Commentary

"Future" Threat of Gram-negative Resistance in Singapore

Thuan Tong Tan,[1] *MBBS, MRCP (UK), PhD (Lund, Sweden)*

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 baumannii*. 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.

Ann Acad Med Singapore 2008;37:884-90

Key words: Outcomes, Predictors, *Stenotrophomonas maltophilia*

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 signicant 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*, *Acinetobacter baumannii* and extended-spectrum β-lactamase (ESBL) producing organisms. Hence in this review, we will look at these specific pathogens common in the nosocomial setting to appreciate how they raise real concerns to the future of therapeutic options. A basic understanding of various resistance mechanisms is necessary and the reader is referred to excellent reviews published elsewhere.[5,6] Table 1 provides a glossary of various terms discussed here for easy referencing.

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

[1] Consultant, Infectious Diseases, Department of Infectious Diseases, Singapore General Hospital
Address for Correspondence: Dr Tan Thuan Tong, Department of Infectious Diseases, Singapore General Hospital, Outram Road, Singapore 169608.
Email: tan.thuan.tong@sgh.com.sg.

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.[7] 18.2% of all *Acinetobacter* spp. were susceptible to polymyxins alone. Pan-drug resistant *Acinetobacter baumannii* strains have been reported locally too.[11] 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. aeruginos*a with metallo-β-lactamases such as the IMP and VIM can lead to resistance to imipenem and meropenem plus the antipseudomonal cephalopsporins, 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

a. 3rd generation cephalosporins: These were able to overcome resistance caused by common β-lactamases when they first came into use. Examples, ceftriaxone (Rocephin) and ceftazidime (Fortum).

b. 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.

c. 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 clavulanate, sulbactam, or tazobactam.

d. 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.

e. 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 carbapenem and they are integron-encoded metallo-β-lactamase; and KPC is derived from *Klebsiella pneumoniae* carbapenemases.

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

g. 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.

h. Carbapenems: Structurally like penicillins but a carbon atom replaces a sulphur 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.

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

j. 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.

k. 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.

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

m. Plasmid: An extrachromosomal DNA. They often carry resistance genes.

Table 2. Antibiograms of Multidrug-resistant *P. aeruginosa* and *A. baumannii* that are seen in Tertiary Units in Singapore not Infrequently

Pseudomonas aeruginosa	
	Susceptibility
Piperacillin/Tazobactam	R
Cefepime	R
Aztreonam	R
Imipenem	R
Amikacin	R
Gentamicin	R
Netilmicin	R
Ciprofloxacin	R
Polymyxin B	S
Acinetobacter baumannii	
	Susceptibility
Ampicillin/Sulbactam	R
Piperacillin/Tazobactam	R
Cefepime	R
Aztreonam	R
Imipenem	R
Amikacin	R
Gentamicin	R
Netilmicin	R
Ciprofloxacin	R
Cotrimoxazole	R
Minocycline	R
Polymyxin B	S

combinations for which the supporting clinical data are sparse and sketchy.[20]

Polymyxin B has, by default, become our last line antibiotic (and for many other centres in the world) for this pathogen. The polymyxins are old antibiotics active against some gram-negative bacteria, including *Acinetobacter* species, *Pseudomonas aeruginosa*, *Klebsiella* species and *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.

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

Acinetobacter baumannii

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.[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.[22] Carbapenemases resistant strains and clones are now common. It is endemic in many intensive care units (ICU) in different geographic locations.[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.[26] Infections are associated with increased ICU and hospital stay as well as increased hospitalised mortality.[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.[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.[21] As mentioned above, our local carbapenem resistance rates are very high especially in intensive care units and 18.2% of all *Acinetobacter* spp. local isolates are susceptible to polymyxin B alone.[7] Pan-drug resistance isolates (i.e., resistant to polymyxin B as well) have now been reported several times elsewhere and locally too.[11,31]

Like *P. aeruginosa*, *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 *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.[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.[33]

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

multiple classes of antibiotics.[34,35] It also has different chromosomal β-lactamases and acquired β-lactamases, including those that can hydrolyse carbapenems.[36,37] Fortunately, the relatively new antibiotic, tigecycline is active against it with activity in strains that are resistant to imipenem and other antibiotics.[38,39] Local data suggest good in vitro activity against multi-resistant strains of *Enterobacteriaceae*, with but more variable activity against multi-resistant strains of *Acinetobacter* spp.[40] Clinical experience is, however, modest and the low achievable serum concentrations of tigecycline at recommended doses is a concern.[41] The emergence of resistant strains of *A. baumannii* while on therapy highlights the risks.[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 *P. aeruginosa* and *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.[2,8,9]

The ESBL family is heterogeneous and is found exclusively in gram-negative organisms, primarily in *Klebsiella pneumoniae* and *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).[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[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.[45] These CTX-M β-lactamases have now been found in many different *Enterobacteriaceae*, including *Salmonella*, and are the most prevalent ESBL type worldwide.[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.[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.[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.[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 *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).[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 *Klebsiella pneumoniae* from Singapore as well![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.[52] The soon-to-be available doripenem is also warmly welcomed. However, the use of carbapenem will favour the development of carbapenem resistant isolates.[53,54] Finally, among extended-spectrum β-lactamase producing *Enterobacteriaceae* isolates, some can carry the carbapenem-hydrolysing β-lactamase KPC.[55] *Enterobacteriaceae* with KPC has now been reported in geographically diverse locations spanning North and South America to the Middle East.[56,57] Organisms reported to harbour these plasmid-borne enzymes include *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Salmonella enterica*.[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.[58] Furthermore, laboratories may mistakenly report them as being susceptible to imipenem because it is difficult to detect with automated susceptibility testing methods[58] (fortunately, 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 *Enterobacteriaceae* is now found in a significant portion of ESBL-producing isolates.[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![60] Hence, options are becoming limited. More recently, ESBL-producing *Enterobacteriaceae* have

emerged in the community setting as well.[61]

Discussion

From the above data presented, one can see that multidrug-resistant non-fermenters are not uncommon in Singapore.[7] Extended-spectrum β-lactamase carrying bacteria are also widely encountered locally.[2,8,9] Several outbreaks have occurred with *P. aeruginosa,* and *XA. baumannii* isolates, which were sensitive to polymyxin B alone (author's unpublished data). Studies have also shown that the isolation of a pan-drug resistant organism is preceded by the isolation of an organism susceptible only to polymyxin alone.[62] The treatment of those gram-negative bacteria with almost pan-resistant phenotype is also difficult. Hence, it is appropriate to highlight the precarious position we are in and the therapeutic options or the lack of it now and in the near future.

What can we do to retard the relentless progress of these bacteria that are developing resistance? Experts are of the opinion that antimicrobial stewardship is the key in the prevention of antimicrobial resistance in hospitals.[63] Indeed, the appropriate and optimised use of antibiotics and proper duration will minimise the selection of resistant bacterial strains. Some of the measures suggested in stewardship programmes include antibiotic cycling, education/formulary restriction and automated computer-assisted programmes.

However, it is known that measures such as the rotation of antibiotic classes (antibiotic cycling) has not been shown convincingly in systematic reviews to be useful.[64] In theory, it may be a tool for limiting the selective pressures and that resistance to any single agent and emergence of resistance may be retarded.[64,65] However, some have argued that cycling is unlikely to reduce antibiotic resistance and may actually promote higher selection pressure according to mathematical models.[66,67] Others suggest that antibiotic heterogeneity may be more effective in slowing the spread of resistance but all these are options that need to be further explored. Formulary restriction may sometimes promote the emergence of pathogens with new resistance profiles. Automated protocols such as computer-assisted programmes have been used to improve antibiotic utilisation.[68] However, the possible effect of antibiotic resistance in such programme is unclear at this point in time.

Treatment options ahead may be in combination therapy and this will be discussed in depth in a separate paper in this edition of the *Annals*. Distant in the future, our hope rests on novel approaches to augment the rapidly depleting antibiotic "pipeline". There may also be some help coming from vaccines of which several against *P. aeruginosa* are under development. The immunodominant surface-expressed epitopes could be the targets of these vaccines. 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

1. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control 2004;32:470-85.
2. 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.
3. Falagas ME, Bliziotis IA. Pandrug-resistant gram-negative bacteria: the dawn of the post-antibiotic era? Int J Antimicrob Agents 2007;29: 630-6.
4. Talbot G, Bradley J, Edwards J, John E, Gilbert D, Scheld M, et al. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis 2006;42:657-68.
5. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med 2005;352:380-91.
6. Paterson DL. Resistance in gram-negative bacteria: *Enterobacteriaceae*. Am J Infect Control 2006;34:S20-S28; discussion S64-73.
7. Hsu LY, Tan TY, Jureen R, Koh TH, Krishnan P, Tzer-Pin Lin R, et al. Antimicrobial drug resistance in Singapore hospitals. Emerg Infect Dis 2007;13:1944-7.
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. 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.

10. Goossens H, Grabein B. Prevalence and antimicrobial susceptibility data for extended-spectrum beta-lactamase and AmpC-producing *Enterobacteriaceae* from the MYSTIC Program in Europe and the United States (1997-2004). Diagn Microbiol Infect Dis 2005;53:257-64.
11. Ang SW, Lee ST. Emergence of a multiply-resistant strain of Acinetobacter in a burns unit. Ann Acad Med Singapore 1992;21: 660-3.
12. Department of Pathology. Singapore General Hospital, Annual Report 2006:172.
13. Bonomo R, Szabo D. Mechanisms of multi-drug resistance in acinetobacter species and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43:S49-S56.
14. Rice L. Challenges in identifying new antimicrobial agents effective for treating infections with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43:S100-S105.
15. Nordmann P, Poirel L. Emerging carbapenemases in gram-negative aerobes. Clin Microbiol Infect 2002;8:321-31.
16. Andrade SS, Jones RN, Gales AC, Sader HS. Increasing prevalence of antimicrobial resistance among *Pseudomonas aeruginosa* isolates in Latin American medical centres: 5-year report of the SENTRY Antimicrobial Surveillance Program (1997-2001). J Antimicrob Chemother 2003;52:140-1.
17. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol 2000;3:489-95.
18. Koh TH, Wang GC, Sng LH. Clonal spread of IMP-1-producing *Pseudomonas aeruginosa* in two hospitals in Singapore. J Clin Microbiol 2004;42:5378-80.
19. Dubois V, Arpin C, Melon M, Melon B, Andre C, Frigo C, et al. Nosocomial outbreak due to a multiresistant strain of *Pseudomonas aeruginosa* P12: efficacy of cefepime-amikacin therapy and analysis of beta-lactam resistance. J Clin Microbiol 2001;39:2072-8.
20. Landman D, Bratu S, Alam M, Quale J. Citywide emergence of *Pseudomonas aeruginosa* strains with reduced susceptibility to polymyxin B. J Antimicrob Chemother 2005;55:954-7.
21. Falagas ME, Karveli EA. The changing global epidemiology of *Acinetobacter baumannii* infections: a development with major public health implications. Clin Microbiol Infect 2007;13:117-9.
22. Falagas ME, Bliziotis IA, Siempos II. Attributable mortality of *Acinetobacter baumannii* infections in critically ill patients: a systematic review of matched cohort and case-control studies. Crit Care 2006;10:R48.
23. Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. J Hosp Infect 2006;64:7-15.
24. Zarrilli R, Casillo R, Di Popolo A, Tripodi MF, Bagattini M, Cuccurullo S, et al. Molecular epidemiology of a clonal outbreak of multi-drug-resistant *Acinetobacter baumannii* in a university hospital in Italy. Clin Microbiol Infect 2007;13:481-9.
25. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-36.
26. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005;41:848-54.
27. Playford EG, Craig JC, Iredell JR. Carbapenem-resistant *Acinetobacter baumannii* in intensive care unit patients: risk factors for acquisition, infection and their consequences. J Hosp Infect 2007;65:204-11.
28. Jones M, Draghi D, Thornsberry C, Karlowsky J, Sahm D, Wenzel R. Emerging resistance among bacterial pathogens in the intensive care unit – a European and North American Surveillance study (2000-2002). Ann Clin Microbiol Antimicrob 2004;3:14.
29. Tognim MC, Andrade SS, Silbert S, Gales AC, Jones RN, Sader HS. Resistance trends of *Acinetobacter* spp. in Latin America and characterization of international dissemination of multi-drug resistant strains: five-year report of the SENTRY Antimicrobial Surveillance Program. Int J Infect Dis 2004;8:284-91.
30. Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. Antimicrob Agents Chemother 2007;51:4022-8.
31. Wang SH, Sheng WH, Chang YY, Wang LH, Lin HC, Chen ML, et al. Healthcare-associated outbreak due to pan-drug resistant *Acinetobacter baumannii* in a surgical intensive care unit. J Hosp Infect 2003;53: 97-102.
32. Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, et al. Comparative genomics of multi-drug resistance in *Acinetobacter baumannii*. PLoS Genetics 2006;2:e7.
33. Wendt C, Dietze B, Dietz E, Ruden H. Survival of *Acinetobacter baumannii* on dry surfaces. J Clin Microbiol 1997;35:1394-7.
34. Chau SL, Chu YW, Houang ET. Novel resistance-nodulation-cell division efflux system AdeDE in Acinetobacter genomic DNA group 3. Antimicrob Agents Chemother 2004;48:4054-5.
35. Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in Acinetobacter baumannii strain BM4454. Antimicrob Agents Chemother 2001;45: 3375-80.
36. Heritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49: 3198-202.
37. Heritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49:4174-9.
38. Milatovic D, Schmitz F-J, Verhoef J, Fluit AC. Activities of the glycylcycline tigecycline (GAR-936) against 1,924 recent European clinical bacterial isolates. Antimicrob Agents Chemother 2003;47: 400-4.
39. Pachon-Ibanez ME, Jimenez-Mejias ME, Pichardo C, Llanos AC, Pachon J. Activity of tigecycline (GAR-936) against *Acinetobacter baumannii* strains, including those resistant to imipenem. Antimicrob Agents Chemother 2004;48:4479-81.
40. 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.
41. Anthony K, Fishman N, Linkin D, Gasink L, Edelstein P, Lautenbach E. Clinical and microbiological outcomes of serious infections with multi-drug resistant gram-negative organisms treated with tigecycline. Clin Infect Dis 2008;46:567-70.
42. Schafer JJ, Goff DA, Stevenson KB, Mangino JE. Early experience with tigecycline for ventilator-associated pneumonia and bacteremia caused by multi-drug-resistant *Acinetobacter baumannii*. Pharmacotherapy 2007;27:980-7.
43. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. Drugs 2003;63:353-65.
44. Turner PJ. Extended-spectrum beta-lactamases. Clin Infect Dis 2005;41 Suppl 4:S273-5.
45. Canton R, Coque TM. The CTX-M [beta]-lactamase pandemic. Curr Opin Microbiol 2006;9:466-75.
46. Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol 2001;39:2206-12.
47. Tumbarello M, Spanu T, Sanguinetti M, Citton R, Montuori E, Leone F, et al. Bloodstream infections caused by extended-spectrum-{beta}-

lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. Antimicrob Agents Chemother 2006;50:498-504.

48. Peterson LR. Antibiotic policy and prescribing strategies for therapy of extended-spectrum B-lactamase-producing *Enterobacteriaceae*: the role of piperacillin-tazobactam. Clin Microbiol Infect 2008;14:181-4.
49. Ramphal R, Ambrose PG. Extended-spectrum beta-lactamases and clinical outcomes: current data. Clin Infect Dis 2006;42 Suppl 4: S164-72.
50. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. Clin Infect Dis 2006;42Suppl 4:S153-S163.
51. 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.
52. Teng CP, Chen HH, Chan J, Lye DC. Ertapenem for the treatment of extended-spectrum beta-lactamase-producing gram-negative bacterial infections. Int J Antimicrob Agents 2007;30:356-9.
53. Akinci E, Colpan A, Bodur H, Balaban N, Erbay A. Risk factors for ICU-acquired imipenem-resistant gram-negative bacterial infections. J Hosp Infect 2005;59:317-23.
54. Lee SO, Kim NJ, Choi SH, Hyong Kim T, Chung JW, Woo JH, et al. Risk factors for acquisition of imipenem-resistant *Acinetobacter baumannii*: a case-control study. Antimicrob Agents Chemother 2004;48:224-8.
55. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing {beta}-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2001;45:1151-61.
56. Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli Hospital. Antimicrob Agents Chemother 2007;51:3026-9.
57. Villegas MV, Lolans K, Correa A, Suarez CJ, Lopez JA, Vallejo M, et al. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. Antimicrob Agents Chemother 2006;50:2880-2.
58. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, et al. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch Intern Med 2005;165:1430-5.
59. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. Clin Infect Dis 2000;30: 473-8.
60. Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the qnr gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob Agents Chemother 2004;48:1295-9.
61. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother 2005;56:52-9.
62. Beno P, Krcmery V, Demitrovicova A. Bacteraemia in cancer patients caused by colistin-resistant gram-negative bacilli after previous exposure to ciprofloxacin and/or colistin. Clin Microbiol Infect 2006;12:497-8.
63. Shlaes DM, Gerding DN, John JF Jr, Craig WA, Bornstein DL, Duncan RA, et al. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: guidelines for the prevention of antimicrobial resistance in hospitals. Clin Infect Dis 1997;25:584-99.
64. Brown EM, Nathwani D. Antibiotic cycling or rotation: a systematic review of the evidence of efficacy. J Antimicrob Chemother 2005; 55:6-9.
65. Kollef M. Is antibiotic cycling the answer to preventing the emergence of bacterial resistance in the intensive care unit? Clin Infect Dis 2006;43:S82-S88.
66. Magee JT. The resistance ratchet: theoretical implications of cyclic selection pressure. J Antimicrob Chemother 2005;56:427-30.
67. Bergstrom CT, Lo M, Lipsitch M. Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals. Proc Natl Acad Sci U S A 2004;101:13285-90.
68. Evans R, Pestotnik S, Classen D, Burke J. Evaluation of a computer-assisted antibiotic-dose monitor. Ann Pharmacother 1999; 33:1026-31.
69. DiGiandomenico A, Rao J, Harcher K, Zaidi TS, Gardner J, Neely AN, et al. Intranasal immunization with heterologously expressed polysaccharide protects against multiple *Pseudomonas aeruginosa* infections. Proc Natl Acad Sci U S A 2007;104:4624-9.
70. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. The biology and future prospects of antivirulence therapies. Nat Rev Microbiol 2008;6: 17-27.
71. James JS. Empty antibiotic pipeline critically endangers public: IDSA report. AIDS Treat News 2004:7.
72. MacKenzie FM, Struelens MJ, Towner KJ, Gould IM. Report of the Consensus Conference on Antibiotic Resistance; Prevention and Control (ARPAC). Clin Microbiol Infect 2005;11:938-54.