-4.12:1998, Water Quality: Detection and Enumeration of *Legionella*). Positive isolates were typed using the SBT method.

SBT analysis

All pure isolates were grown on buffered charcoal yeast extract (BCYE) agar and DNA was extracted using Qiagen DNeasy blood & tissue kit. Polymerase chain reaction (PCR) was performed on the DNA using 7 pairs of primers: *flaA, pilE, asd, mip, mompS, proA* and *neuA*. The PCR products were purified using Qiagen QIAquick PCR purification kit and sequencing was performed.

Analysis of sequences

The sequences were submitted into the EWGLI database. The sequences of each isolate's alleles were compared with the allele sequences in the database. Allelic profile (a string of 7 allele numbers) was allocated to each individual isolate in a pre-determined order (i.e. *flaA, pilE, asd, mip, mompS, proA* and *neuA*). The allelic profiles of the isolates were compared with the existing profiles in the database. Matched allelic profile was given a sequence type (ST) number. Non-matching allelic profiles were considered novel, and were designated with an EHI number.

Results and discussions

Tables 3 and 4 show the allelic profiles from the water sources studied. Both published and novel allelic profiles of *L. pnuemophila* have been found in Singapore. Interestingly, among the published allelic profiles found, all were associated with clinical cases and disease, as shown in Table 5. This suggests that these strains found in Singapore's spa pools and cooling towers may be disease causing. Therefore, the surveillance of *Legionella* in the environment is essential to assess the risk of *Legionella* infection so that adequate timely environmental control can be implemented.

It is unknown if the novel isolates discovered can cause disease. Local clinical isolates may be able to shed light on some characteristics of these novel environmental isolates.

Table 3

Allelic profile from potable water sources

Sample ID	SG	Sampling site	Allelic profile	Novel	Non-novel
PL 001	1	Spa pool	7,6,31,3,48,15,11		ST 469
PL 002	1	Spa pool	7,6,31,3,48,15,11		ST 469
PL 003	1	Spa pool	7,6,17,3,13,11,11		ST 59
PL 004	1	Spa pool	7,6,17,3,13,11,11		ST 59
PL 005	1	Spa pool	7,6,17,3,13,11,11		ST 59
PL 006	1	Spa pool	7,6,17,3,13,11,11		ST 59
PL 007	1	Hot water tank	1,4,3,1,1,1,1		ST 1
PL 008	1	Spa pool	7,6,31,3,48,15,11		ST 469
PL 009	2-14	Hot water tank	-1, 21,33,37,41,1,11	EHI 1	
PL 010	2-14	Cold water tank	-1,21,33,37,41,1,11	EHI 1	
PL 011	2-14	Unknown	3,10,1,28,14,9,3		ST 187
PL 012	1	Unknown	1,4,3,1,1,1,1		ST 1
PL 013	1	Spa pool	6,10,19,3,19,4,11		ST 345
PL 014	1	Spa pool	22,4,3,1,1,30,1		ST 252
PL 015	1	Spa pool	7,6,31,3,48,15,11		ST 469
FS3WS1 (3)	2-14	Mist fan	-1,21,33,37,41,1,1	EHI 2	
FS18WS2	2-14	Mist fan	16,21,33,37,41,1,3	EHI 3	

Table 4

Allelic profile from non-potable water sources

Sample ID	SG	Sampling sites	Allelic profile	Novel	Non-novel
NPL 001	1	Cooling towers	7,6,17,1,48,11,1	EHI 4	
NPL 002	1	Cooling towers	7,6,17,1,48,11,1	EHI 4	
NPL 003	1	Cooling towers	1,4,3,1,1,1,1		ST 1
NPL 004	1	Cooling towers	7,6,17,1,48,11,1	EHI 4	
NPL 005	1	Cooling towers	1,4,3,1,1,1,1		ST 1
NPL 006	1	Cooling towers	16,4,3,19,1,30,1	EHI 5	
NPL 007	1	Cooling towers	1,4,3,1,1,1,1		ST 1
NPL 008	2-14	Cooling towers	3,6,1,6,14,11,9		ST 114
NPL 009	2-14	Cooling towers	8,6,34,9,53,8,1	EHI 6	
NPL 010	2-14	Cooling towers	1,4,3,19,1,1,1	EHI 7	
NPL 011	2-14	Cooling towers	7,6,17,3,1,11,1	EHI 8	
NPL 012	2-14	Cooling towers	-1, 21,33,37,41,1,11	EHI 1	
NPL 013	2-14	Cooling towers	1,4,3,19,1,30,3	EHI 9	
NPL 014	1	Cooling towers	1,4,3,1,1,1,1		ST 1
NPL 015	2-14	Cooling towers	7,6,31,19,48,11,1	EHI 10	

Table 5

Information on the 7 published isolates found in Singapore.

ST number of Singapore's environmental isolates	No. of isolates in EWGLI database	Clinical	Environment
1	150	59	91
59	9	6	3
114	4	2	2
187	2	1	1
252	2	2	0
345	5	2	3
469	1	1	0

The rapid SBT method allows a highly specific differentiation of *L. pneumophila* strains. The establishment of this database and typing capacity will serve as a platform to assist in epidemiological investigations to link any future clinical isolates to the potential environmental sources.

(Reported by Lim PY, Kek R., Yap J. and Ng L.C, Environmental Health Institute, National Environment Agency, Singapore)

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Surveillance of mould flora in shopping malls in Singapore

Introduction

As people spend more time in the indoor environment, the quality of indoor air may significantly impact an individual's exposure to environmental contaminants. It has been reported that the presence of excessive mould could contribute to poor indoor air quality (IAQ) [1]. In normal circumstances, presence of moulds in low concentration usually will not pose any health hazard. However, in water-damaged areas, excessive moisture con-

tent may enhance the growth of moulds and increase the risk of potential health hazard [2].

To understand the mould flora in our environment, we have adopted the patented US Environment Protection Agency (EPA) mould specific quantitative PCR technology for rapid identification of targeted mould species. This molecular detection allows the detection of 36 species at one time, of which 26 species frequently occur in water- damaged environment and 10 species in typical environment. Subsequent computation of relative mould index (RMI) using data on the 36 species, determines the likelihood of having mould problems of an indoor environment. A pilot project monitoring the indoor mould flora in Singapore's shopping malls is described in this paper.

Investigation methodology

Sample collection

A total of 15 shopping malls, consisting of 2 water-damaged and 13 non water-damaged shopping malls, were sampled. Three sampling locations were chosen for each shopping mall; *viz* the reception counter, midpoint of the mall as well as food court. As most reception counters are located near the entrance, this location represents the point of infiltration of outdoor air into the shopping mall. The midpoint was chosen to represent the general mould flora in the shopping centre. Food court was also selected, as this is an environment with high nutrient content and high level of humidity due to cooking activities.

Dust from the air filters corresponding to the sampling points were collected from each of the shopping malls. Air sampling was also done but the results were not significant. Data presented is based on analysis done on air filters.

Laboratory analysis

Dust samples were extracted from filters through soaking in sterile water, centrifugation and drying. DNA was extracted from 5 mg of dry dust from each filter using GeneRite EZ 01 kit. Quantitative PCR, using the 36 pairs of species-specific primers, was then performed on each DNA sample.

Data analysis

Relative mould index (RMI) for shopping malls was calculated using the formula:

RMI = Σ of log [concentration of water damaged species] - Σ of log [concentration of non-water damaged species]

Results and discussions

Findings from the study showed that the mould concentrations of the 3 most dominant species for shopping malls follow the trend of *Aspergillus penicilliodes, Aureobasidium pullulans* and *Wallemia sebi*, respectively (*Fig. 4*). However, variations in the order were observed for 3 out of 15 shopping malls surveyed. *Aureobasidium pullulans* replaced *Aspergillus penicilliodes* as the top dominant species in shopping mall SC06, while *Wallemia sebi* became the second dominant species in shopping malls SC04 and SC15. All 3 species have been found to be associated with asthma in water-damaged homes in the US [2].

Many studies for mould exposure in indoor environment in the USA were traced to the dampness in homes [3]. *Aspergillus penicillioides* and *Wallemia sebi* are found in higher amounts in water-damaged homes in the US, and have been implicated to play a role in childhood asthma. Interestingly, the common species in Singapore's shopping malls were also found in

water-damaged homes of asthmatics in the US [2] (*Table 6*). The health implication in our context needs to be further studied.

The study showed that the RMI for Singapore's shopping malls fall within the range of 1.70 to 50.53. RMI of a building, based on the relative mould indices calculated for all surveyed shopping malls, is categorized into 4 tiers, which correspond to their likelihood of having mould problem (*Fig. 5*). Most of the Singapore's shopping malls (33.3%) fell under Tier 3, which has an RMI range of 20.7 to 30.09, indicating a high likelihood of having mould problems.

The average relative mould index for a water-damaged shopping mall is 37.3, which is approximately 2 times higher than that for typical shopping malls (*Fig. 6*). Similar trend was observed in the US where the RMI for water-damaged homes was higher than that of a typical home [2]. Results also showed that 22 out of the 36 mould species have a higher concentration in water-damaged shopping malls. This is probably due to the high relative humidity in water-damaged shopping malls, providing an environment conducive for mould growth.

Further study is required to refine the variation between the flora present in water- damaged and typical buildings so as to further refine the RMI that suits Singapore's context.

While there is no observed illness linked to the current findings, further studies are also required to investigate its implications to human health. Nevertheless, this data calls for proper maintenance and rectification of buildings, so as to prevent any excessive mould proliferation.

Aspergillus niger was found to replace *Aurobasidium pullulans* as the second dominant species in water-damaged shopping malls. This could be due to the opportunistic and high water activity a characteristic of A*spergillus niger,* taking advantage of the excessively high moisture content in water-damaged

Figure 4
The concentrations of top 3 mould species in individual shopping mall

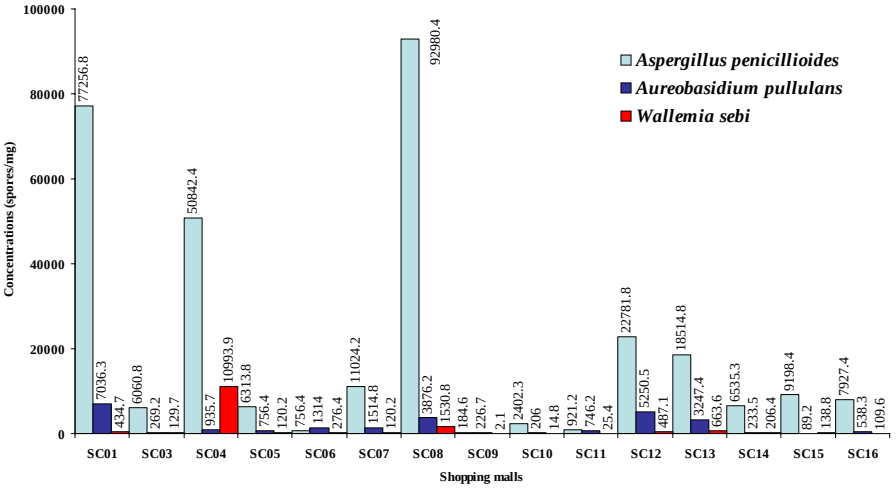


Table 6

Comparison of common mould species between Singapore's shopping malls and homes in the US

Singapore	United States	
Shopping malls	**Typical homes**	**Homes of asthmatics**
Aspergillus penicillioides	*Epicoccum nigrum*	*Eurotium amstelodami*
Aureobasidium pullulans	*Aureobasidium pullulans*	*Aspergillus penicillioides*
Wallemia sebi	*Cladosporium cladosporioides 1*	*Wallemia sebi*

Figure 5
Relative mould index (RMI) of Singapore's shopping malls

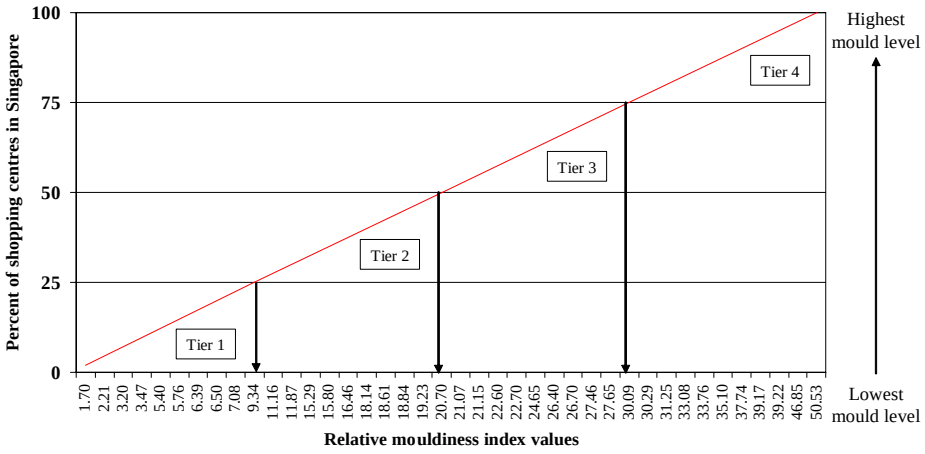
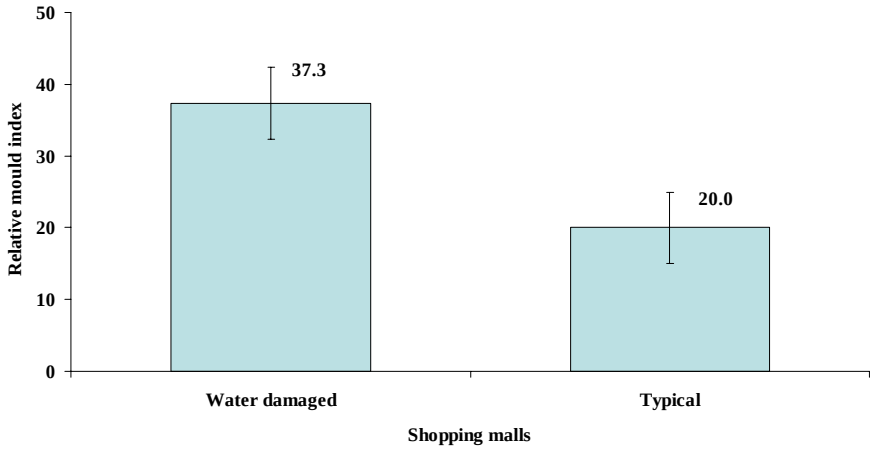


Figure 6
Comparison of relative mould index between water-damaged and typical shopping malls



buildings. While it is common knowledge that *Aspergillus fumigatus* accounts for 80% to 90% of human aspergillosis, a few other species of *Aspergillus* are also capable of causing invasive disease (e.g. *A niger*, *A flavus*) [4].

Although no mould-related health effects have been reported by tenants or alike from the shopping malls, monitoring of these mould species is important to understand the common flora in our local indoor environments and for linking future clinical cases to environmental contaminants. Mould specific quantitative PCR and the RMI have the potential to be used for mould investigation, and rapid assessment of extent of water damage in a building.

(Reported by Toh ZA, Goh V, Yap J and Ng LC, Environmental Health Institute (EHI), National Environment Agency, Singapore)

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