Introduction

Standardised antimicrobial susceptibility testing was first introduced to Singapore in the mid-1970s. The earliest Singapore antibiogram the author is aware of was published in 1974 by Tan et al (Fig. 1) based on a limited number of isolates from sterile and non-sterile sites at the Department of Pathology, Ministry of Health. Today’s workhorse antibiotics, such as the extended-spectrum cephalosporins (ESCs, ceftriaxone and ceftazidime) and carbapenems (imipenem, meropenem and ertapenem) were unavailable in 1974 for comparison. However, it is interesting to note that almost all hospital Escherichia coli were susceptible to simple antibiotics like nalidixic acid (94%), sulfamethoxazole-trimethoprim (95%) and gentamicin (97%), though susceptibility to ampicillin was only 55%. The corresponding figures in 2006 (with a much larger sample) were ciprofloxacin (61.3%), sulfamethoxazole-trimethoprim (59.6%), gentamicin (84.6%) and ampicillin (38.7%). For the purpose of this review, the discussion will be confined to ESC, carbapenem, and fluoroquinolone resistance as these are the most pressing gram-negative resistance issues confronting the local clinician.

Extended-spectrum β-lactamases (ESBLs)

In terms of scale, the single largest gram-negative resistance problem in Singapore hospitals is ESBL-producing Klebsiella spp and E. coli. ESBLs were first described in Germany in 1985. They are defined as enzymes with the ability to hydrolyse ESCs and are inhibited by clavulanate. The original ESBLs were point-mutation derivatives of the restricted-spectrum TEM and SHV enzymes commonly found in ampicillin resistant E. coli and Klebsiella spp.

Mulgrave described a resistant strain of Klebsiella pneumoniae isolated in 1988 from a patient in the Charles Gairdner Hospital, Perth “who had recently undergone extensive surgery in Singapore”. The ESBL from this isolate had an isoelectric point of 8.2, hydrolysed ceftazidime preferentially to cefotaxime, and was eventually identified as SHV-12. Because sporadic ESBL-producing bacteria had already been isolated in Western Australia, it could not be ascertained with certainty that the strain had been acquired in Singapore (personal communication, Leigh Mulgrave). Nevertheless, by 1994 it was noted that all ceftriaxone-resistant Klebsiella spp. in the Singapore General Hospital (SGH) were ESBL-producers (personal communication A/Prof Raymond Lin, Department of Laboratory Medicine, National University Hospital).

The next publication describing ESBLs in Singapore was that by Inglis et al based on Enterobacteriaceae at the National University Hospital (NUH) in 1993. When they reviewed the laboratory records retrospectively to 1985, they found that the first ESC-resistant Klebsiella spp was isolated in blood culture in their hospital in 1986.

We have since sequenced SHV-5/SHV-12-like ESBL genes in 2 meropenem-resistant, imipenem-susceptible K. pneumoniae from KK Women’s and Children’s Hospital (KKWCH) and SGH (unpublished data). These remain the

Abstract

In the past 3 decades, classical extended-spectrum β-lactamases (ESBLs) have probably been the main contributors to gram-negative antimicrobial resistance in Singapore. These appear to be being replaced by the newer CTX-M ESBLs. Metallo-β-lactamases are found in Pseudomonas aeruginosa but do not seem to have spread widely in Acinetobacter spp. and Enterobacteriaceae. Carbapenem-hydrolysing oxacillinases are prevalent in multidrug-resistant Acinetobacter spp. More insidious developments include the emergence of plasmid AmpC β-lactamases and multifactorial quinolone resistance in Enterobacteriaceae.

Key words: AmpC, Carbapenemase, Extended-spectrum β-lactamase, Oxacillinase, Quinolone
**Fig. 1.** The first published Singapore antibiogram.

**Fig. 2.** Percentage of *E. coli* resistant to extended-spectrum cephalosporins.

**Fig. 3.** Percentage of *Klebsiella* spp. resistant to extended-spectrum cephalosporins.
only classical ESBLs in Singapore to be characterised.

The increase in ESC-resistant *Klebsiella* spp. and *E. coli* is charted in Figures 2 and 3. These studies are not strictly comparable because they have different sampling criteria and study populations but have been juxtaposed to give a sense of how things have developed.13-14

ESC resistance in *Klebsiella* spp. increased rapidly up to 1990 and seems to have stabilised at 35% to 40% of isolates. In contrast, the increase in resistance in *E. coli* has been more gradual but may not have peaked yet. This increase may be due to the introduction of new types of ESBLs. The initial ESBL isolates in Singapore tended to be equally if not more resistant to ceftazidime compared to ceftriaxone and were therefore likely to be the classical TEM or SHV-type.8,10,12,15

The SENTRY study is an international antimicrobial resistance surveillance programme that collects bacteria isolated from diverse body sites. In the first survey period from 1998 to 1999, the percentage of ESBL producers among *E. coli* (78 isolates) from all sites in TTSH was 5.1%. The corresponding figure in *Klebsiella* spp. (65 isolates) was 41.5%. The proportion of ESBLs in *Klebsiella* spp. from Singapore was the second highest among the survey countries (Australia, Hong Kong, Japan, China, Philippines, South Africa and Taiwan). There was no difference between ceftazidime and ceftriaxone as the preferred substrate although the numbers were small.16

When this study was extended to 2002, the proportion of *E. coli* (318 isolates) that produced ESBLs had increased to 11.3% whereas the proportion in *K. pneumoniae* (225 isolates) had fallen to 35.6%. Ceftriaxone was now the preferred substrate of ESBLs in *E. coli* (97.2% versus 83.3%).14

Local microbiologists had also started to notice a subtle change in the resistance pattern of ESBL-producing *Enterobacteriaceae* in the late 1990s. *E. coli* and *Klebsiella* spp. were starting to emerge as being resistant to ceftriaxone but apparently susceptible to ceftazidime. In 2003, 2 *K. pneumoniae* and 1 *E. coli* were isolated in Changi General Hospital (CGH) with this resistance phenotype. The *K. pneumoniae* were found to be carrying genes for CTX-M-9 type and CTX-M-1 type ESBLs, and the *E. coli* a CTX-M-2 type ESBL.17

TEM/SHV or CTX-M ESBL, does it make a difference? CTX-M enzymes originate from the chromosomal β-lactamases of *Klobrera* spp.18,19 They have recently emerged in many countries and have even started to replace incumbent TEM and SHV enzymes as the predominant ESBL type.20

The earliest report of community-acquired bacteraemia with an ESBL-producing strain in Singapore was an *E. coli* in a 50-year-old woman who presented with uncomplicated septicaemia to casualty in CGH in 2000. Her only healthcare contact was a hospitalisation 1 year before.21 That was a sporadic case and the type of ESBL was never characterised. However, unlike classical TEM and SHV ESBLs that are typically associated with nosocomial spread, CTX-M ESBLs seem to be capable of spreading in outbreaks outside the hospital as well. In the United Kingdom, community spread of CTX-M-15-producing *E. coli* (CTX-M-1 group) has emerged as a particular problem.22 A recent study concluded that the worldwide spread of the gene for the β-lactamase CTX-M-15 (bla<sub>CTX-M-15</sub>) is due to 2 epidemic *E. coli* clones belonging to multi-locus sequence types ST131 and ST405.21 It would be interesting to investigate if the same phenomenon is responsible for the increase in prevalence of ESBLs among *E. coli* in Singapore.

Finally, some CTX-M ESBLs may also be associated with carbapenem resistance in combination with porin loss or efflux.24

ESBLs are not confined to *E. coli* and *Klebsiella* spp. In the SENTRY studies, 44% of *Enterobacter cloacae* (27 strains collected from 1998 to 2001) and 17.9% of *Proteus mirabilis* (39 isolates collected from 1998 to 2002) from Tan Tock Seng Hospital (TTSH) were ESBL positive.24,25 Eight per cent of *Serratia* spp. (12 isolates), 44% of *Enterobacter* spp. (18 isolates), and 11% of *Citrobacter* spp. (18 isolates) at NUH in 1998 were ESBL producers.12

**Plasmid AmpC β-lactamases (pAmpCs)**

Up to the turn of the millennium it could be assumed that a *Klebsiella* spp. or *E. coli* isolated in Singapore that was resistant to ESCs would be an ESBL producer. For example in 2000, Chiew found that all 145 *Klebsiella* spp. and 52 *E. coli* in CGH that were resistant to ceftazidime were ESBL producers.26

Shortly afterwards, local microbiologists started noticing *Enterobacteriaceae* that were showing reduced susceptibility to ESCs but were negative for ESBL production by routine laboratory tests. This coincided with a worldwide increase in the prevalence of pAmpC. These are related to the cephalosporinas that are found on the chromosomes of *Enterobacter* spp., *Serratia* spp., *Citrobacter freundii*, *Proteus vulgaris*, *Providencia* spp. and *Morganella morganii*. They confer resistance to the ESCs and β-lactam inhibitor combinations but remain susceptible to cefepime.27

The presence of pAmpC in Singapore was first confirmed when 2 ESC-resistant, ESBL-negative *E. coli* isolated at CGH in 2003 were found to have CMY-2-like genes (bla<sub>CMY-2_like</sub>).17
This resistance pattern was studied in SGH from 2004 to 2005. Of the 48 cefoxitin resistant ESBL-negative *E. coli*, 96% were positive for bla$_{CMY}^*$ by polymerase chain reaction. In a collection of 110 ESBL-producing *E. coli*, 7% were also positive for bla$_{CMY}^*$. A representative strain was sequenced and confirmed the presence of bla$_{CMY}^*$. From our experience, any *E. coli* that shows reduced susceptibility to an ESC but is ESBL negative is almost certainly going to be carrying bla$_{CMY}^*$.

The situation is surprisingly different in *Klebsiella* spp. Fifty-three per cent of 17 cefoxitin resistant ESBL-negative *Klebsiella* spp. isolated in SGH between 2004 and 2005 contained a different pAMPC gene, bla$_{DHA}$. Six per cent of 104 strains of ESBL-producing *Klebsiella* spp. also contained bla$_{DHA}$. Three representative strains were sequenced and were shown to carry bla$_{DHA}$. We have since also found inducible DHA β-lactamases in *E. coli* and *Salmonella* spp.28

In 2004, microbiologists in NUH found that about 21% of *E. coli* and *Klebsiella* spp. had pAmpC (personal communication – Dr G Kumarsinghe, Department of Laboratory Medicine, National University Hospital).

Returning to CGH, Tan et al found that from 2005 to 2006, 30% of *Klebsiella* spp. (of 43 isolates), 23% of *E. coli* (of 153 isolates) and 80% of *P. mirabilis* (of 5 isolates), that were resistant to amoxicillin-clavulanate, cefuroxime and cephalaxin but showed no phenotypic evidence of ESBL activity, were positive for pAmpC. Genes coding for CMY-2 like enzymes were present in *E. coli* predominantly (34 isolates), but were also present in *Klebsiella* spp. (3 isolates), and *P. mirabilis* (4 isolates). Genes coding for DHA-1-like enzymes were found in 10 *K. pneumoniae* and 1 *E. coli*.29

CMY-2 is similar to the chromosomal AmpC from *C. freundii* and is the most common and widely distributed pAmpC worldwide. DHA-1 is similar to the chromosomal AmpC from *M. morganii* and is best described in Korea and Taiwan.30,31 Recently, Kurupati et al described a *Klebsiella pneumoniae* which contained the restricted-spectrum β-lactamases TEM-1, SHV-11 and the pAmpC DHA-1.32 *Klebsiella* spp. containing the exact same complement of β-lactamases have been described in Taiwan.31

The impact of pAmpC in Singapore is still relatively small compared to that of ESBLs but they have the potential to become a significant problem for the following reasons.

Firstly, they are difficult for the laboratory to detect. No major standardised susceptibility testing method specifically addresses pAmpC. In the CGH study, up to 34% of pAmpC-positive isolates appeared to be susceptible to ceftriaxone by standard susceptibility testing.29 This is a particular problem with DHA as it is not constitutively expressed like CMY. DHA is strongly induced by antibiotics like clavulanate, cefoxitin and imipenem but not by ceftriaxone and aztreonam. Therefore, a number of isolates have been found which appear susceptible to ceftriaxone and aztreonam at the dilutions performed during routine testing. However, these isolates readily develop mutants at higher inoculums which constitutively hyper-produce the β-lactamase, giving rise to a >30-fold increase in minimal inhibitory concentration (MIC). This may be clinically relevant to infection at sites with poor antimicrobial penetration and high bacterial cell densities.28 The use of cephalosporins to treat infections with bacteria known to produce AmpC β-lactamases (*Enterobacter* spp., *Serratia* spp. *C. freundii*, *P. vulgaris*, *Providencia* spp. *M. morganii*, and now pAmpC-producing *E. coli* and *K. pneumoniae*) is prone to failure regardless of the in-vitro susceptibility result and alternative antimicrobials (possibly a carbapenem or fluroquinolone) should be preferred.33

Second, because they are weak carbapenemases, hyper-production of pAmpC in association with porin loss may even lead to carbapenem resistance.34 Forty per cent of 20 carbapenem-resistant *K. pneumoniae* isolated in SGH between 2004 and 2006 were positive for bla$_{DHA}$.28 Such isolates are sporadic but have been increasing in recent years.

Lastly, CMY-2 may be associated with community spread and may enter into the food chain.35 In the CGH study, 35% of pAmpC positive strains were isolated within the first 48 hours of admission and had no previous record of hospitalisation in that hospital in the previous 90 days suggesting the possibility of community acquisition.29

**Inhibitor-resistant Phenotype**

The late 1990s or early 2000s saw the emergence of *E. coli* which were resistant to piperacillin-tazobactam and amoxicillin-clavulanate but retained susceptibility to ceftriaxone (this is the exact opposite of the resistance pattern seen with ESBLs). This phenotype can be caused by any one of 4 mechanisms, none of which hydrolyse ceftriaxone. These include hyper-production of a restricted-spectrum TEM β-lactamase like TEM-1, or a derivative that has acquired mutations which are sufficient to alter the substrate specificity of the enzyme to include β-lactamase inhibitors.28 In a small study in SGH, all 17 *E. coli* isolates with reduced susceptibility to amoxicillin-clavulanate and piperacillin-tazobactam, but susceptible to ESBLs contained bla$_{TEM-1}$ (personal communication – Dr LY Hsu, Assistant Professor, Yong Loo Lin School of Medicine, National University of Singapore). Another mechanism that gives rise to this phenotype is the production of an oxacillinase. About 8% of *E. coli* in SGH may carry the bla$_{OXA-1}$ gene. An unusual feature of OXA-1 is that it may rarely confer high-level resistance to ceftazidime while retaining susceptibility.
to ESCs (the opposite of pAmpC β-lactamases). Three E. coli with this unusual phenotype were recently isolated in SGH and have not been described anywhere else in the world. Two of the isolates also showed diminished susceptibility to ertapenem (8 to >32 mg/L) though the mechanism for this is unknown.

For one of these isolates, \( \text{bla}_{\text{OXA-1}} \) was found on a 3.7 kb integron together with the plasmid-mediated aminoglycoside resistance gene \( \text{aac(6')-Ib-cr} \) that is also associated with quinolone resistance (see below). An integron is a DNA element that acts as an assembly framework for gene cassettes that are usually antimicrobial resistance genes. While they are not mobile in themselves, integrons may be incorporated into plasmids and transposons and therefore have the potential to transfer many resistance genes simultaneously. Interestingly, the sequence of this particular integron was virtually identical to that found in an E. coli described in Shanghai.

**Carbapenemases**

ESBLs and pAmpCs are able to hydrolyse ESCs, but remain susceptible to carbapenem antibiotics. The carbapenemases have consequently become the treatment of last resort. The metallo-β-lactamases (MBLs) are able to hydrolyse even the carbapenems but until recently were only found in the chromosomes of relatively uncommon and less pathogenic gram-negative bacilli like Stenotrophomonas maltophilia and Elizabethkingia meningoseptica (formerly Flavobacterium meningosepticum).

The first transferable MBL IMP-1 was found on plasmids in Pseudomonas aeruginosa in Japan in 1988. The first description of IMP-1 outside Japan was in a K. pneumoniae strain isolated from a haematology patient in SGH in 1996. When the \( \text{bla}_{\text{IMP-1}} \) gene was transferred by conjugation to an E. coli recipient, the transconjugant showed an 8-fold rise in imipenem MIC from 0.25 mg/L to 2 mg/L. This was much lower than the imipenem MIC of the original K. pneumoniae isolate (>128 mg/L). However on repeated subculture, the K. pneumoniae isolates became imipenem susceptible again (4 mg/L). Further investigation showed that a porin that was not expressed in the resistant isolate was now being expressed. Taken together, these 2 findings imply that Enterobacteriaceae that carry MBL genes may appear carabapenem susceptible, while still retaining the potential for developing full-blown carbapenem resistance. The anticipated spread of MBLs in K. pneumoniae does not seem to have materialised as carbapenem resistance in this species in Singapore seems to be largely due to pAmpC (see above).

Carbapenem resistance is more common in P. aeruginosa than in the Enterobacteriaceae. As early as 1991, 10% of 243 isolates of P. aeruginosa in NUH were resistant to imipenem. Because \( \text{bla}_{\text{IMP-1}} \) had been found in P. aeruginosa in Japan, it was logical to see if these genes could also be found in this species in Singapore.

Ninety-six imipenem-resistant P. aeruginosa were collected in SGH from 1999 to 2001. Thirty-six isolates were MBL producers by phenotypic testing (37.5%). MBLs therefore represented 1.7% of all P. aeruginosa isolates in SGH in this study. This compared with 1.3% of all P. aeruginosa in Japan from 1996 to 1997. Thirty-five isolates had \( \text{bla}_{\text{IMP-1}} \) with some sharing the same nucleotide sequence as isolates in Japan, and others having a local variant which had a number of silent mutations. There was evidence of clonal spread in both SGH and a 200-bed community hospital with some clones common to both suggesting cross-institutional spread. One isolate had the gene for IMP-7, a relative of IMP-1 with which it shares 91% amino acid identity. IMP-7 was first described in P. aeruginosa in Japan from Canada (Calgary) and Malaysia. Interestingly, \( \text{bla}_{\text{IMP-7}} \) was recently described in P. aeruginosa in Japan from a patient who developed interstitial pneumonia while living in Singapore in 2005. This patient had been hospitalised and received artificial ventilation in Singapore before being transferred to Japan (personal communication – Prof M Sugai, Department of Bacteriology, Hiroshima University Graduate School of Biomedical Sciences).

\( \text{bla}_{\text{IMP-7}} \) and another acquired MBL gene \( \text{bla}_{\text{VIM-6}} \) have also been found in multiresistant Pseudomonas putida and Pseudomonas fluorescens. While these are relatively non-pathogenic, they may serve as reservoirs of resistance genes which may eventually be transferred to more pathogenic bacteria.

Another group of significant multiresistant gram-negative bacilli in the local context are the Acinetobacter spp. Multidrug-resistant Acinetobacter emerged as important pathogens in the Burns Unit of SGH from November 1990 onwards, with a strain that was resistant to all antibiotics (including amikacin, ampicillin-sulbactam, ceftaxone, ceftazidime, gentamicin, netilmicin, perfloxacin, ciprofloxacin, minocycline, imipenem) except polymyxin B isolated from 4 patients. In 1992, 2 strains were reported as being resistant to even polymyxin B. If it had been confirmed, this would probably be among the first reports of pan-resistant Acinetobacter spp. in the world. Unfortunately, these strains were not archived and are therefore unavailable for further study.

In 1991, 6% of 165 Acinetobacter spp. isolated in NUH were resistant to imipenem. This had increased to 12.9% of 70 isolates by 1993 to 1994. Sng and Yeo found that 16% of 99 clinical isolates of Acinetobacter baumannii-calcoaceticus complex isolated...
in SGH in 1993 were resistant to imipenem. Since multiresistant Acinetobacter spp. and P. aeruginosa are often found in the same setting, it would not be unreasonable to expect to find IMP-1 to be also responsible for carbapenem resistance in Acinetobacter spp. Surprisingly, this is not the case.

The first transferable carbapenemase to be described in Acinetobacter spp., OXA-23 (then called ARI-1) was found in an isolate in Scotland in 1985. An Acinetobacter spp. from Singapore in 1995 to 1997 was found to have a related oxacillinase, OXA-27, that shares 99% amino acid similarity to OXA-23.


We now know that there are 4 distantly-related families of OXA carbapenemases found in Acinetobacter spp. OXA-23-type, OXA-24-type, and OXA-58-type are transferable, whereas OXA-51-type appears to be specific to A. baumannii. These are even more remotely related to the non-carbapenem hydrolysing oxacillinases like OXA-1 (see above).

In SGH, 114 carbapenem-resistant Acinetobacter spp. were isolated over two 5-month periods between 1996 and 2001. The incidence of imipenem resistant Acinetobacter spp rose from 1.1 per 1000 admissions in 1996 to 2.3 per 1000 admissions in 2001. All A. baumannii that showed carbapenemase activity contained blaOXA-51-type and blaOXA-23 genes. There was a great diversity of blaOXA-51-type genes. Many were blaOXA-66 and blaOXA-69 as had been described by Brown but in addition there was blaOXA-68 and the novel blaOXA-88, blaOXA-91, and blaOXA-97. Since blaOXA-51-type genes are now thought to be intrinsic to A. baumannii, it was not surprising to also find them (blaOXA-93, blaOXA-94) in isolates which were susceptible to imipenem.

Two Acinetobacter genomospecies 3 and 2 Acinetobacter genomospecies 13TU isolates had blaOXA-28 and the blaIMPA4 MBL gene. Three of these were isolated from haematology patients from Indonesia. When the flanking regions were sequenced, blaIMPA4 was found to be on a 2.8 kb integron which was essentially identical to that found in blaIMPA4 bearing Acinetobacter spp. and Enterobacteriaceae in Hong Kong and Australia.

All 4 of these strains also contained a separate integron carrying blaPSE-1, a β-lactamase gene which is found in P. aeruginosa and E. coli and has not been described in Acinetobacter spp. before.

The problem with multiresistant Acinetobacter spp. is compounded by their ability to rapidly develop resistance to new antimicrobials. Tigecycline is one of the few recently developed novel antimicrobials with activity against gram-negative bacilli. However, in a recent study, 9% of 55 Acinetobacter spp. collected since 2004 in CGH already showed elevated MICs to tigecycline.

Quinolone Resistance in Enterobacteriaceae

While attention tends to focus on β-lactam resistance, quinolone resistance is also increasing to become a significant problem. In 1991, 12% of Klebsiella spp in NUH were resistant to ciprofloxacin. Hirataka noted in the 1998-2002 SENTRY study that ciprofloxacin co-resistance (46%) in ESBL-producing Klebsiella spp. in Singapore (TTSH) was high compared to most other countries. During a recent survey of local hospitals in the public sector, we found that 42% of Klebsiella spp were ciprofloxacin resistant.

Scheiders et al studied 19 ESC-resistant K. pneumoniae that were also resistant to fluoroquinolones from KKWH (23 isolates), SGH (9 isolates) and Alexandra Hospital (1 isolate). Strains with a ciprofloxacin MIC of ≥0.5 mg/L had a mutation in DNA gyrase A (Ser83 → Tyr, Leu, or Ile), and some also had a second Asp87 → Asn mutation. Isolates that had an MIC of 16 mg/L had an additional mutation in the ParC subunit of topoisomerase IV (Ser803 → Ile, Trp, or Arg). Some of these isolates also showed over-expression of the AcrAB-TolC efflux pump.

The newly described plasmid-mediated quinolone resistance determinants qnrA, qnrB, qnrS and aac(6’)-Ib-cr have all been found in local isolates of E. coli and Klebsiellasp. (personal communication – A/Prof Raymond Lin, Department of Laboratory Medicine, National University Hospital and Dr Deepak N Rama, Department of Pathology, Singapore General Hospital).

Conclusions

So what can we conclude from looking back at the evolution of gram-negative resistance in Singapore? Two themes quickly become apparent.

Firstly, life has become increasingly complicated for microbiologists. Multiple factors may act in concert to bring about antimicrobial resistance. It is no longer sufficient to detect a particular β-lactamase or to develop a drug that only targets 1 mechanism of resistance.

Secondly, there is circumstantial evidence to support the theory that resistant bacteria are constantly being imported (and exported). Medical tourism, travel in general and the increase in the local expatriate population may all contribute to this. The possibility of resistance genes in food and animals also needs to be explored.

Finally, big problems often start small. Our ESBL rates in E. coli in 1981 were similar to those in the Netherlands (3.2%) in 2006. Change may be imperceptible. This underlines the importance of a systematic national
antimicrobial resistance surveillance programme. To this end, an informal collection of microbiologists, pharmacists and infectious disease physicians have formed the Network for Antimicrobial Resistance Surveillance (Singapore). However, surveillance by itself is passive. Over the years, high rates of gram-negative resistance have become established in Singapore and new forms are rapidly emerging. Action needs to be taken now.

REFERENCES