Review Article

Gram-negative Resistance in Singapore: A Historical Perspective

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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*.

Ann Acad Med Singapore 2008;37:847-54

Key words: AmpC, Carbapenemase, Extended-spectrum β-lactamase, Oxacillinase, Quinolone

Introduction

Standardised antimicrobial susceptibility testing was first introduced to Singapore in the mid-1970s.[1] 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.[2] 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%).[3] 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.[4] 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.[5]

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.[6,7] 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.[8]

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

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TABLE 2. Summary of results of sensitivity testing for each micro-organism isolated from clinical specimens

	Staph. Aureus	E. Coli	Proteus Spp.	Pseudomonas Spp.	Klebsiella Spp.	Aerobacter Spp.
Total number	162	148	48	150	64	23
Percentage of sensitive cultures:						
Carbenicillin	–	(55)	(75)	(73)	(20)	(83)
Penicillin	(24)	–	–	–	–	–
Ampicillin	(27)	(55)	(69)	(3)	(17)	(52)
Cephaloridine	(99)	(88)	(65)	(1)	(75)	(43)
Doxycycline	(79)	(47)	(4)	(5)	(66)	(87)
Methicillin	(91)	–	–	–	–	–
Erythromycin	(81)	–	–	–	–	–
Chloramphenicol	(83)	(73)	(83)	(5)	(72)	(52)
Co-trimoxazole	(99)	(95)	(73)	(10)	(92)	(83)
Sulphonamide	–	(7)	(10)	(7)	(3)	(39)*
Tetracycline	(63)	(28)	(6)	(5)	(58)	(57)
Streptomycin	(60)	(68)	(54)	(37)	(59)	(39)
Gentamicin	(98)	(97)	(100)	(99)	(98)	(100)
Kanamycin	(91)	(94)	(77)	(37)	(92)	(96)
Polymyxin B	–	(95)	(6)	(92)	(95)	(96)
Naladixic Acid	–	(94)*	–	–	–	–
Nitrofurantoin	–	(100)*	–	–	–	–

Percentage of sensitive cultures for each antimicrobial agent is shown in parentheses.
* Tests were performed on only 67 strains of the total of 148. Percentage is calculated on number of positives out of 67.

Fig. 1. The first published Singapore antibiogram.

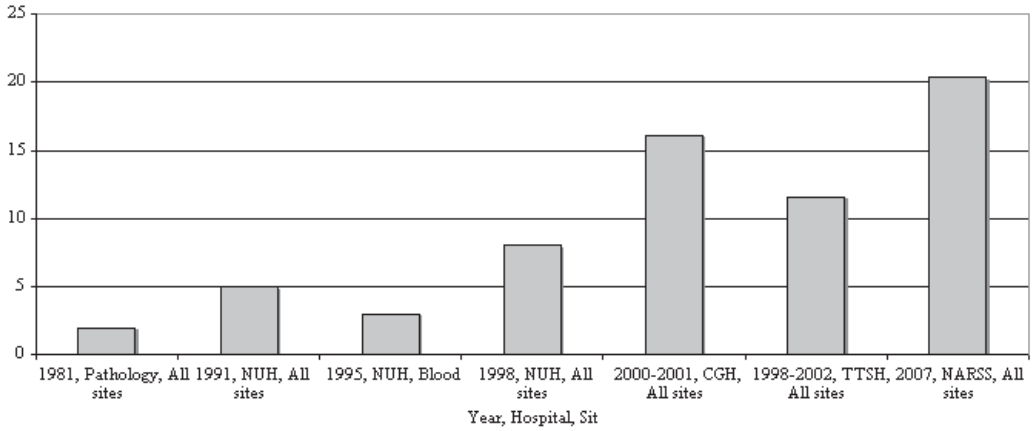

Fig. 2. Percentage of *E. coli* resistant to extended-spectrum cephalosporins.

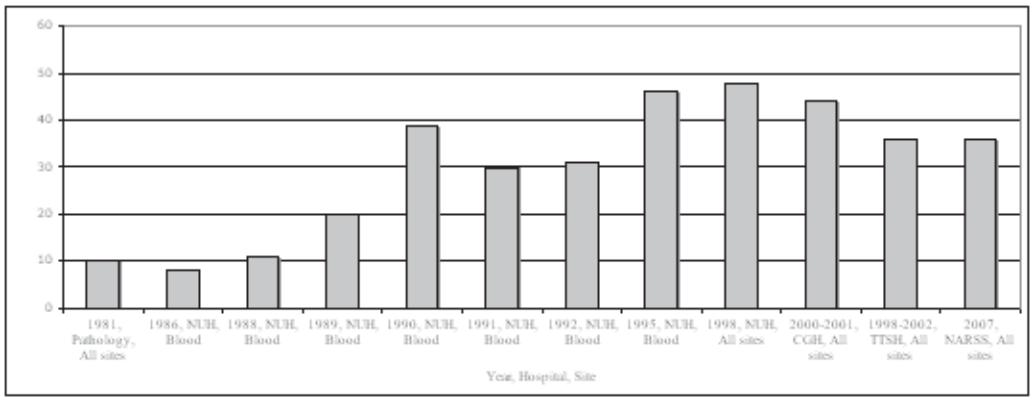

Fig. 3. Percentage of *Klebsiella* spp. resistant to extended-spectrum cephalosporins.

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.[3,8-14]

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 β-lactam substrate though 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 *Kluyvera* 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 SGH 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 ($bla_{\text{CTX-M-15}}$) is due to 2 epidemic *E. coli* clones belonging to multi-locus sequence types ST131 and ST405.[23] 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.[14,25] 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 cephalosporinases 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 ($bla_{\text{CMY-2-}}$like).[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-2}. 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. in SGH isolated 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-1}. 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 Kumarasinghe, 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 cephalexin 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.[36] In a small study in SGH, all 17 *E. coli* isolates with reduced susceptibility to amoxicillin-clavulanate and piperacillin-tazobactam, but susceptible to ESCs 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 cefepime while retaining susceptibility

to ESCs (the opposite of pAmpC β-lactamases).[37] 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, bla_{OXA-1} was found on a 3.7 kb integron together with the plasmid-mediated amino-glycoside resistance gene *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.[38] 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 carbapenems 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 meningo-septicum*).[39]

The first transferable MBL IMP-1 was found on plasmids in *Pseudomonas aeruginosa* in Japan in 1988.[40] The first description of IMP-1 outside Japan was in a *K. pneumoniae* strain isolated from a haematology patient in SGH in 1996.[41] When the bla_{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 become imipenem susceptible again (4 mg/L). Further investigation showed that a porin that was not expressed in the resistant isolate was now being expressed.[42] Taken together, these 2 findings imply that *Enterobacteriaceae* that carry MBL genes may appear carpabenem 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.[10] Because bla_{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.[43] Thirty-five isolates had bla_{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* from Canada (Calgary) and Malaysia.[44,45] Interestingly, bla_{IMP-7} was recently described in *P. aeruginosa* in Japan from a patient who developed interstitial pneumonia while living in Singapore in 2005.[46] 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).

bla_{IMP-1} and another acquired MBL gene bla_{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.[47]

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, ceftriaxone, ceftazidime, gentamicin, netilmicin, perfloxacine, ciprofloxacin, minocycline, imipenem) except polymyxin B isolated from 4 patients. In 1992, 2 strains were reported as being resistant to even polymixin 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.[48]

In 1991, 6% of 165 *Acinetobacter* spp. isolated in NUH were resistant to imipenem.[10] This had increased to 12.9% of 70 isolates by 1993 to 1994.[49]

Sng and Yeo found that 16% of 99 clinical isolates of *Acinetobacter baumannii-calcoaceticus* complex isolated

in SGH in 1993 were resistant to imipenem.[50] 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.[51] An *Acinetobacter* spp. from Singapore isolated in 1995 to 1997 was found to have a related oxacillinase, OXA-27, that shares 99% amino acid similarity to OXA-23.[52]

Brown et al described 2 new OXA-51-type β-lactamases (OXA-66 and OXA-69) in *Acinetobacter baumannii* collected from KKWCH between 1996 and 2000.[53]

We now know that there are 4 distantly-related families of OXA carbapenemases found in *Acinetobacter* spp.[54] 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.[55] 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 $bla_{\text{OXA-51}}$-type and $bla_{\text{OXA-23}}$ genes. There was a great diversity of $bla_{\text{OXA-51}}$-type genes. Many were $bla_{\text{OXA-66}}$ and $bla_{\text{OXA-69}}$ as had been described by Brown but in addition there was $bla_{\text{OXA-64}}$ and the novel $bla_{\text{OXA-88}}$, $bla_{\text{OXA-91}}$, and $bla_{\text{OXA-95}}$. Since $bla_{\text{OXA-51}}$-type genes are now thought to be intrinsic to *A. baumannii*, it was not surprising to also find them ($bla_{\text{OXA-93}}$, $bla_{\text{OXA-94}}$) in isolates which were susceptible to imipenem.

Two *Acinetobacter* genomospecies 3 and 2 *Acinetobacter* genomospecies 13TU isolates had $bla_{\text{OXA-58}}$ and the $bla_{\text{IMP-4}}$ MBL gene. Three of these were isolated from haematology patients from Indonesia. When the flanking regions were sequenced, $bla_{\text{IMP-4}}$ was found to be on a 2.8 kb integron which was essentially identical to that found in $bla_{\text{IMP-4}}$-bearing *Acinetobacter* spp. and *Enterobacteriaceae* in Hong Kong and Australia.[56,57]

All 4 of these strains also contained a separate integron carrying $bla_{\text{PSE-1}}$, 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 tigecyline.[58]

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.[10] 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.[14] During a recent survey of local hospitals in the public sector, we found that 42% of *Klebsiella* spp were ciprofloxacin resistant.[59]

Scheiders et al[60] studied 19 ESC-resistant *K. pneumonia* that were also resistant to fluoroquinolones from KKWCH (23 isolates), SGH (9 isolates) and Alexandra Hospital (1 isolate). Strains with a ciprooxacin MIC of ≥0.5 mg/L had a mutation in DNA gyrase A (Ser83 → Tyr, Leu, or IIe), 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 → IIe, 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 *Klebsiella* spp. (personal communication – A/Prof Raymond Lin, Department of Laboratory Medicine, National University Hospital and Dr Deepak N Rama, Department of Pathology, Singapore General Hospital).[61]

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.[62]

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.[63] 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

1. Tay L. Microbiology in Singapore. Ann Acad Med Singapore 1989;18:465-76.
2. Tan RJS, Seto WH, Sng EH, Tay L, Wee R. Antimicrobial sensitivity testing of hospital isolates using a standardised disc method. Asian J Mod Med 1974;10:361-3.
3. Network for Antimicrobial Resistance Surveillance (Singapore). Singapore: NARSS Report, Feb 2007.
4. Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother 1985;28:302-7.
5. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933-51.
6. Mulgrave L. Extended broad-spectrum beta-lactamases in Australia. Med J Aust 1990;152:444-5.
7. Howard C, van Daal A, Kelly G, Schooneveldt J, Nimmo G, Giffard PM. Identification and minisequencing-based discrimination of SHV beta-lactamases in nosocomial infection-associated *Klebsiella pneumoniae* in Brisbane, Australia. Antimicrob Agents Chemother 2002;46:659-64.
8. Inglis TJ, Kumarasinghe G, Chow C, Liew HY. Multiple antibiotic resistance in *Klebsiella* spp. and other *Enterobacteriaceae* isolated in Singapore. Singapore Med J 1994;35:602-4.
9. Annual Report. Singapore: Department of Pathology, Singapore General Hospital, 1982.
10. Kumarasinghe G, Chow C, Koh BL, Chiang KL, Liew HY, Ti TY. Antimicrobial resistance problem in a university hospital. Pathology 1995;27:67-70.
11. Kumarasinghe G, Chow C, Chiu C. In vitro activity of meropenem against organisms causing serious infections in a Singapore hospital. Int J Antimicrob Agents 1997;9:121-5.
12. Kumarasinghe G, Chow C, Tambyah PA. Widespread resistance to new antimicrobials in a university hospital before clinical use. Int J Antimicrob Agents 2001;18:391-3.
13. Chlebicki MP, Oh HM. Extended-spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella* spp. in a Singapore hospital: clinical spectrum. Ann Acad Med Singapore 2004;33:302-6.
14. Hirakata Y, Matsuda J, Miyazaki Y, Kamihira S, Kawakami S, Miyazawa Y, et al. Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). Diagn Microbiol Infect Dis 2005;52:323-9.
15. Biedenbach DJ, Lewis MT, Jones RN. In vitro evaluation of cefepime and other broad-spectrum beta-lactams for isolates in Malaysia and Singapore medical centers. The Malaysia/Singapore Antimicrobial Resistance Study Group. Diagn Microbiol Infect Dis 1999;35:277-83.
16. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN. Prevalence of extended spectrum beta-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998-99). Diagn Microbiol Infect Dis 2002;42:193-8.
17. Koh TH, Wang GC, Sng LH, Koh TY. CTX-M and plasmid-mediated AmpC-producing *Enterobacteriaceae* Singapore. Emerg Infect Dis 2004;10:1172-4.
18. Rodríguez MM, Power P, Radice M, Vay C, Famiglietti A, Galleni M, et al. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. Antimicrob Agents Chemother 2004;48:4895-7.
19. Olson AB, Silverman M, Boyd DA, McGeer A, Willey BM, Pong-Porter V, et al. Identification of a progenitor of the CTX-M-9 group of extended-spectrum beta-lactamases from *Kluyvera georgiana* isolated in Guyana. Antimicrob Agents Chemother 2005;49:2112-5.
20. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165-74.
21. Tan BH, Tan AL. Community-acquired bacteremia with an ESBL-producing *Escherichia coli* strain – a case report. Int J Infect Dis 2002;6:318-9.
22. Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother 2004;54:735-43.
23. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis 2008;14:195-200.
24. Woodford N, Dallow JW, Hill RL, Palepou MF, Pike R, Ward ME, et al. Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. Int J Antimicrob Agents 2007;29: 456-9.
25. Bell JM, Turnidge JD, Jones RN. Prevalence of extended-spectrum beta-lactamase-producing *Enterobacter cloacae* in the Asia-Pacific region: Results from the SENTRY Antimicrobial Surveillance Program, 1998 to 2001. Antimicrob Agents Chemother 2003;47:3989-93.
26. Chiew YF. Detection of extended-spectrum beta-lactamases in a Singapore routine clinical microbiology laboratory. J Hosp Infect 2004;56:328-9.
27. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type beta-lactamases. Antimicrob Agents Chemother 2002;46:1-11.
28. Koh TH, Sng LH, Wang G, Hsu LY, Lin RT, Tee NW. Emerging problems with plasmid-mediated DHA and CMY AmpC beta-lactamases in *Enterobacteriaceae* in Singapore. Int J Antimicrob Agents 2007;30:278-80.
29. Tan TY, Ng SY, Teo L, Koh Y, Teok CH. Detection of plasmid-mediated AmpC in *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. J Clin Pathol 2008;61:642-4
30. Yong D, Lim Y, Song W, Choi YS, Park DY, Lee H, et al. Plasmid-mediated, inducible AmpC beta-lactamase (DHA-1)-producing Enterobacteriaceae at a Korean hospital: wide dissemination in *Klebsiella pneumoniae* and *Klebsiella oxytoca* and emergence in *Proteus mirabilis*. Diagn Microbiol Infect Dis 2005;53:65-70.
31. Yan JJ, Ko WC, Jung YC, Chuang CL, Wu JJ. Emergence of *Klebsiella pneumoniae* isolates producing inducible DHA-1 beta-lactamase in a university hospital in Taiwan. J Clin Microbiol 2002;40:3121-6.
32. Kurupati P, Tan KS, Kumarasinghe G, Poh CL. Inhibition of gene expression and growth by antisense peptide nucleic acids in a multiresistant beta-lacamase-producing *Klebsiella pneumoniae* strain. Antimicrob Agents Chemother 2007;51:805-11.
33. BSAC Methods for Antimicrobial Susceptibility Testing Version 7.1. British Society for Antimicrobial Chemotherapy; 2008. Available at: http://www.bsac.org.uk/_db/_documents/version_7_1_february_2008.pdf. Accessed 12 April 2008.
34. Lee K, Yong D, Choi YS, Yum JH, Kim JM, Woodford N, et al. Reduced imipenem susceptibility in *Klebsiella pneumoniae* clinical isolates with plasmid-mediated CMY-2 and DHA-1 beta-lactamases co-mediated by porin loss. Int J Antimicrob Agents 2007;29:201-6.
35. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother 2001;45:2716-22.
36. Chaibi EB, Sirot D, Paul G, Labia R. Inhibitor-resistant TEM beta-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother 1999;43:447-58.
37. Koh TH, Wang G, Koh T. High-level cefepime resistance in *Escherichia coli* from Singapore producing OXA-1 beta-lactamase. Int J Antimicrob Agents 2008;31:382-3.
38. Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 1989 3:1669-83.
39. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases:

the quiet before the storm? Clin Microbiol Rev 2005;18:306-25.
40. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991;35:147-51.
41. Koh TH, Babini GS, Woodford N, Sng LH, Hall LM, Livermore DM. Carbapenem-hydrolysing IMP-1 beta-lactamase in *Klebsiella pneumoniae* from Singapore. Lancet 1999;353:2162.
42. Koh TH, Sng LH, Babini GS, Woodford N, Livermore DM, Hall LM. Carbapenem-resistant *Klebsiella pneumoniae* in Singapore producing IMP-1 beta-lactamase and lacking an outer membrane protein. Antimicrob Agents Chemother 2001;45:1939-40.
43. Kurokawa H, Yagi T, Shibata N, Shibayama K, Arakawa Y. Worldwide proliferation of carbapenem-resistant gram-negative bacteria. Lancet 1999;354:955.
44. Gibb AP, Tribuddharat C, Moore RA, Louie TJ, Krulick W, Livermore DM, et al. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla(IMP) allele, bla(IMP-7). Antimicrob Agents Chemother 2002;46:255-8.
45. Ho SE, Subramaniam G, Palasubramaniam S, Navaratnam P. Carbapenem-resistant *Pseudomonas aeruginosa* in Malaysia producing IMP-7 beta-lactamase. Antimicrob Agents Chemother 2002;46:3286-7.
46. Kouda S, Kuwahara R, Ohara M, Shigeta M, Fujiwara T, Komatsuzawa H, et al. First isolation of bla(IMP-7) in a *Pseudomonas aeruginosa* in Japan. J Infect Chemother. 2007;13:276-7.
47. Koh TH, Wang GC, Sng LH. IMP-1 and a novel metallo-beta-lactamase, VIM-6, in fluorescent pseudomonads isolated in Singapore. Antimicrob Agents Chemother 2004;48:2334-6.
48. Ang SW, Lee ST. Emergence of a multiply-resistant strain of *Acinetobacter* in a burns unit. Ann Acad Med Singapore 1992;21:660-3.
49. Kuah BG, Kumarasinghe G, Doran J, Chang HR. Antimicrobial susceptibilities of clinical isolates of *Acinetobacter baumannii* from Singapore. Antimicrob Agents Chemother 1994;38:2502-3.
50. Sng LH, Yeo M. Changing trends in the antimicrobial susceptibility of clinical isolates of *Acinetobacter baumannii*. Ann Acad Med Singapore 1996;25:179-83.
51. Paton R, Miles R, Hood J, Amyes S. ARI 1: beta-lactamase-mediated imipenem resistance in *Acinetobacter baumannii*. Int J Antimicrob Agents 1993;2:81-8.
52. Afzal-Shah M, Woodford N, Livermore DM. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2001;45:583-8.
53. Brown S, Amyes SG. The sequences of seven class D beta-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. Clin Microbiol Infect 2005;11:326-9.
54. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. J Antimicrob Chemother 2006;57:373-83.
55. Koh TH, Sng LH, Wang GC, Hsu LY, Zhao Y. IMP-4 and OXA beta-lactamases in *Acinetobacter baumannii* from Singapore. J Antimicrob Chemother 2007;59:627-32.
56. Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, Woodford N, et al. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob Agents Chemother 2001;45:710-4.
57. Thomas L, Espedido B, Watson S, Iredell J. Forewarned is forearmed: antibiotic resistance gene surveillance in critical care. J Hosp Infect 2005;60:291-3.
58. Tan TY, Ng LS. Susceptibility of multi-resistant gram-negative bacilli in Singapore to tigecycline as tested by agar dilution. Ann Acad Med Singapore 2007;36:807-10.
59. National Antimicrobial Resistance Surveillance Workgroup. Singapore: NARSW Report, Jan-Mar 2006.
60. Schneiders T, Amyes SG, Levy SB. Role of AcrR and ramA in fluoroquinolone resistance in clinical *Klebsiella pneumoniae* isolates from Singapore. Antimicrob Agents Chemother 2003;47:2831-7.
61. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis 2006;6: 629-40.
62. Warren RE, Ensor VM, O'Neill P, Butler V, Taylor J, Nye K, et al. Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother 2008;61:504-8.
63. European Antimicrobial Resistance Surveillance System. Available at: http://www.rivm.nl/earss/database. Accessed 2 February 2008.