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“Future” Threat of Gram-negative Resistance in Singapore
Thuan Tong Tan, MBBS, MRCP (UK), PhD (Lund, Sweden)

Introduction
Gram-negative bacteria are important causes of urinary tract infections, bloodstream infections, healthcare-associated pneumonia, and intra-abdominal infections. The increasing resistance of Enterobacteriaceae is a significant challenge. Worldwide, an increasing prevalence of gram-negative bacteria with multi-drug resistance profiles is now recognised.1-3 In this paper, available local data are reviewed and one will see that our situation is approaching those seen elsewhere. To further compound the problem, there is also a dwindling of the antibiotic “pipeline”, with no new antibiotics targeting gram-negative bacteria available in the near future.4 The threat is particularly serious with regard to nosocomial pathogens like Pseudomonas aeruginosa and Acinetobacter baumanii. This is followed closely by the Enterobacteriaceae family with their array of extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases. There are also resistance mechanisms such as efflux pumps and porins down-regulation that effect resistance against multiple classes of agents. Potentiating these developments is the dwindling “pipeline” of new agents. Hence, there is a real concern that we are running out of options for our patients. Novel antibiotic combinations, enhanced infection control, antibiotic cycling, computer-assisted programmes, and maybe in the distant future, non-antimicrobial agents is all that we have.

Incidence and Prevalence of Multidrug-resistant Gram-negative Bacilli
The incidence and prevalence of multidrug-resistant gram-negative bacilli in Singapore were not previously collected systematically until a laboratory-based surveillance programme was established in 2006. This attempted to monitor the antimicrobial drug-resistance trends of pathogens in public hospitals. It found disturbing data. Of all hospital isolates of Klebsiella pneumoniae in 2006, 35.9% were resistant to third-generation cephalosporin.7 Data extracted from various publications from various individual hospitals in earlier years also supported the fact that our rates for ESBL carrying organisms are very high (around 40%).2,8,9 Comparing with data from the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection), a resistance surveillance programme in Europe, our ESBL rates are higher then most European centres except for those from Eastern European countries such as Russia (nearly 50%) and Poland (nearly 40%).10 A study conducted in Tan Tock Seng Hospital in 2006 revealed that almost 10% of more than 1800 emergency department attendees were colonised by third generation cephalosporin-resistant Escherichia coli (Personal

Commentary

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Abstract
The emergence of multidrug-resistant gram-negative bacteria is challenging the treatment of serious nosocomial infections. This is an international trend that is mirrored in Singapore too. Reports of strains resistant to all currently available agents have surfaced here and possibly have taken root here as well. The direst situation is among the non-fermenters, Pseudomonas aeruginosa and Acinetobacter baumanii. This is followed closely by the Enterobacteriaceae family with their array of extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases. There are also resistance mechanisms such as efflux pumps and porins down-regulation that effect resistance against multiple classes of agents. Potentiating these developments is the dwindling “pipeline” of new agents. Hence, there is a real concern that we are running out of options for our patients. Novel antibiotic combinations, enhanced infection control, antibiotic cycling, computer-assisted programmes, and maybe in the distant future, non-antimicrobial agents is all that we have.

Key words: Outcomes, Predictors, Stenotrophomonas maltophilia


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communication – Dr Brenda Ang).

Even more disturbing was the fact that 69% of all *Acinetobacter* spp. isolates at 1 intensive care unit was carbapenem resistant. Overall, resistance to carbapenems was 49.6% among all *Acinetobacter* spp. isolates.15 18.2% of all *Acinetobacter* spp. were susceptible to polymyxins alone. Pan-drug resistant *Acinetobacter baumannii* strains have been reported locally too.10 The situation with *P. aeruginosa* is only slightly better. Considering that this agent is virulent and deadly in immunocompromised hosts who are often exposed heavily to antibiotics (and thus at risk for more resistant organisms), the occurrence of carbapenem resistance at 9.6% of all *P. aeruginosa* isolates and in up to 27.2% of ICU isolates is much cause for concern.7 It is no longer unusual to see antibiograms in tertiary units in Singapore that look like those in Table 2. To appreciate the situation, it is necessary to go into further detail with regard to some of these organisms to understand the threat we face.

**Pseudomonas aeruginosa**

*P. aeruginosa* is a common cause of morbidity and mortality in hospitalised patients. In the Singapore General Hospital, it is the third most common gram-negative isolate.12 It is a common pathogen among hematological units and an important nosocomial pathogen. This agent is particularly fearsome because it is virulent and infects mainly immunocompromised hospitalised patients. It is intrinsically resistant to antibiotics and is able to acquire resistance determinants leading to the development of multiply resistant strains.13 The diverse array of mechanisms includes an ability to alter its permeability via the down regulation of porins. It also has efflux pumps as well as a wide variety of β-lactamases and aminoglycoside-modifying enzymes.14

Carbapenem resistance in *P. aeruginosa* with metallo-β-lactamases such as the IMP and VIM can lead to resistance to imipenem and meropenem plus the antipseudomonal cephalosporins, including cefepime and antipseudomonal penicillins.15 There has been an increase in carbapenem resistance seen in various locales in the world with the IMP genes being reported in increasingly more centres.16,17 Locally, almost 10% of all *P. aeruginosa* isolates and up to 27% of ICU isolates are carbapenem resistant.7 IMP-1 producing *Pseudomonas aeruginosa* has been found here and apparent clonal spread has been documented.18 Fortunately, at this point, it is not common.18 Be it an IMP/VIM carbapenemases or porins changes (and more commonly, a multitude of resistant determinants), multidrug-resistant strains are beginning to show their mark in hospitals. In these circumstances, the options can be limited to antibiotics that still have moderate activity.19 When there are no such options, various groups have tried

Table 1. Glossary of Selected Terms

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<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tr>
<td>IMP</td>
<td>Imperonin</td>
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<td>VIM</td>
<td>Verona</td>
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<td>KPC</td>
<td>Klebsiella pneumoniae carbapenemase</td>
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<tr>
<td>ESBL</td>
<td>Extended-spectrum β-lactamase</td>
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<tr>
<td>β-lactamase</td>
<td>Enzymes that hydrolyse β-lactam antibiotics</td>
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## References

1. October 2008, Vol. 37 No. 10

2. AmpC β-lactamase: This type of broad-spectrum enzyme is usually encoded on the bacterial chromosome and are inducible by β-lactams. Mutations result in increased expression and broad-spectrum cephalosporin resistance in *Enterobacter cloacae*. Plasmid borne versions may be responsible for similar broad-spectrum resistance in klebsiella and salmonella species.

3. Extended-spectrum β-lactamase (ESBL): Originally termed to reflect the expanded substrate spectrum of enzymes derived from narrower-spectrum TEM, SHV, or OXA β-lactamases. They are typically not active against cephamycins (e.g., cefoxitin) or carbapenems (imipenem, ertapenem, and meropenem), and can generally be inhibited by inhibitors such as clavulatane, sulbactam, or tazobactam.

4. CTX-M-type ESBLs: Another type of ESBL that has different lineage from the TEM and SHV type. The clinical implication is the same as the usual ESBL where carbapenems are the only reliable antibiotic in serious infection.

5. SHV, TEM, IMP, VIM and KPC: They are all β-lactamase related by amino acid substitutions. The nomenclature is not standardised and often confusing. SHV denotes a variable response to sulfhydryl inhibitors; TEM was named after the patient (Temoneira) from whom the first sample was obtained; IMP/VIM enzymes are able to hydrolyse carbapenems and they are integron-encoded metallo-β-lactamase; and KPC is derived from *Klebsiella pneumoniae* carbapenemases.

6. Pan-drug resistant: Refers to resistance to all antibiotics usually active against the pathogen.

7. β-lactam β-lactamase inhibitor combinations: Clavulanic acid, sulbactam, and tazobactam are inhibitory β-lactams that bind to and block the action of β-lactamases. Available in combinations with other β-lactams.

8. Carbenpem: Structurally like penicillins but a carbon atom replaces the sulpha atom at one position. It has the broadest antibacterial spectrum compared to other penicillins and cephalosporins and is generally resistant to ESBL. Examples include imipenem, meropenem, and ertapenem. Doripenem (the most recent one) is not currently available in Singapore.

9. Inoculum effect: Increased resistance with increasing numbers of infecting bacteria as a result of larger inocula of β-lactamase producing organisms.

10. Porins: In gram-negative bacteria, the inner membrane is the major permeability barrier. The outer membrane contains proteins that form channels and these are permeable to antibiotics.

11. Efflux pumps: An energy dependent system of extruding toxic substance from the bacteria. Some are molecule specific while others are not, thus contributing to resistance across multiple classes of antibiotics.

12. Monobactam: A monocyclic β-lactam. The only locally available example is aztreonam.

13. Plasmid: An extrachromosomal DNA. They often carry resistance genes.
combinations for which the supporting clinical data are sparse and sketchy.\textsuperscript{20} Polymyxin B has, by default, become our last line antibiotic (and for many other centres in the world) for this pathogen.\textsuperscript{20} The polymyxins are old antibiotics active against some gram-negative bacteria, including \textit{Acinetobacter} species, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella} species and \textit{Enterobacter} species. In the early days, there were common reports of nephrotoxicity and neurotoxicity. As such, parenteral use became unpopular. Since the late 1990s, it has become more widely used again because of the emergence of these multidrug-resistant organisms.

\textit{P. aeruginosa} with reduced susceptibility to polymyxin B have recently been reported in New York hospitals.\textsuperscript{20} Fortunately, many of the polymyxin B-resistant \textit{P. aeruginosa} isolates were susceptible to other anti-pseudomonal agents. For now, pan-resistant isolates are still uncommon.\textsuperscript{3,20} However, a greater use of polymyxin B in centres with high multidrug-resistant \textit{Acinetobacter baumannii} can lead to the kind of environment where polymyxin resistant \textit{P. aeruginosa} is selected. An analysis of the above New York hospitals revealed that the increased use against \textit{A. baumannii} was likely responsible for the development of polymyxin B resistant \textit{P. aeruginosa}.\textsuperscript{20}

\textbf{Acinetobacter baumannii}

\textit{Acinetobacter baumannii} commonly causes nosocomial infections, particularly ventilator-associated pneumonia (VAP). It is responsible for outbreak situations in neurosurgical intensive care units, in burns units and traumatic wounds among others.\textsuperscript{21} Many clinical isolates in such a nosocomial setting are often resistant to many antibiotics. Although there are doubts about the attributable mortality of this agent, it is becoming clear that this pathogen can cause significant morbidity.\textsuperscript{22} Carbapenemases resistant strains and clones are now common. It is endemic in many intensive care units (ICU) in different geographic locations.\textsuperscript{23-25} Analysis of the United States National Nosocomial Infections Surveillance (NNIS) System showed an increase from 4\% in 1986 to 7.0\% in 2003.\textsuperscript{26} Infections are associated with increased ICU and hospital stay as well as increased hospitalised mortality.\textsuperscript{27} Intensive care isolates in some countries including those of some Asian hospitals already have susceptibility patterns that meant little available treatment options except with the toxic polymyxin and colistin.\textsuperscript{28-30} Sporadic outbreaks with such strains have occurred when patients treated in endemic countries return to their homeland highlighting the rapidity of spread of these agents in this age of international travel and globalisation.\textsuperscript{21} As mentioned above, our local carbapenem resistance rates are very high especially in intensive care units and 18.2\% of all \textit{Acinetobacter spp}. local isolates are susceptible to polymyxin B alone.\textsuperscript{11,31}

Like \textit{P. aeruginosa}, \textit{A. baumannii} too has a diverse array of resistance mechanisms. It is “naturally transformable” and possesses genetic elements that facilitate the acquisition of resistant genes. A recent analysis of a multi-drug-resistant \textit{A. baumannii} strain revealed that it had an extra 86-kb region that encoded many resistance determinants that probably came from other gram-negative bacteria.\textsuperscript{32} Its unique ability to survive on dry inanimate surfaces for a long duration will also potentiate this ability to acquire resistance genes in a nosocomial setting.\textsuperscript{33}

Like \textit{P. aeruginosa}, its efflux pumps in \textit{A. baumannii} have broad spectrum of activity and are able to extrude

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<thead>
<tr>
<th>\textbf{Pseudomonas aeruginosa}</th>
<th>\textbf{Susceptibility}</th>
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<td>Piperacillin/Tazobactam</td>
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<td>Cefepime</td>
<td>R</td>
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<tr>
<td>Aztreonam</td>
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<td>Imipenem</td>
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<td>Gentamicin</td>
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<td>Netilmicin</td>
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<td>Ciprofloxacin</td>
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<td>Polymyxin B</td>
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<th>\textbf{Acinetobacter baumannii}</th>
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<td>Ciprofloxacin</td>
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<td>Cotrimoxazole</td>
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<td>Minocycline</td>
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<td>Polymyxin B</td>
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multiple classes of antibiotics.\textsuperscript{34,35} It also has different chromosomal β-lactamases and acquired β-lactamases, including those that can hydrolyse carbapenems.\textsuperscript{36,37} Fortunately, the relatively new antibiotic, tigecycline is active against it with activity in strains that are resistant to imipenem and other antibiotics.\textsuperscript{38,39} Local data suggest good in vitro activity against multi-resistant strains of \textit{Enterobacteriaceae}, with but more variable activity against multi-resistant strains of \textit{Actinobacter} spp.\textsuperscript{40} Clinical experience is, however, modest and the low achievable serum concentrations of tigecycline at recommended doses is a concern.\textsuperscript{41} The emergence of resistant strains of \textit{A. baumannii} while on therapy highlights the risks.\textsuperscript{41,42} Some experts have suggested using combinations of different antibiotics, which individually is ineffective. Preclinical data, however, are mixed and uncertain and clinical evidence is even more limited.

**Other Gram-negative Bacteria**

Besides \textit{P. aeruginosa} and \textit{A. baumannii}, other ESBL producing organisms also pose a threat in our public hospitals. These organisms are frequent causes of infections in hospitalised patients. The ESBL they produced are enzymes that confer resistance to most β-lactam antibiotics, including penicillins, cephalosporins and monobactam aztreonam. High rates of ESBL-producing isolates are already found in Singapore hospitals as mentioned earlier.\textsuperscript{28,29}

The ESBL family is heterogeneous and is found exclusively in gram-negative organisms, primarily in \textit{Klebsiella pneumoniae} and \textit{Escherichia coli}, but also in others. They are frequently plasmid encoded and these plasmids carry genes encoding resistance to other drug classes (i.e. aminoglycosides, quinolones, trimethoprim-sulfamethoxazole).\textsuperscript{43} Therefore, antibiotic options in the treatment of ESBL-producing pathogens are extremely limited.

For the usual TEM or SHV-related ESBL β-lactamases (derivatives of the originally described enzymes\textsuperscript{44}), β-lactamase inhibitors such as tazobactam do have in vitro activity but such activity is influenced by the bacterial inoculums and dosing of the drug. There are several other varieties of ESBLs, such as the CTX-M β-lactamases which have a similar spectrum of activity but are not derivatives of the original TEM or SHV.\textsuperscript{45} These CTX-M β-lactamases have now been found in many different \textit{Enterobacteriaceae}, including \textit{Salmonella}, and are the most prevalent ESBL type worldwide.\textsuperscript{45} Other types of ESBLs are less common.

Currently, carbapenems are considered the best treatment option for infections caused by such organisms, especially in treating serious infections.\textsuperscript{6,46} Some experts are of the opinion that piperacillin/tazobactam should be deemed as an alternative treatment option in the light of limited options available.\textsuperscript{47,48} Cefepime too has also been advocated as the probability of attaining the time above the minimum inhibitory concentration is higher than with other antimicrobials.\textsuperscript{49} Nevertheless, because the activities of these 2 drugs are influenced by factors such as the bacterial inoculums and dosing of the drug, carbapenems still need to be used in an unstable patient. Complicating the issue is that \textit{Enterobacteriaceae} with ESBL type antibiograms may express AmpC β-lactamases, which may in turn confer resistance to β-lactam β-lactamase inhibitor combinations (such as piperacillin-tazobactam).\textsuperscript{50} Finally, while carbapenems seem to be the answer for all these difficult infections, it must be noted that carbapenem-hydrolysing IMP-1 β-lactamase has been reported in \textit{Klebsiella pneumoniae} from Singapore as well.\textsuperscript{51} Carbapenems will be ineffective if these IMP-1 carrying isolates become more common.

In recent years, the availability of ertapenem to both imipenem and meropenem has meant the ability to sparingly use the latter 2 in non-pseudomonal settings.\textsuperscript{52} The soon-to-be available doripenem is also warmly welcomed. However, the use of carbapenem will favour the development of carbapenem resistant isolates.\textsuperscript{53,54} Finally, among extended-spectrum β-lactamase producing \textit{Enterobacteriaceae} isolates, some can carry the carbapenem-hydrolysing β-lactamase KPC.\textsuperscript{55} \textit{Enterobacteriaceae} with KPC has now been reported in geographically diverse locations spanning North and South America to the Middle East.\textsuperscript{56,57} Organisms reported to harbour these plasmid-borne enzymes include \textit{Escherichia coli}, \textit{Klebsiella} species, \textit{Enterobacter} species, and \textit{Salmonella enterica}.\textsuperscript{58} Such dissemination of KPC β-lactamases will have grave implications in the management of nosocomial infections. The mortality associated with such infections is significant and outbreaks have been reported.\textsuperscript{58} Furthermore, laboratories may mistakenly report them as being susceptible to imipenem because it is difficult to detect with automated susceptibility testing methods\textsuperscript{59} (fortuitously, KPC harbouring isolates have yet to be reported here in Singapore).

Quinolones have been advocated for infections due to ESBL-producing organisms in selective settings such as urinary tract infections. However, quinolone resistance in \textit{Enterobacteriaceae} is now found in a significant portion of ESBL-producing isolates.\textsuperscript{59} The usual mechanisms have been selective enzymes alterations or as part of other non-selective changes such as efflux or porins changes. These changes have been chromosomal mutations; but more recently, plasmid-mediated quinolone resistance has been reported!\textsuperscript{60} Hence, options are becoming limited. More recently, ESBL-producing \textit{Enterobacteriaceae} have
It is thus theoretically possible to target these multidrug-resistant organisms when they first colonise the human host.69 Inhibition of virulence factors and inhibition of signalling (i.e. quorum sensing) are among other ways to combat these pathogens but none of these are anywhere near advanced clinical trials.70 In the meantime, we will have to rely on good infection control measures and appropriate antibiotics usage.

**Conclusion**

The worst consequence of increasing antimicrobial resistance is therapeutic failure. If we do not turn this tide of increasing resistance around, there will be perilously few antibiotic choices left. We will be forced to use more and more antimicrobial agents to cover the possibility that we may be dealing with resistant organisms, especially in intensive care units among critically ill patients, adding substantially to the cost of patient care and possibly creating more selective pressure.

Without a doubt, we are approaching the “post-antibiotic era” and the threat from these organisms is not quite in the future but in the present moment. The 2 most influential infectious disease societies today, the Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) are both deeply worried.71,72 We should be equally concerned too.

**REFERENCES**


