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◆ REVIEW ARTICLE ◆

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The threat from the pink corner

David M Livermore

In terms of numbers of isolates, the greatest present resistance problems arise with gram-positive pathogens (which stain purple/black in Gram's method), especially methicillin-resistant *Staphylococcus aureus* (MRSA). But, is the MRSA problem — by its size — blinding us to something ultimately more dangerous: the slow emergence of gram-negative pathogens (which stain pink) with resistance to *all* reliable antibiotics? Although presently rare, 'pan-resistant' gram-negative bacteria — predominantly strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*- have more comprehensive resistance than any gram-positive pathogen. They already pose treatment problems in compromised hospital patients, especially in specialist units, as well in particular groups, such as cystic fibrosis patents. Disturbingly, there is a near-total lack of developmental antibiotics active against gram-negative pathogens.

Keywords: *Acinetobacter*; antibiotic resistance; β-lactamase; gram-negative bacteria; metallo-β-lactamases; multi-resistance; Pseudomonas

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Introduction

Most clinicians and microbiologists would agree that the greatest antibiotic resistance problems occur with gram-positive pathogens, which stain blue-black by Gram's method, and not with gram-negative bacteria, which stain pink. But, is this a case of dreading the dinosaurs and ignoring the mammals? Superficially, the dominance of gram-positive resistance is overwhelming, with staphylococci, enterococci and pneumococci all being frequent multi-resistant isolates in many countries. Ten years ago MRSA accounted for 2% of *S. aureus* isolates from in-patients in the UK: nowadays this proportion has risen to 42%, with MRSA accounting for 14.7% of *all* non-replicate bacteria isolated from in-patients (1, 2). MRSA also account for more than 25% of *S. aureus* isolates from bacteraemias in much of southern Europe (http://www.earss.rivm.nl), with many epidemic MRSA resistant also to multiple non-β-lactam agents. Enterococci are much less pathogenic than *S. aureus*, but are important opportunists with inherent resistance to cephalosporins and most quinolones and with increasing rates of acquired resistance to other drugs. The therapy of enterococcal infections has become harder with the spread of high-level aminoglycoside resistance (thus precluding the β-lactam/aminoglycoside/synergy needed for optimal treatment) and with the emergence of vancomycin resistance. Vancomycin-resistant enterococci were first recorded only 15 years ago but now account for 25% of clinical *Enterococcus faecium* in the UK and 47%–70% of those in the USA (2, 3). Their resistance has recently also spread to a few MRSA isolates in the USA (4). In the case of pneumococci, multi-resistant strains of serotypes 6, 9, 14, 15, 19 and 23 have disseminated internationally, with penicillin resistance in 70% of isolates from several Far Eastern countries and in 30%–40% of those the USA, Spain, France and Portugal (5).

By contrast, imipenem retains near universal activity against the Enterobacteriaceae, even after 17 years of use, giving a sense of security. Among 1.42 million Enterobacteriaceae (*Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Morganella* spp., *Proteus* spp., *Serratia* spp.) reported to The Surveillance Network® (TSN®) Database USA between 1 January 1996 and 30 November 2002 from >250 hospitals, just 59 (0.005%) were resistant to imipenem.

In reality, the dominance of gram-positive resistance is not so clear, for three reasons. First, new anti-gram positive agents are being launched, whereas new anti-gram-negative agents are conspicuously absent.

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Secondly, many multi-resistant gram-positive bacteria remain susceptible to old agents. Thirdly, although carbapenems retain near universal activity against Enterobacteriaceae, resistance is accumulating in *Pseudomonas* and *Acinetobacter* and, increasingly, this resistance is caused by acquired carbapenem-hydrolysing β-lactamases, of which there are a growing number recorded (Table 1) (6). New anti-gram-positive agents already launched include linezolid and quinupristin/dalfopristin, and these should be followed by daptomycin, tigecycline and oritavancin (7). New ketolides (e.g., telithromycin) and fluoroquinolones (e.g., moxifloxacin) provide further options against pneumococcal infections. As regards susceptibility to old agents, nearly all MRSA are still susceptible to vancomycin and teicoplanin and many also to fucidin, rifampicin, and minocycline. Most 'penicillin-resistant' pneumococci remain susceptible to high-dose penicillin or amoxicillin, except in meningitis (8).

In five years of running England's national reference laboratory for antibiotic resistance, I have yet to see any gram-positive pathogen resistant to all 'good' antibiotics. On the other hand, I do see a steady trickle of gram-negative isolates that are resistant to all good antibiotics. Almost all are non-fermenters of the genera *Pseudomonas, Acinetobacter, Burkholderia* and *Stenotrophomonas,* and Table 2 summarises several recent submissions. In each case there was no good antibiotic, and the agents recommended (bold font) lacked bactericidal activity (e.g., minocycline), were unpleasantly toxic (e.g., polymyxin), had only marginal activity, or, at best, represented monotherapy in settings where synergistic combinations are considered mandatory.

Key messages

- **Resistance to all good antibiotics, including carbapenems, is accumulating in non-fermentative gram-negative bacteria, in particular *Pseudomonas aeruginosa* and *Acinetobacter baumannii.***
- **Many Enterobacteriaceae are susceptible only to carbapenems, meaning that any emergence of resistance to these drugs would swiftly give a pool of pan-resistance.**
- **Very few anti-gram-negative antibiotics are in advanced development.**

Pan-resistant non-fermenters

P. aeruginosa

Most *P. aeruginosa* isolates remain susceptible to multiple β-lactams, aminoglycosides and fluoroquinolones, but the prevalence of isolates resistant to three or more of the most relevant antibiotics (amikacin, ceftazidime, ciprofloxacin, gentamicin, carbapenems and piperacillin) has increased steadily

Table 1. Acquired carbapenemases.

Class	Enzyme(s)[a]	Distribution	Comments
A	SME-1 and -2	*Serratia* spp. from USA and UK	Reported on three occasions since 1982; minimal significance
A	IMI-1/NMC-A	*E. cloacae* France and USA	Almost identical; Reported twice since 1986; minimal significance
A	KPC-1 to -3	*K. pneumoniae*, USA and Greece	Reported in three US cities in past 2 years, with one sizeable outbreak. May warrant concern
B	IMP-1 to -12	Mostly non-fermenters from Far East, more rarely Enterobacteriaceae (esp. *Serratia* in Japan); also a few *P. aeruginosa* and *A. baumannii* from Europe and Canada	Increasing in host range, geographic distribution and number of variants described. Major concern.
B	VIM-1 to -7	Mostly in *P. aeruginosa* and *P. putida* from Far East and Southern Europe; rarely in Enterobacteriaceae	Increasing in host range, geographic distribution and number of variants described. Major concern.
B	SPM-1	*P. aeruginosa* in Brazil	One recent report, significance uncertain
D	OXA-24, 25, 26 & 40	*Acinetobacter* spp. in, or epidemiologically linked to, Iberia	Several reports of major nosocomial outbreaks. Significant concern.
D	OXA-23 and 27	*Acinetobacter* spp. in Brazil, UK and Singapore	Single reports from each country; with spread between two hospitals in Brazil; only 60% amino-acid homology to OXA-24 etc. cluster above. Significant concern.
D	OXA-unsequenced	*Acinetobacter* spp. in Argentina, Kuwait, UK, France and elsewhere	Numerous reports. Significant concern.

[a] β-Lactamases are designated by three-letter, one-number codes alluding to source, substrate preference and /or genetic relatedness. IMP, for example, stands for imipenemase, and VIM for Verona imipenemase, but the derivations, many of them less clear than these examples, need not trouble readers!

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Table 2. Multi-resistant non-fermenters recently submitted to the Antibiotic Resistance Monitoring and Reference Laboratory

		Minimum inhibitory concentration — MIC (mg/L)												
Organism	**Clinical setting**	**CAZ**	**CTX**	**IMP**	**MEM**	**PTZ**	**TIM**	**AK**	**GM**	**CIP**	**SXT**	**COL**	**MIN**	**SUL**
P. aeruginosa	CF sputum	>256	—	>32	>32	>256	—	64	8	>32	—	**1**[b]	—	—
P. aeruginosa[a]	Catheter urine	16	—	>32	>32	32	—	>256	>256	>32	>32	**4**	>256	—
Acinetobacter	Respiratory disease/ ITU	256	>256	32	32	>256	—	**2**	16	>32	—	—	—	—
Acinetobacter	Ventilator pneumonia	>256	>256	64	32	>256	—	16	64	>32	8	**0.125**	16	16
S. maltophilia	?	>256	—	IR[c]	IR	—	32	>256	>256	32	**2**	>1024	**0.5**	—
S. maltophilia	?	32	—	IR	IR	—	16	8	32	32	**1**	>1024	16	—
S. maltophilia	CF sputum	>256	—	IR	IR	—	>256	32	—	4	**1**	32	—	—
B. cepacia	CF sputum	**8**	—	>32	8	64	—	>256	>256	>32	>32	>1024	64	—
B. gladioli	CF sputum	>256	—	32	8	>256	—	>256	32	4	**1**	32	32	

[a] Had VIM β- lactamase.
[b] Values in **bold** font indicate susceptibility; all others indicate resistance. Interpretations based on British Society for Antimicrobial Chemotherapy breakpoints (http://www.bsac.org.uk).
[c] IR, species intrinsically resistant, drug therefore not tested.
Abbreviations: AK = amikacin; CAZ = ceftazidime; CIP = ciprofloxacin; COL = colistin; CTX = cefotaxime; GENT = gentamicin; IMP = imipenem; MEM = meropenem; MIN = minocycline; PIP/TAZ = piperacillin/tazobactam; SUL = sulbactam; SXT = co-trimoxazole; TIM = ticarcillin/clavulanate.

over the past 5 years, with approximately 1% of isolates in the USA resistant to *all* these six agents (9). The prevalence of multi-resistance is much higher in chronic infections, especially pulmonary disease in cystic fibrosis patients, and may also be increased in intensive care and burns units (10, 11).

P. aeruginosa has two routes to pan-resistance (9). The first, and more frequent, is by accumulation of successive mutations that variously up-regulate chromosomal (AmpC) β-lactamase expression and multidrug efflux, reduce permeability of the outer and cytoplasmic membranes, and diminish fluoroquinolone sensitivity of the DNA gyrase. Antibiotics do not cause mutations, but many select hypermutable variants that arise randomly within any pseudomonal population. These lack the DNA repair mechanisms that normally correct most mutations as they arise. If hypermutability is co-selected with resistance, the result is a population that is primed to develop further resistances more rapidly than normal (12).

The other route to pan-resistance in *P. aeruginosa* is by acquisition of DNA elements encoding potent β-lactamases together with aminoglycoside-modifying enzymes. Most acquired β-lactamases in *P. aeruginosa* attack only penicillins and cefoperazone, sparing ceftazidime, aztreonam and carbapenems, but emerging types have broader spectra. These critically include the VIM and IMP metallo-β-lactamases (Table 1), which hydrolyse all β-lactams except aztreonam (6, 13) — also PER-1 (14) and various extended-spectrum OXA (15) mutants that hydrolyse all β-lactams except carbapenems. Transferable resistance to fluoroquinolones has not been documented in *P. aeruginosa* but pan-resistance ensues if resistances to β-lactams and aminoglycosides are acquired by a strain that already has, or later develops, mutational fluoroquinolone resistance.

The IMP and VIM β-lactamases deserve particular emphasis. They remain infrequent, but have spread among hospitals and *P. aeruginosa* strains in East Asia (6, 13). They are also becoming scattered in Europe, with multiple reports from the south of the Continent, and more recent discovery in Poland and England. Moreover, the lists of known variants is growing rapidly (6, Table 1). In Thessoloniki, a single serotype O12 strain with a VIM enzyme spread among multiple patients, with over 200 representatives collected between 1996 and 1998. Smaller outbreaks of producers are recorded in Italy and Canada (13, 16, 17).

When a *P. aeruginosa* isolate is multi-resistant to standard antibiotics, including carbapenems, a few basic rules should be remembered. First, tobramycin is the aminoglycoside with the best intrinsic activity against the species and often remains active against isolates with low-level, mutational resistance to other aminoglycosides. Secondly, isolates highly resistant to tobramycin and amikacin but with low level resistance to gentamicin may have a 6′ N-acetyl transferase type 1a that spares isepamicin, which can be obtainable for compassionate use (Schering-Plough) even in those countries where it is not licensed. Thirdly, isolates with IMP and VIM metallo-β-lactamases will remain susceptible to aztreonam (9) unless they also have other mechanisms, such as up-regulated efflux or derepression of AmpC chromosomal β-lactamase. Unfortunately many IMP and VIM producers *do* have these mechanisms and are so are aztreonam resistant.

Finally, there are the polymyxins. These were used as antipseudomonal agents before the introduction of

Table 3. Outcomes among patients treated with colistin (polymyxin E) for infections caused by multi-resistant non-fermenters.

	Number of cases treated		
Site of infection	***P. aeruginosa***	***A. baumannii***	**Number with good outcome**
Pneumonia	6	14	5/20
Urinary tract	4	8	10/12
Primary bacteraemia	7	2	7/9
Central nervous system	0	5	4/5
Surgical site	1	4	3/5
Venous catheter	1	3	3/4
Peritonitis	1	3	2/4
Otitis	1	0	1/1
Total	21	39	35/60 = 58%

From Levin *et al.* (19); the multi-resistant *P. aeruginosa* isolates were diverse but the *A. baumannii* were clonal, which may be a biasing factor.

gentamicin and carbenicillin in the 1960s, and retain near-universal activity against nosocomial *P. aeruginosa* (18), although occasional resistance has emerged in isolates from cystic fibrosis patients, who receive nebulised colistin (polymyxin E) at high dosage. The largest recent study on the efficacy of polymyxin against nosocomial infections caused by multi-resistant *P. aeruginosa* and *Acinetobacter* spp. was by Levin *et al.* (19) who, unfortunately, do not split their results by species. Overall, they found 58% efficacy, but only 25% efficacy in nosocomial pneumonias (Table 3). Nephrotoxicity and neurotoxicity — reasons why polymyxin originally fell from use — occurred in 39% of patients, but never necessitated cessation of therapy.

Acinetobacter *spp*.

For over 30 years *Acinetobacter baumannii*, the principal pathogen in the genus, has successively acquired resistance to penicillins, aminoglycosides, cephalosporins and, in many centres, fluoroquinolones (20). Carbapenems have become standard therapy for serious acinetobacter infections, but resistance is emerging to them too, mostly in strains that are already multi-resistant to other treatments. Data collected from >250 hospitals in the USA *via* the TSN® surveillance system show imipenem resistance among *A. baumannii* isolates rising from 1.8% in 1996 to 7.3% in 2002, with intermediate resistance rising from 0.6% to 6.2% (Fig 1). Major outbreaks of

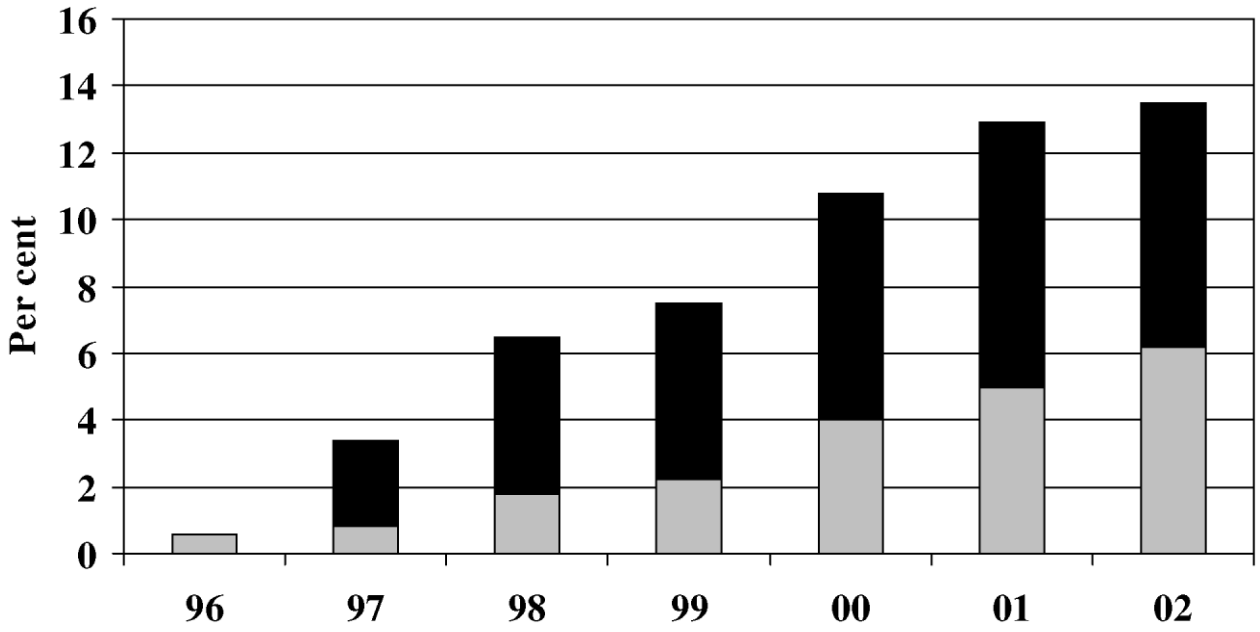

Figure 1. Susceptibility trends to imipenem among *A. baumannii* in the USA: black, resistant; grey intermediate. Based on data reported from >250 hospitals participating in the TSN® surveillance, with at least 2177 reports per annum. Data for 2002 are up to 30 September, other data are for full years.

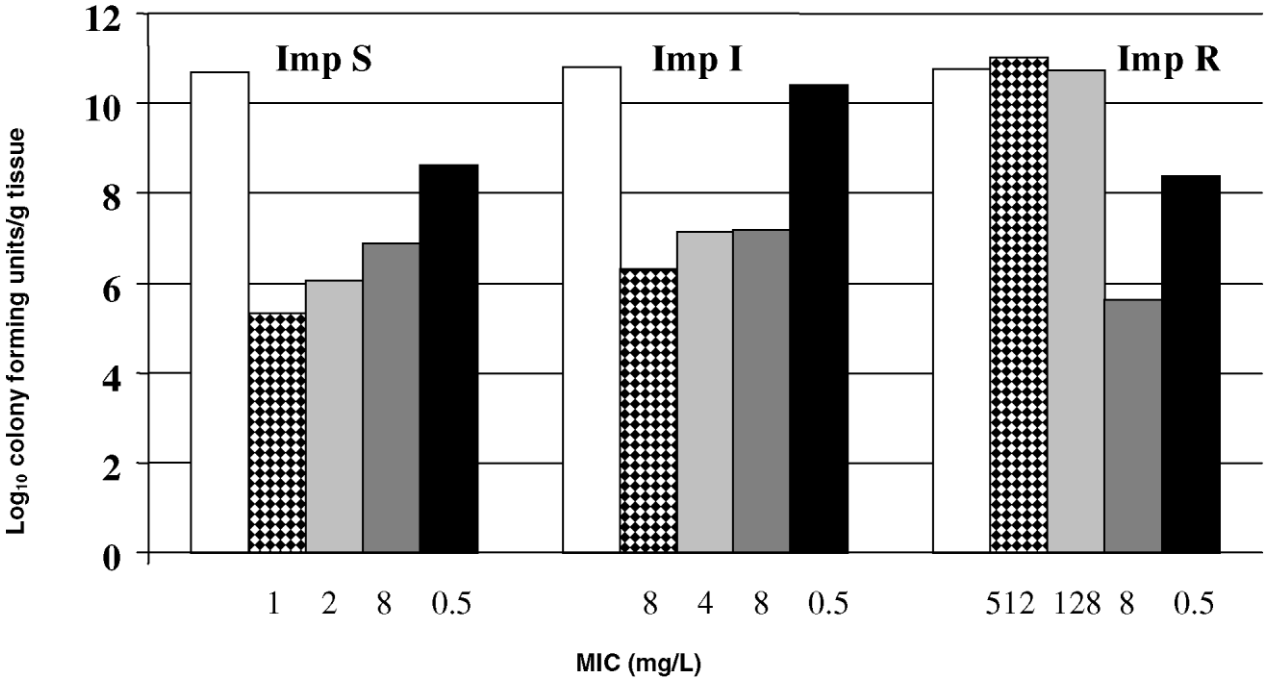

Figure 2. Bacterial counts, 48 h after infection, in the lung tissue of immuno-competent mice given acinetobacter pneumonia and then treated with imipenem (diamonds), sulbactam (light grey), rifampicin (dark grey) or colistin (black). The white bars show the counts for untreated control mice. MICs are shown below the bars. Data are from Moreno *et al.* (25).

carbapenem-resistant acinetobacters have occurred in many centres worldwide. Acquired carbapenem resistance can reflect metallo-β-lactamases of the IMP and VIM families, as in *P. aeruginosa*, but is more often associated with β-lactamase-independent mechanisms, presumably including impermeability, efflux and target changes and/or with unusual zinc-independent β-lactamases belonging to the OXA family (see Table 1) (6, 13). The relevant OXA enzymes have only a very feeble and difficult-to-detect carbapenemase activity *in vitro* but can be associated with high MICs (minimum inhibitory concentrations, the lowest drug level needed to stop bacterial growth) and clinical resistance, perhaps because their catalytic efficiency is greater in the living bacterium, or because many host strains are impermeable, or have up-regulated efflux (21, 22). At a genetic level these enzymes form at least two clusters. The OXA-24, -25, -26 and -40 types differ only by point mutations and are epidemiologically linked to strains from Iberia, whereas OXA-23 and -27, which have 40% amino acid divergence from the OXA-24 cluster, have been recorded once or twice each in the UK, Brazil and Singapore (21–23).

Acinetobacter spp. isolates resistant to carbapenems are mostly already resistant to penicillins, cephalosporins, aminoglycosides and fluoroquinolones. Nevertheless, treatment options are not quite so limited as against pan-resistant *P. aeruginosa*. Not only do the polymyxins remain *in vitro* active but so also — often — do minocycline, doxycycline and sulbactam (24). Clinical data on the relative merits of these options are sketchy. Levin's (19) use of polymyxin against multi-resistant *P. aeruginosa* and Acinetobacter infections was reviewed above (Table 3) making the point that, although overall efficacy was 58%, this fell to 25% for pneumonias. The latter figure is disturbing because of the importance of *A. baumannii* in ventilator-associated pneumonias. Poor activity by colistin (polymyxin E) in acinetobacter pneumonias was also seen in immuno-competent mice, where, despite in-vitro susceptibility, bacterial counts were reduced by ≤ 2 $\log_{10}$, compared with 3 to 4 $\log_{10}$ for imipenem, sulbactam and (more surprisingly) rifampicin (Fig 2) (25). These data suggest that sulbactam might be a better choice than polymyxin against carbapenem-resistant acinetobacters, if they remain susceptible. The efficacy of sulbactam is further supported by human studies. Wood *et al.* (26), found similar efficacy for imipenem and ampicillin/sulbactam in ventilator–associated acinetobacter pneumonias in trauma unit patients, whilst Corbella *et al.* (27) observed cure or improvement among 39/45 patients treated with either sulbactam alone or sulbactam/ampicillin for a variety of non-life-threatening infections caused by carbapenem-resistant *A. baumannii*.

A problem with all these clinical studies (also Levin's on polymyxin (19)) is that they were performed at single sites with acinetobacters that

were largely or entirely clonal. Results with particular strains may not have a global validity. Another reservation is that sulbactam resistance (tentatively defined as an MIC >16 mg/L) is widespread in *A. baumannii* and is more frequent than resistance to carbapenems (24). It may be increasing in some centres (28), leading to doubts about long-term efficiency. High-level sulbactam resistance (MIC >512 mg/L) was associated with failure in the immuno-competent mouse model (Fig 2) (25).

Doxycycline and minocycline are active against many multi-resistant acinetobacters *in vitro* (24) but clinical data are lacking. Likewise, although animal data suggest that rifampicin may be a useful adjunct in acinetobacter infections (Fig 2), there are no systematic clinical studies to confirm this view. Rifampicin monotherapy is contra-indicated by the risk of mutational resistance.

Stenotrophomonas maltophilia

S. maltophilia is a frequent clinical isolate, most often as a colonist but sometimes as a pathogen. Most strains appear resistant to all β-lactams on Mueller-Hinton agar, as used in the USA, but may appear susceptible to various penicillins, cephalosporins and meropenem on the DST ('Diagnostic Sensitivity Test') or Iso-Sensitest agars predominantly used in the UK and Scandinavia (29). It is unclear which medium better reflects clinical conditions. There are anecdotal reports of clinical efficacy by cephalosporins that appear inactive on Mueller-Hinton agar, but there are also data suggesting that these agents select for overgrowth by *S. maltophilia* in patients, implying inactivity *in vivo* (30).

What is certain is that *S. maltophilia* has two inducible β-lactamases, L-1 and L-2, which collectively hydrolyse every available β-lactam. Fluoroquinolone susceptibility is borderline at best and aminoglycoside resistance is near-universal. The drug which does remain active, *in vitro* and *in vivo,* is co-trimoxazole, which should be first-line therapy whenever *S. maltophilia* is implicated in significant infections (see also Table 2). Resistance is rare, but does occur (31): more often, effective resistance arises because a patient is intolerant of sulphonamides. In this instance, therapeutic choices lie between ticarcillin/clavulanate, as the least-inactive of the β-lactams, and chloramphenicol, which retains activity against about half of the isolates (31). There is a rationale for using aztreonam with a clavulanate combination, since aztreonam is stable to the L-1 metallo-β-lactamase and is protected from the L-2 by clavulanate (32). Clinical data for this combination are scanty, but favourable (33, 34).

Burkholderia *spp.*

Burkholderia pseudomallei is the agent of meliodosis, a pulmonary and septic disease principally seen in Southeast Asia and occasionally encountered in returning travellers. *B. cepacia* is an important pulmonary opportunist in cystic fibrosis patients. Resistance mechanisms in these organisms are less comprehensively understood than in *P. aeruginosa* and *A. baumannii,* but both are frequently multi-resistant and have potent combinations of β-lactamase and efflux-mediated resistance (35–39). They are resistant to aminoglycosides and have borderline susceptibility, at best, to fluoroquinolones, tetracyclines, chloramphenicol and co-trimoxazole. Genes for at least three β-lactamases occur in *B. pseudomallei* and altered expression or sequence mutation can cause resistance to ceftazidime (35, 36), which is the standard therapy. Meropenem is a frequent therapy for *B. cepacia* infection of cystic fibrosis patients, but its MICs typically are only just below the breakpoint. Some strains have a carbapenemase (40) and are resistant, as are others without obvious carbapenemase activity (41). Where resistance to 'standard' drugs (i.e., meropenem and ceftazidime) is seen, the best that can be done is to seek among the tetracyclines, chloramphenicol and co-trimoxazole, in the hope that one or more of these will retain activity. Owing to their lipopolysaccharide structure *Burkholderia* spp. are inherently resistant to polymyxins. There is interest in using multiple-antibiotic mixtures for potential synergy against *B. cepacia* and in optimising these to the phenotypes predominating in individual cystic fibrosis patients (42). At present, however, the costs of testing multiple drug combinations, and the uncertainties of extrapolating in-vitro synergy to the patient, are more apparent than any proven clinical benefit.

Pan-resistance in Enterobacteriaceae

Pan-resistance is much rarer in Enterobacteriaceae than in non-fermenters, primarily because — as already noted — carbapenems retain near-universal activity. Resistance to all agents except carbapenems is, however, widespread, mostly in *Klebsiella, Enterobacter* and *Serratia* spp. If carbapenem resistance does spread there is consequently a potential for a sudden and dramatic emergence of pan-resistance.

A few carbapenemase-producing *Enterobacter cloacae* and *Serratia marcescens* isolates have been known since before imipenem was launched in the mid-1980s (6, 13). Such strains include *Enterobacter* spp. with the IMI-1/NMC-A enzymes and *Serratia* spp. with SME enzymes (Table 1). These have not subsequently accumulated and, in any event, their enzymes are not transferable and do not confer

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resistance to third-generation cephalosporins. More disturbingly, IMP and VIM metallo-β-lactamases have reached Enterobacteriaceae. Their dissemination is not widespread so far, but is difficult to monitor since they can occur in Enterobacteriaceae without causing frank resistance unless permeability is restricted by mutation (43). Resistant or not, bla_{IMP}- and bla_{VIM}-positive Enterobacteriaceae isolates, including *Klebsiella* spp., *Enterobacter* spp, *Serratia* spp. and *Citrobacter* spp., have been found in several Far Eastern countries, including Japan, Taiwan, Singapore and China (43–46). Clonal spread of bla_{VIM}–positive *Enterobacter* and *Citrobacter* spp. strains that lacked obvious carbapenem resistance has occurred in parts of Taiwan (44, 45). There also are recent reports of *K. pneumoniae* with 'KPC' β-lactamases from three cities on the Atlantic coast of North America; KPC-types are not metallo-β-lactamases (Table 1) but do confer resistance to carbapenems and all other β-lactams (47). The host organisms were resistant also aminoglycosides and fluoroquinolones and, at one site, caused a major outbreak. The spread of all these enzymes will need careful monitoring, especially with the launch of ertapenem, as a carbapenem for front-line use in community-acquired infections.

On the rare occasions when resistance that encompasses carbapenems is found in Enterobacteriaceae, the advice is much as for *Acinetobacter* spp. Isepamicin may be active against strains resistant to other aminoglycosides; minocycline and doxycycline evade most tetracycline-resistance and aztreonam is stable to metallo-β-lactamases, though it may to be compromised by permeability mutations or strongly-expressed AmpC β-lactamases. Polymyxin activity is species-specific, with resistance ubiquitous in Proteeae but susceptibility general in most other Enterobacteriaceae.

New treatment options

Of the new treatments emerging for gram-positive infections, only tigecycline (48) — a tetracycline that evades both efflux and ribosomal modification — offers any real promise against multi-resistant gram-negative pathogens. And, that promise is largely limited to Enterobacteriaceae: *P. aeruginosa* is inherently resistant and, although tigecycline overcomes acquired minocycline resistance in *Acinetobacter* spp., it is intrinsically less active than minocycline against this genus (24). Newer fluoroquinolones such as moxifloxacin and gemifloxacin do not, in general, offer any advance over ciprofloxacin against most multi-resistant gram-negative bacteria, although *S. maltophilia* may be an exception to this generalisation (49). Ertapenem, a recently launched carbapenem, has weaker activity against non-fermenters than established carbapenems and does not overcome resistance to them: rather, its advantages are pharmacological.

New drugs that do offer further advances against gram-negative bacteria are not even in Phase I development, and their ultimate commercialisation remains very uncertain. One class of interest are dihydroxypyrridone β-lactams. Like catechol β-lactams — which were investigated previously but not developed – dihydroxypyrridones exploit the bacterial ferric iron uptake pathway to achieve efficient accumulation in the bacterial cell (50). This uptake allows very low MICs under the iron-deficient conditions believe to apply *in vivo*. If a dihydroxypyridone is linked to a monobactam, as with PTX 2416 (PanTherix) there is a potential for good penetration of non-fermenters and stability to metallo-β-lactamases.

Other areas of interest include antibacterial peptides and inhibitors of multidrug efflux pumps and metallo-carbapenemases. Natural antibacterial peptides have been investigated as therapeutic agents for over 20 years. Several, including buforin II (from toad skin), magainin (from frog skin) and cecropin (from moths) achieve worthwhile in-vitro activity against key non-fermenters, with MICs of 0.25 to 16 mg/L for *Acinetobacter* spp., 1 to 16 mg/L for *S. maltophilia* and 4–64 mg/L for *P. aeruginosa* (51–53). They disorganise bacterial membranes and so are unlikely to be readily compromised by resistance, but their development has been bedevilled by high costs and rapid metabolism. Efflux inhibitors may be useful against those organisms, notably *P. aeruginosa*, where up-regulated multidrug efflux is a major component in resistance. Potential inhibitors were investigated by Essential Therapeutics (formerly Microcide) of Mountain View, California (54), who found agents that substantially reduced the MICs of levofloxacin for normal *P. aeruginosa* and for mutants with up-regulated efflux.

Inhibitory activity against Class B β-lactamases, including VIM and IMP types, has been found in a range of β-lactam and (mostly) non-β-lactam compounds (55) but none is under active development. Even if a lead was identified, trials would be extremely difficult allowing the present rarity of acquired metallo-enzymes, and the doubt as to whether they, or the KPC and OXA types, will ultimately pose the greatest threat.

Conclusion

Much has been written in the medical and lay press on the 'end of antibiotics'. This literature stresses the most frequent problem organisms, principally MRSA, and often fails to emphasise those — *P.*

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aeruginosa and *Acinetobacter* spp. — where the end is nearest. In both these latter organisms there is widespread resistance to drugs except carbapenems and significant erosion of carbapenem activity. Among Enterobacteriaceae there is an accumulation of strains resistant to all drugs except carbapenems, meaning that any wide emergence of carbapenemases could swiftly generate a large pool of pan-resistance.

When gram-negative isolates appear resistant to all relevant β-lactams, aminoglycosides and fluoroquinolones, it is usually possible to identify some obscure agent — often polymyxin, that retains *in-vitro* activity. *In-vivo* efficacy is however uncertain and toxicity significant. Less toxic agents that often retain against *Acinetobacter* spp. and Enterobacteriaceae activity include minocycline and doxycycline, also, in the case of *Acinetobacter* spp., sulbactam. Among agents recently launched, or shortly to be launched, only tigecycline has anything to offer against multiresistant gram-negative bacteria, and it is limited by a lack of anti-pseudomonas activity. Long-term possibilities include dihydroxypyridone monobactams, peptides, efflux inhibitors and carbapenemase inhibitors.

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References

1 **Livermore DM, Mushtaq S, James D, Potz N, Walker RA, Charlett A, et al.** In-vitro activity of piperacillin/tazobactam and other broad-spectrum antibiotics against bacteria from hospitalised patients in the British Isles. *Int J Antimicrob Agents* 2003: in press.

2 **Reacher MH, Shah A, Livermore DM, Wale MC, Graham C, Johnson AP, et al.** Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. *BMJ* 2000; 320: 213–6.

3 **Jones RN.** Resistance patterns among nosocomial pathogens: trends over the past few years. *Chest* 2001; 119(2 Suppl): 397S–404S.

4 **Centers for Disease Control and Prevention.** *Staphylococcus aureus* resistant to vancomycin — United States, 2002. *MMWR* 2002; 51: 565–7.

5 **Gruneberg RN.** Global surveillance through PROTEKT: the first year. *J Chemother* 2002; 14 Suppl 3: 9–16.

6 **Livermore DM.** The impact of carbapenemases on antimicrobial development and therapy. *Curr Opin Investig Drugs* 2002; 3: 218–24.

7 **Johnson AP, Livermore DM, Tillotson GS.** Antimicrobial susceptibility of gram-positive bacteria: what's current, what's anticipated? *J Hosp Infect* 2001; 49 Suppl A: S3–11.

8 **Garau J.** Treatment of drug-resistant pneumococcal pneumonia. *Lancet Infect Dis* 2002; 2: 404–15.

9 **Livermore DM.** Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*, our worst nightmare? *Clin Infect Dis* 2002; 34: 634–40.

10 **Henwood CJ, Livermore DM, James D, Warner M, Pseudomonas Study Group.** Antimicrobial susceptibility of *Pseudomonas aeruginosa*: results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test. *J Antimicrob Chemother* 2001; 47: 789–99.

11 **Chen HY, Yuan M, Ibrahim-Elmagboul IB, Livermore DM.** National survey of susceptibility to antimicrobials amongst clinical isolates of *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1995; 35: 521–34.

12 **Oliver A, Canton R, Campo P, Baquero F, Blazquez J.** High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000; 288: 1251–4.

13 **Nordmann P, Poirel L.** Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect* 2002; 8: 321–31.

14 **Danel F, Hall LM, Gür D, Akalin HE, Livermore DM.** Transferable production of PER-1 β-lactamase in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1995; 35: 281–94.

15 **Naas T, Nordmann P.** OXA-type β-lactamases. *Curr Pharm Des* 1999; 5: 865–79.

16 **Tsakris A, Pournaras S, Woodford N, Palepou MF, Babini GS, Douboyas J, et al.** Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. *J Clin Microbiol* 2000; 38: 1290–2.

17 **Gibb AP, Tribuddharat C, Moore RA, Louie TJ, Krulicki 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.

18 **Evans ME, Feola DJ, Rapp RP.** Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. *Ann Pharmacother* 1999; 33: 960–7.

19 **Levin AS, Barone AA, Penco J, Santos MV, Marinho IS, Arruda EA, et al.** Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999; 28: 1008–11.

20 **Bergogne-Berezin E, Towner KJ.** *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* 1996; 9: 148–65.

21 **Afzal-Shah M, Woodford N, Livermore DM.** Characterization of: OXA-25, -26 and -27 molecular Class D β-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2001; 45: 583–8.

22 **Bou G, Cervero G, Dominguez MA, Quereda C, Martinez-Beltran J.** Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in *A. baumannii* is not due solely to the presence of β-lactamases. *J Clin Microbiol* 2000; 38: 3299–305.

23 **Lopez-Otsoa F, Gallego L, Towner KJ, Tysall L, Woodford N, Livermore DM.** Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in Northern Spain. *J Clin Microbiol* 2002; 40: 4741–3.

24 **Henwood CJ, Gatward T, Warner M, James D, Stockdale MW, Spence R, et al.** Antibiotic resistance among clinical isolates of *Acinetobacter* in the United Kingdom and *in-vitro* evaluation of tigecycline (GAR-936). *J Antimicrob Chemother* 2002; 49: 479–87.

25 **Montero A, Ariza J, Corbella X, Domenech A, Cabellos C, Ayats J, et al.** Efficacy of colistin versus β-lactams, aminoglycosides, and rifampin as monotherapy in a mouse model of

 ISSN 0785-3890

pneumonia caused by multiresistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2002; 46: 1946–52.

26 **Wood GC, Hanes SD, Croce MA, Fabian TC, Boucher BA.** Comparison of ampicillin-sulbactam and imipenem-cilastatin for the treatment of acinetobacter ventilator-associated pneumonia. *Clin Infect Dis* 2002; 34: 1425–30.

27 **Corbella X, Ariza J, Ardanuy C, Vuelta M, Tubau F, Sora M, et al.** Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multiresistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 1998; 42: 793–802.

28 **Bello H, Dominguez M, Gonzalez G, Zemelman R, Mella S, Young HK, et al.** In-vitro activities of ampicillin, sulbactam and a combination of ampicillin and sulbactam against isolates of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex isolated in Chile between 1990 and 1998. *J Antimicrob Chemother* 2000; 45: 712–3.

29 **Bonfiglio G, Livermore DM.** Effect of media composition on the susceptibility of *Xanthomonas maltophilia* to β-lactam antibiotics. *J Antimicrob Chemother* 1991; 28: 837–42.

30 **VanCouwenberghe CJ, Farver TB, Cohen SH.** Risk factors associated with isolation of *Stenotrophomonas (Xanthomonas) maltophilia* in clinical specimens. *Infect Control Hosp Epidemiol* 1997; 18: 316–21.

31 **Tsiodras S, Pittet D, Carmeli Y, Eliopoulos G, Boucher H, Harbarth S.** Clinical implications of *Stenotrophomonas maltophilia* resistant to trimethoprim-sulfamethoxazole: a study of 69 patients at 2 university hospitals. *Scand J Infect Dis* 2000; 32: 651–6.

32 **Munoz Bellido JL, Garcia-Rodriguez JA.** Aztreonam-clavulanic acid synergy does not mean extended-spectrum β-lactamase in *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 1998; 41: 493–4.

33 **Garcia Sanchez JE, Vazquez Lopez ML, Blazquez de Castro AM, Perez Simon JA, Gutierrez NG, Martin IT, et al.** Aztreonam/clavulanic acid in the treatment of serious infections caused by *Stenotrophomonas maltophilia* in neutropenic patients: case reports. *J Chemother* 1997; 9: 238–40.

34 **Downhour NP, Petersen EA, Krueger TS, Tangella KV, Nix DE.** Severe cellulitis/myositis caused by *Stenotrophomonas maltophilia*. *Ann Pharmacother* 2002; 36: 63–6.

35 **Ho PL, Cheung TK, Yam WC, Yuen KY.** Characterization of a laboratory-generated variant of BPS β-lactamase from *Burkholderia pseudomallei* that hydrolyses ceftazidime. *J Antimicrob Chemother* 2002; 50: 723–6.

36 **Niumsup P, Wuthiekanun V.** Cloning of the class D β-lactamase gene from *Burkholderia pseudomallei* and studies on its expression in ceftazidime-susceptible and -resistant strains. *J Antimicrob Chemother* 2002; 50: 445–55.

37 **Cheung TK, Ho PL, Woo PC, Yuen KY, Chau PY.** Cloning and expression of class A β-lactamase gene *blaA* (BPS) in *Burkholderia pseudomallei*. *Antimicrob Agents Chemother* 2002; 46: 1132–5.

38 **Poole K.** Multidrug efflux pumps and antimicrobial resistance in Pseudomonas aeruginosa and related organisms. *J Mol Microbiol Biotechnol* 2001; 3: 255–64.

39 **Moore RA, DeShazer D, Reckseidler S, Weissman A, Woods DE.** Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. *Antimicrob Agents Chemother* 1999; 43: 465–70.

40 **Baxter IA, Lambert PA.** Isolation and partial purification of a carbapenem-hydrolysing metallo-β-lactamase from *Pseudomonas cepacia*. *FEMS Microbiol Lett* 1994; 122: 251–6.

41 **Pitt TL, Kaufmann ME, Patel PS, Benge LC, Gaskin S, Livermore DM.** Type characterisation and antibiotic susceptibility of *Burkholderia (Pseudomonas) cepacia* isolates from patients with cystic fibrosis in the United Kingdom and the Republic of Ireland. *J Med Microbiol* 1996; 44: 203–10.

42 **Aaron SD, Ferris W, Henry DA, Speert DP, Macdonald NE.** Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *Am J Respir Crit Care Med* 2000; 161: 1206–12.

43 **Koh TH, Sng LH, Babini GS, Woodford N, Livermore DM, Hall LMC.** Carbapenem-resistant *Klebsiella pnuemoniae* in Singapore producing IMP-1 β-lactamase and lacking an outer membrane protein. *Antimicrob Agents Chemother* 2001; 45: 1939–40.

44 **Yan JJ, Ko WC, Chuang CL, Wu JJ.** Metallo-β-lactamase-producing Enterobacteriaceae isolates in a university hospital in Taiwan: prevalence of IMP-8 in *Enterobacter cloacae* and first identification of VIM-2 in *Citrobacter freundii*. *J Antimicrob Chemother* 2002; 50: 503–11.

45 **Yan JJ, Ko WC, Tsai SH, Wu HM, Wu JJ.** Outbreak of infection with multidrug-resistant *Klebsiella pneumoniae* carrying *bla*(IMP-8) in a university medical center in Taiwan. *J Clin Microbiol* 2001; 39: 4433–9.

46 **Hawkey PM, Xiong J, Ye H, Li H, M'Zali FH.** Occurrence of a new metallo-β-lactamase IMP-4 carried on a conjugative plasmid in *Citrobacter youngae* from the People's Republic of China. *FEMS Microbiol Lett* 2001; 194: 53–7.

47 **Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al.** Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001; 45: 1151–61.

48 **Chopra I.** New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. *Drug Resist Updat* 2002; 5: 119–25.

49 **Weiss K, Restieri C, De Carolis E, Laverdiere M, Guay H.** Comparative activity of new quinolones against 326 clinical isolates of *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 2000; 45: 363–5.

50 **Critchley IA, Basker MJ, Edmondson RA, Knott SJ.** Uptake of a catecholic cephalosporin by the iron transport system of *Escherichia coli*. *J Antimicrob Chemother* 1991; 28: 377–88.

51 **Giacometti A, Cirioni O, Del Prete MS, Barchiesi F, Paggi AM, Petrelli E, et al.** Comparative activities of polycationic peptides and clinically used antimicrobial agents against multidrug-resistant nosocomial isolates of *Acinetobacter baumannii*. *J Antimicrob Chemother* 2000; 46: 807–10.

52 **Giacometti A, Cirioni O, Del Prete MS, Barchiesi F, Fortuna M, Drenaggi D, et al.** In vitro activities of membrane-active peptides alone and in combination with clinically used antimicrobial agents against *Stenotrophomonas maltophilia*. *Antimicrob Agents Chemother* 2000; 44: 1716–9.

53 **Giacometti A, Cirioni O, Barchiesi F, Fortuna M, Scalise G.** In-vitro activity of cationic peptides alone and in combination with clinically used antimicrobial agents against *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1999; 44: 641–5.

54 **Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, et al.** Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. *Antimicrob Agents Chemother* 2001; 45: 105–16.

55 **Payne DJ, Du W, Bateson JH.** β-Lactamase epidemiology and the utility of established and novel β-lactamase inhibitors. *Expert Opin Investig Drugs* 2000; 9: 247–61.

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