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Spatial and temporal distribution of dengue serotypes in Singapore

Background

Dengue is a disease caused by four serotypes of a flavivirus of the same name. Serotype shifts have been associated with outbreaks and significant increases in the incidence of dengue hemorrhagic fever (DHF). An example is the 2001 incident in Venezuela, where dengue (DEN)3 re-appeared after 32 years of absence, causing an enormous increase in the number of cases¹. The relation between the resurgence of new serotypes and possible severity of dengue related diseases stress the importance of close monitoring of emerging serotypes. This can only be done by continuous monitoring of different circulating serotypes, which could serve as an early warning for dengue outbreaks.

Singapore is hyperendemic for dengue, with all four serotypes circulating. However, a predominant serotype is usually apparent (*Fig 1*). Since 2002, the Environmental Health Institute (EHI), National Environmental Agency, has initiated a dengue serotype surveillance programme in support of Ministry of Health, through analysis of clinical samples collected from private hospitals and general practitioners. In 2005, the effort was enhanced to include the monitoring of spatial and temporal distribution of the 4 serotypes.

Methods and materials

Serotyping of dengue was previously done by isolation of virus, following by immunofluorescence assay. Since 2004, in-house developed duplex and multiplex RT-PCR techniques have enabled high throughput and more real-time results to be generated².

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Results and comments

In 2003, we detected a transition from DEN 2 to DEN 1³ (*Fig 1*), which was followed by a huge increase in the number of cases in Singapore in 2004. The DEN1 outbreak prolonged to 2005, leading to an unprecedented level of 14,209 cases within the year.

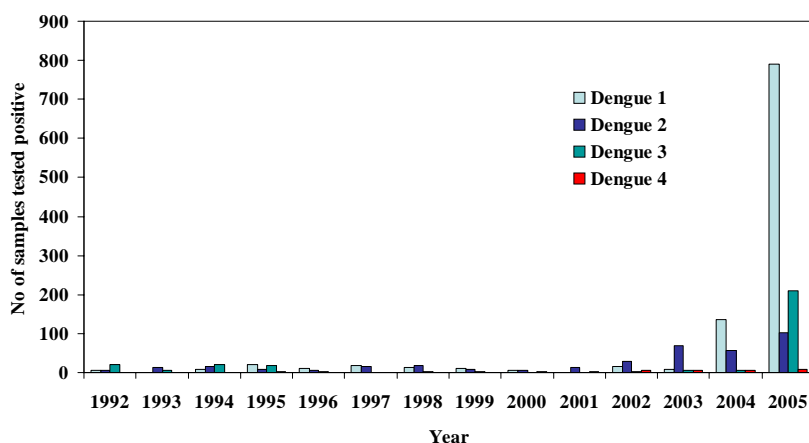
The serotype surveillance result showed DEN 1 to be the predominant serotype for 2005-2006. While the proportion of DEN 2, the predominant serotype prior to 2004, had been reduced, DEN 3 appeared to be an emerging serotype in the height of the outbreak in 2005 (*Fig 1*). Through spatial analysis of serotype distribution, we detected an emergence of DEN 3 from certain foci of Singapore (*Fig 2*). Singapore has not seen much of DEN 3 in the last decade, suggesting that a large part of population may be susceptible to it. In other parts of the world, DEN 3 has previously caused an epidemic in Havana city in 2001-2002, resulting in 13,000 cases, including 81 DHF cases⁴. In Venezuela, DEN 3 re-appeared in July 2000 after 32 years of absence and produced a prolonged major

outbreak – by end 2001, there were 83 000 cases of DF¹. WHO had also warned of the emergence of DEN 3 in South East Asia. An emergence of DEN 3 in Singapore, in the midst of a DEN1 outbreak, could aggravate the situation.

Local serotype information was passed on to the vector control operational department of the National Environment Agency, which beefed up vector control efforts in these areas to prevent the spread of the emerging serotype. The success in smothering the emergence of DEN 3 demonstrates the importance of serotype epidemiology for our vector control effort.

Since Nov 2005, the number of dengue cases has dropped significantly. Lately, an emergence of DEN 2 has also been detected and the serotype situation is under close monitoring (*Fig 3*). In this lull period, surveillance effort will continue, and much effort is being put into “recruiting” participating hospitals and clinics. At the same time, sequencing effort is also ongoing to determine the genotype of the dengue viruses circulating in Singapore.

Figure 1
Prevalence of the four dengue serotypes in Singapore, 1992 – 2005



Source: Ministry of Health



Vector control strategy based on serotype distribution forms an additional novel approach in dengue control in the National Environment Agency. Evaluation of the approach to determine its effectiveness, is ongoing. Private clinics could partici-

pate in the effort by sending blood samples of suspected dengue cases to the Environment Health Institute (For more information, please contact Ms Cheryl Cheng at Cheryl_Cheng@nea.gov.sg or 67719106).

Figure 2
Spatial distribution of dengue serotype, Jul-Sep 2005

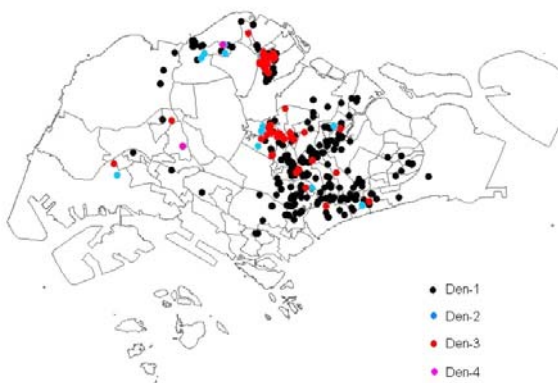
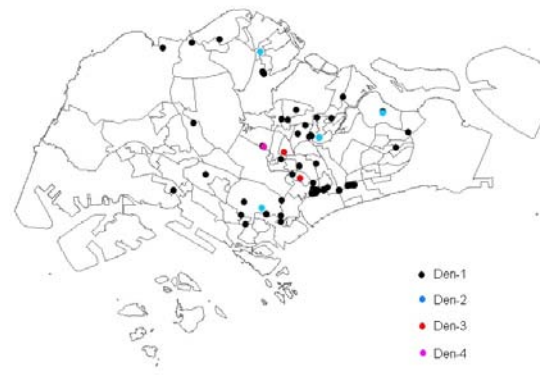


Figure 3
Spatial distribution of dengue serotype, Jul-Dec 2006



Acknowledgement

We are grateful to the hospitals and clinics that have contributed to the surveillance effort by sending clinical samples to the laboratory.

(Reported by Ng LC, Lai Y L, Cheng C Environmental Health Institute, National Environment Agency and Barkham T, Tan Tock Seng Hospital)

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Invasive pneumococcal disease in Singapore and pneumococcal vaccines

Introduction

Streptococcus pneumoniae is a gram-positive encapsulated coccus. Based on the differences in the composition of the polysaccharide capsule, about 90 serotypes have been identified. The capsule is an essential virulence factor. ¹

Pneumococci are transmitted by direct contact with respiratory secretions from both infected ill persons and healthy carriers. Pneumococci do not usually cause outbreaks. Invasive pneumococcal infections, defined as a clinical condition in which *Streptococcus pneumoniae* is isolated from a normally sterile site, include pneumonia, meningitis and febrile bacteremia. The common non-invasive pneumococcal infections are otitis media, sinusitis and bronchitis. ¹

Global disease burden

It is estimated that about 700,000 to 1 million children die of pneumococcal disease every year, most of whom are young children in developing countries. In terms of age-distribution, the disease is common among children below 2 years of age in both developed and developing countries. Elderly persons also experience relatively high rates of invasive pneumococcal disease. Growing resistance of *S. pneumoniae* to essential antibiotics underlines the urgent need for vaccines to control pneumococcal disease. ²

Situation in Singapore

From 1995 to 2004, 4,272 cases of invasive pneumococcal disease were admitted to hospitals in

Singapore. 98% of these were cases of pneumococcal pneumonia. During this period, the mean annual hospitalization rate for pneumococcal disease was 11 per 100,000 population. This was similar to those of the United Kingdom ³ and Canada ⁴, which had annual incidence rates of 9 per 100,000 to 15 per 100,000 population. However, our rate was lower than that in the USA ⁵ (24/ 100,000).

Over the period 1995-2004, the average number of cases of invasive pneumococcal disease that were hospitalised each year were :

- ~ 15 cases among infants (below 1 year of age) (rate: 30 per 100,000)
- ~ 75 cases among young children aged 1-4 years (rate: 40/100,000)
- ~ 60-70 cases among older children aged 5-14 years (12.4/100,000)
- ~ 150 cases among teens and adults (15-64 years) (4.5/100,000)
- ~ 140 cases among the elderly (>65 years) (67/ 100,000)

The median length of stay in hospital for all ages was 4 days. The overall case fatality was 3.2%. The highest case fatality rate occurred in the elderly above 75 years of age (8.4%) while infants (<1 year) had a case fatality rate of 3.6%.

A study on 147 cases of invasive pneumococcal disease was conducted in Kangar Kerbau Hospital (KKH) from 1997 – 2004. The median age of the



cases was 3 years (10% under 1 year, 14% aged 1-2 years, 46% aged 2-5 years). It was found that 22% required intensive care unit (ICU) admission, 24% developed complications and 6% died. In addition, 40% of the strains were resistant to penicillin, which was formerly the drug of choice for pneumococcal disease.

Local studies highlight the increasing resistance of *S. pneumoniae* strains to penicillin (25% in 1995⁶ and 63% in 1997 to 1999⁷). There has also been a high prevalence of resistance of *S. pneumoniae* to trimethoprim-sulfamethoxazole, tetracycline, erythromycin and chloramphenicol. The most prevalent serotypes encountered in our local pediatric population are 19F, followed by 23F, 6B, 14, 6A, and 15. Most of the penicillin-resistant as well as multi-drug resistant strains were of serotype 19F.⁷

Pneumococcal vaccines

Two types of pneumococcal vaccines are currently available in Singapore:

- a 23-valent pneumococcal polysaccharide vaccine (PPV) which contains polysaccharides derived from 23 most frequent or most virulent capsular types of *S. pneumoniae*. It targets children from the age of 2 years and adults who are at risk of invasive pneumococcal disease (IPD). The 23-valent pneumococcal vaccines currently registered with the Health Sciences Authority (HSA) are the *Pneumo 23* vaccine (Sanofi-Aventis) and *Pneumovax 23* (MSD).
- b 7-valent pneumococcal conjugate vaccine (PCV) which contains saccharides of the capsular antigen of *S. pneumoniae* serotypes 4, 6B, 9V, 14,

18C, 19F and 23F. It is marketed as *Prevenar* by Wyeth and targets the infants and children aged 6 weeks to 9 years.

23-valent pneumococcal polysaccharide vaccine (PPV)

In 2003, at least 17 European countries recommended that the PPV vaccine be administered to all those more than 65 years of age⁸. The recommended vaccination schedule is generally a single dose. In 1997, the US Advisory Committee on Immunisation Practices (ACIP) recommended the use of PPV for persons aged 65 years and above, and persons aged 2 years and above with certain risk factors.⁹ Australia¹⁰, Canada¹¹ and United Kingdom^{8,12} have also recommended the use of PPV.

The effectiveness of 23-valent PPV in the elderly and high-risk groups is controversial. According to a meta-analysis, the effectiveness of 23-valent PPV has not been demonstrated in elderly subjects or high-risk individuals.¹³ However, some observational studies have consistently shown protection (with the exception of immunocompromised persons) against IPD.¹⁴⁻¹⁶ In immunocompetent patients aged 65 to 74 years, vaccination effectiveness over a 5-year period was 71% (95% CI, 30% to 88%), and for those 75 to 84 years of age, its effectiveness over 3 years was 67% (95% CI, 20% to 87%).¹⁷

PPV is generally considered safe. Approximately one-half of persons who receive PPV develop mild, local side effects (e.g. pain at the injection site, erythema, and swelling). These reactions usually persist for less than 48 hours. Moderate and systemic reactions (e.g. fever and myalgias) and more severe local reactions (e.g. local induration) are rare.¹⁸



7-valent pneumococcal conjugate vaccine (PCV)

The efficacy of PCV against vaccine serotypes for invasive pneumococcal disease (IPD) (mainly pneumonia, also bacteraemia and meningitis) is about 94%, based on the randomised control trial and US surveillance data. Overall effectiveness can be expected to vary according to pattern of local serotypes causing IPD. The KKH study found that 84% of IPD is caused by vaccine serotypes; hence, with universal vaccination, PCV can be expected to prevent about 79% of IPD locally in children under 5 years of age.

In 2000, the US ACIP recommended that the 7-valent PCV be used for all children aged 2-23 months and for children aged 24-59 months who are at increased risk for pneumococcal disease.¹⁹ Australia¹⁰, Canada¹¹ and the UK²⁰ also have incorporated the use of pneumococcal conjugate vaccines in their national childhood immunization programme. The dosing schedule by age is shown below:

Age at 1st dose	Total number of 0.5ml doses
7 – 11 months of age	3*
12 – 23 months of age	2**
> 24 months through 9 years of age	1

* 2 doses at least 4 weeks apart; third dose after age one, separated from the 2nd dose by at least 2 months

** 2 doses at least 2 months apart

Since its incorporation into the childhood immunisation programme in the USA in 2000, the rate of invasive pneumococcal disease due to vaccine serotypes in children under 5 years of age have declined by 94%.²¹ Vaccination may also have protective effects on other age groups indirectly. The

duration of immunity following PCV is not yet established.

The majority of adverse events following PCV use were minor. A few children experienced allergic reactions, prolonged or abnormal crying, fussiness, dyspnea and gastrointestinal distress. There are also reports of rare but potentially serious events such as seizures, anaphylactic or anaphylactoid reactions, serum sickness, and thrombocytopenia.²²

Pneumococcal vaccination

Although studies have found mixed results of effectiveness of 23-valent PPV against pneumococcal diseases, the vaccine is generally safe and has been recommended in many countries. The Ministry of Health's Expert Committee on Immunisation (ECI) in May 2003 recommended that pneumococcal vaccination be provided to all elderly persons in institutionalized settings. The ECI also recommended that PPV be given to high-risk adults with chronic conditions such as diabetes mellitus and the immunocompromised.

The 7-valent pneumococcal conjugate vaccine (PCV) has been found to be effective in preventing pneumococcal diseases in young children. Many countries have incorporated the implemented PCV (*Prevenar*), into their childhood immunization programme. These countries include the United States, United Kingdom, Australia and Canada.

In the KKH study, it was found that the 7-valent PCV would cover 84% of all invasive strains. However, there are some recent concerns about replacement by non-vaccine serotypes causing infections.²³ It would be useful to conduct studies on the cost-effectiveness of vaccination programmes with PCV in the local context.



(Reported by Chan F, Ong G, and Cutter J, Communicable Diseases Division, Ministry of Health)

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Sequence-based typing for *Legionella pneumophila*

Introduction

Legionellosis is caused by an infection of members of the *Legionella* genus. The manifestation of the disease ranges from mild flu-like Pontiac fever to an acute fatal pneumonia¹. The majority of human legionellosis is caused by *Legionella pneumophila* serogroup 1. However, in recent years, there has been an increase in the number of human infections caused by other serogroups of *Legionella pneumophila*^{2, 3}. Considering that *Legionella pneumophila* consists of 15 or more serogroups, there is a need for techniques that could efficiently and accurately characterize clinical and environmental *Legionella* isolates. Such techniques could reveal the prevalence of each serogroup in the environment and among clinical cases. Information obtained with such high-resolution tool could aid in epidemiological investigation in case of outbreaks⁴.

Owing to its clinical significance, numerous methods have been used to identify and analyze the different species and strains of this genus. Molecular techniques, which have been employed for these purposes, include Amplified Fragment Length Polymorphism (AFLP), Variable Nucleotide Tandem Repeats (VNTR) and sequencing. The European Working Group for *Legionella* Infections (EWGLI), a consortium that is actively involved in *Legionella* surveillance, has recently developed the Sequence Based Typing (SBT) system.

We have adopted the SBT methodology to study the diversity of *Legionella* in water of cooling towers in Singapore. This method allows for excellent

typeability, reproducibility, and epidemiological concordance of *Legionella* serogroups 1-14⁴.

Methodology

Sample collection

200 ml of water was collected from the basin of each cooling tower. Water samples were sent to accredited laboratories for *Legionella* testing, according to the British Standards Institute Method (Water Quality: Detection and Enumeration of *Legionella*. 6068-4.12, 1998). Isolates were identified using the latex agglutination test. Positive isolates were transferred to the Environmental Health Institute (EHI), National Environmental Agency, for purification and further analysis.

DNA purification and molecular analysis

Pure isolates were propagated on BCYE for DNA extraction using Qiagen Dneasy Kit. Polymerase chain reaction (PCR) was performed on the purified DNA using six pairs of primers: proA, flaA, asd, pile, mip and mompS. A collection of six pairs of primers⁴ enables high-resolution differentiation of the isolates. The nucleotide sequence of each PCR product was determined. The sequences were submitted to the EWGLI database. Matching analysis of the six fragments of each isolate identified the isolate to a unique 6-digit profile.

Results and discussions

A total of 120 *Legionella* isolates from cooling towers were sent to EHI. Fig. 4 shows the breakdown



of *Legionella* isolates according to the latex agglutination test.

Preliminary results obtained from SBT analysis of 18 isolates revealed that 56% of our isolates produced novel profile IDs when compared with the EWGLI database (Fig. 5).

Among the novel profile isolates, 40% were *Legionella pneumophila* serogroup 1 while 60% were serogroup 2-14. On the other hand, among the isolates with profiles found in the database, 75% were serogroup 1 while 25% were serogroup 2-14. It is shown that the molecular make-up of the Singapore isolates is different from those of the EWGLI database.

The 18 isolates identified as *Legionella* generated 14 different 6-digit profiles. This shows the diversity of *Legionella* present in cooling towers. The information generated from this study can provide a clearer representation of *Legionella pneumophila* isolates present in Singapore. However, it is essential that the sample number be increased to present a more accurate baseline database. More work is currently being conducted at EHI to include isolates from potable water sources.

Using the SBT method, two isolates identified by test laboratories as *Legionella*, were found to be mis-identifications. It is probable that the polyclonal anti-*Legionella* antibody used in the latex agglutina-

Figure 4
Breakdown of *Legionella* isolates using latex agglutination test

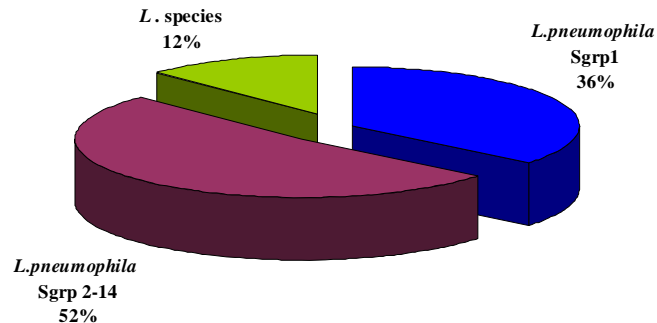
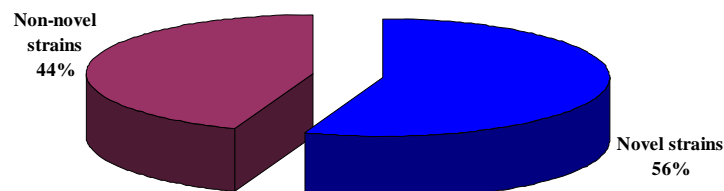


Figure 5
Distribution of novel and non-novel *Legionella* isolates



tion technique cross reacted with other gram-negative bacteria⁵. The specificity of the gene-based method renders it an excellent tool for research and epidemiological investigation. Currently, all isolates analysed are from the environment. In order to establish

associations between clinical cases and specific environments, it is essential to obtain clinical isolates through samples like sputum of patients. EHI welcomes *Legionella* isolates from clinical cases for confirmation and typing.

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

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HIV infection and AIDS in Singapore, 2006

Notifications

In 2006, another 357 Singapore residents (Singapore citizens and permanent residents) were newly reported with HIV infection. About 91% of the new cases detected were males. This brings the total number of HIV-infected Singaporeans to 3,060 as of end 2006. As at 31 Dec 2006, 1,307 persons are asymptomatic carriers, 705 have AIDS-related illnesses and 1,048 have died. The incidence rate of reported HIV/AIDS in 2006

was 98.9 per million population, an 11% increase from 89.2 per million population in 2005 (Fig 6).

Modes of HIV transmission

As in previous years, sexual transmission was the main mode of HIV transmission (Table 1). Of the 357 cases reported in 2006, 92% were infected through sex. Of these, two-thirds occurred via heterosexual



sex. The proportion of cases infected via intravenous drug use remained small (4%). However, the number of such cases increased from just 4 cases in 2005 to 14 cases in 2006.

Distribution by marital status and gender

The majority of HIV infected Singaporeans are males with 2,718 cases; 342 are females, giving a sex ratio of eight males to one female (*Table 2*). Among

the males, 60% were single at the point of diagnosis. For the females, however, the majority (61%) were married. Of the 357 cases reported in 2006, approximately 58% were single, while 30% were married, 10% were divorced and 2% were widowed.

Distribution by age and gender

More than half of all new cases reported in 2006 were aged between 30 and 49 years of age (*Table 3*). Just under one-fifth (57 cases) were aged between 20

Figure 6
Incidence of new HIV/AIDS cases, 1985 – 2006

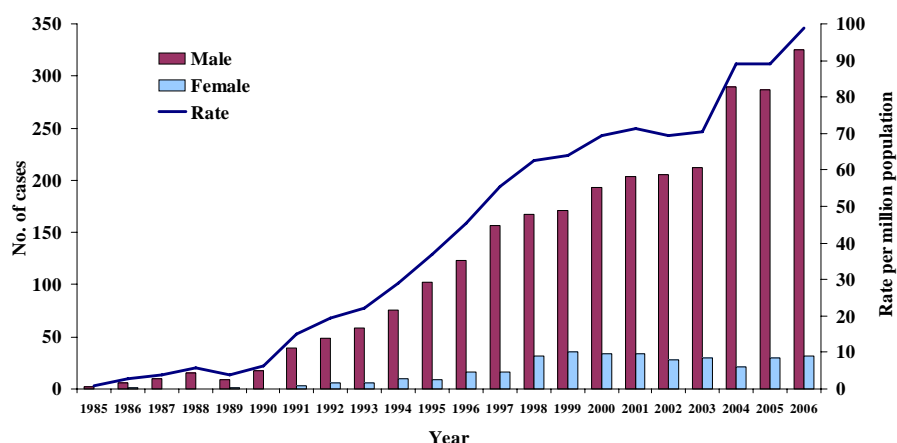


Table 1

Distribution of HIV-infected Singaporeans by mode of transmission, 1985 – 2006

Mode of transmission	1985 – 1999	2000	2001	2002	2003	2004	2005	2006
Sexual orientation								
Heterosexual	789	190	181	181	177	188	185	222
Homosexual	157	12	22	30	40	72	87	94
Bisexual	114	16	16	12	14	22	14	14
Intravenous drug use	23	3	6	6	4	7	4	14
Blood transfusion	3	0	0	0	0	0	0	0
Renal transplant overseas	5	0	0	0	0	0	0	0
Perinatal (mother to child)	12	1	2	2	1	4	3	2
Uncertain	33	4	10	3	6	18	24	11
Total	1136	226	237	234	242	311	317	357



Table 2
HIV-infected Singaporeans by marital status and gender, 1985 – 2006

Marital status	1985 – 1999	2000	2001	2002	2003	2004	2005	2006
Male								
Single	654	104	110	106	112	180	176	198
Married	255	61	60	71	65	74	82	92
Divorced/Separated	70	23	26	25	28	25	27	30
Widowed	22	5	8	4	7	11	2	5
Female								
Single	25	11	4	6	3	7	7	8
Married	93	18	17	15	21	8	19	17
Divorced/Separated	12	1	6	3	5	2	3	6
Widowed	5	3	6	4	1	4	1	1
Total								
Single	679	115	114	112	115	187	183	206
Married	348	79	77	86	86	82	101	109
Divorced/Separated	82	24	32	28	33	27	30	36
Widowed	27	8	14	8	8	15	3	6

Table 3
HIV-infected Singaporeans by age and sex, 1985 – 2006

Age group	1985 – 1999	2000	2001	2002	2003	2004	2005	2006
Male								
0 – 9	6	0	2	1	1	1	0	2
10 – 19	8	1	0	0	1	4	4	1
20 – 29	217	16	17	22	22	41	36	50
30 – 39	432	64	61	53	71	82	85	91
40 – 49	202	71	61	67	66	84	83	90
50 – 59	70	25	34	39	29	44	49	60
60 & above	66	16	29	24	22	34	30	31
Female								
0 – 9	6	1	0	1	0	2	3	1
10 – 19	2	0	1	1	0	0	0	1
20 – 29	56	15	10	10	7	6	8	7
30 – 39	39	8	6	3	12	7	10	8
40 – 49	18	3	7	6	8	5	1	7
50 – 59	9	4	4	6	3	0	7	6
60 & above	5	2	5	1	0	1	1	2
Total								
0 – 9	12	1	2	2	1	3	3	3
10 – 19	10	1	1	1	1	4	4	2
20 – 29	273	31	27	32	29	47	44	57
30 – 39	471	72	67	56	83	89	95	99
40 – 49	220	74	68	73	74	89	84	97
50 – 59	79	29	38	45	32	44	56	66
60 & above	71	18	34	25	22	35	31	33



and 29 years of age. Some of them may have got infected when they were teenagers as HIV may go undetected for several years.

Stage of diagnosis and detection

In 2006, more than half (58%) of the new cases already had late-stage HIV infection (CD4+ cell count of less than 200 per cu mm or AIDS-defining opportunistic infections or both) when they were diagnosed. This was similar to the pattern in previous years. There is thus an urgent need for persons who engage in high risk behaviour such as unprotected casual sex and intravenous drug abuse to test themselves for HIV.

Most of the new cases in 2006 (78%) had their HIV detected when they had HIV testing in the course of some form of medical care. A much smaller proportion were detected as a result of voluntary HIV

screening (13%). The rest were detected through contact tracing and other screening. When differentiated by sexual orientation, a higher proportion of homosexuals had their HIV infection detected via voluntary screening compared to heterosexuals (35% vs 3%).

STI and high-risk sexual behaviour

Persons who have unprotected sex while engaging in high-risk behaviour have a higher risk of HIV and other sexually transmitted infections (STI). In 2006, a total of 7,100 cases of STI were also reported among Singapore residents. Persons engaging in high-risk sexual behaviour should go for HIV testing regularly so that the disease is detected and treated as early as possible. HIV treatment can significantly delay the onset of AIDS and reduce the risk of death.

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