Intensive Care Med (2000) 26: S14–S21

D. M. Livermore

Epidemiology of antibiotic resistance

D. M. Livermore
Antibiotic Resistance Monitoring
and Reference Laboratory,
Central Public Health Laboratory,
61 Colindale Avenue, London NW9 5HT,
UK
e-mail: DLivermore@phls.nhs.uk
Tel.: + 44–181–200–4400 x4223
Fax: + 44-181-200-7449

Abstract Three biological processes contribute to the accumulation of bacterial drug resistance: new selection, gene epidemics and strain epidemics. New resistance emerges by (i) the advantaging of entire species, (ii) by mutation, and (iii) by the escape of resistance genes to mobile DNA. Organisms to have 'benefited' from modern patterns of cephalosporins and quinolone use include enterococci, *Clostridium difficile*, coagulase-negative staphylococci and *Enterobacter* spp. Mutational resistance notoriously occurs with certain antibiotic/organsim combinations and allows rapid multifocal accumulation of resistance. At worst, therapy can fail when resistant mutants are selected in individual patients. Escape of new genes to mobile DNA is rare but, having occurred, permits massive 'gene epidemics', as the same genes and plasmids spread into diverse pathogens. Strain epidemics notoriously occur in individual units, reflecting breakdowns of hygiene. Some strains achieve a much wider distribution: thus, much of the MRSA problem in the UK depends on the dissemination of two epidemic strains, EMRSA15 and 16; penicillin resistant pneumococci of serotypes 6 and 23 have disseminated internationally from Spain and a serotype K25 strain of *Klebsiella pneumoniae* with SHV-4 β-lactamase has spread widely in France. It remains unknown why some strains and genes achieve wide spread whereas others, equally resistant, fail to do so. There is no simple cure for resistance but the best opportunities for control lie in lesser and better use of antibiotics backed by swifter and more accurate microbiology; in developing new antibiotics; and in protecting old ones from resistance determinants. All this must be supported by good local knowledge of the epidemiology of infections and resistance and of the likelihood of particular antibiotics to select resistance.

Key words Antibiotic resistance · Epidemiology

Introduction

Bacterial drug resistance is increasingly prevalent world-wide. Its accumulation reflects the emergence of new resistances, the epidemic transfer of resistance genes among bacteria and the epidemic spread of resistant strains among patients. The relative importance of these processes varies with the combination of species and antimicrobial, but each process is driven by the selective pressure of antimicrobial usage. This contention is supported by the facts that new resistances have repeatedly emerged after the introduction of new antimicrobials; that acquired resistances are absent from bacteria isolated before the antibiotic era and that – critically for this forum – resistance is especially prevalent in settings, for example ICUs, with heavy antibiotic us-

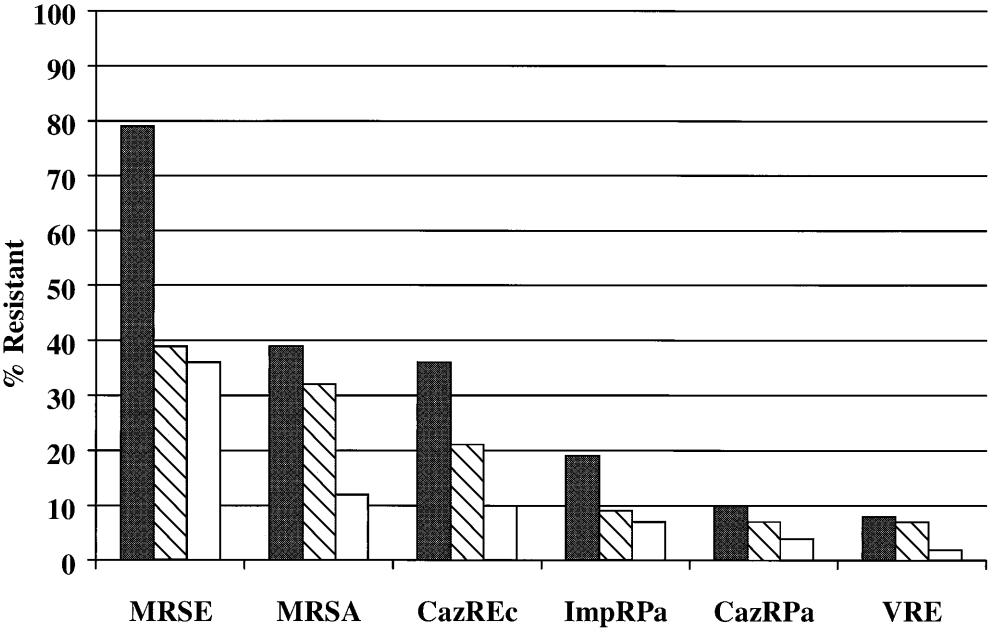

Fig 1 Prevalence of important resistant phenotypes among isolates in the USA: *solid bars* ICU isolates; *hatched bars* isolates from non-ICU in-patients; *open bars* isolates from out-patients. *MRSE* methicillin resistant *Staphylococcus epidermidis*; *MRSA* methicillin-resistant *S. aureus*; *CazREc* ceftazidime-resistant *E. cloacae*; *ImpRPa* imipenem-resistant *P. aeruginosa*; *CazRPa* ceftazidime-resistant *P. aeruginosa*; *VRE* vancomycin-resistant enterococci. Adapted, with permission, from Archibald et al. [2]

age [1]. Thus, Chen et al. [2] found that the prevalence of resistance to penicillins, cephalosporins, ciprofloxacin and aminoglycosides was roughly twice as great among *Pseudomonas aeruginosa* isolates from ICU patients as amongst those from general wards or from out-patients in the UK and Archibald et al. [3] found similar relationships for a broader range of species in US hospitals (Fig. 1).

Selection of new resistance

New resistance problems can emerge via species selection, mutation, or DNA transfer. Species selection changes the relative importance of different pathogens; mutation and DNA transfer yield resistance in previously susceptible species.

Species selection

Shifts in the importance of different opportunist pathogens receive less attention than the emergence of new resistance phenotypes, perhaps because they do not carry the same 'superbug' shock as the vancomycin-intermediate *Staphylococcus aureus* or carbapenem-resistant *Acinetobacter.* Nevertheless, antibiotic usage favours whole species or genera that are resistant, and this process gradually undermines once-valuable antibiotics. Such shifts can occur in individual patients in the community, as exemplified by vaginal candidiasis in those receiving protracted antibacterial therapy, but are a far greater problem in ICU patients, whose impaired defences leave them vulnerable to repeated opportunistic infections. Antimicrobial 'victory' against one pathogen opens an ecological niche to more resistant organisms, both in the individual patient and *in the patient group as a whole.*

Enterococci provide an example, increasingly-important opportunist pathogens in many centres, and it is tempting to speculate that their success reflects the increasing use of quinolones and cephalosporins through the 1980s and 1990s. Enterococci are inherently resistant to these drugs and so enjoy a competitive advantage as the microbial competition is eliminated [4]. A second example of species shift is the increasing dominance of gram-positive organisms – particularly coagulase negative staphylococci and α-haemolytic streptococci – as agents of bacteraemias in haematology patients (Fig. 2) [5]. Once again, changing patterns of antimicrobial usage may be responsible: the streptococci are inherently resistant to fluoroquinolones, which are heavily used as prophylaxis in these patients, and many coagulase-negative staphylococci have developed multiresistance through mutation and plasmid acquisition. As a final example among gram-positive species, *Clostridium difficile* is notoriously isolated from those who have received prior antimicrobials, especially cephalosporins and clindamycin. These drugs disturb the gut microflora, creating an ecological niche for the clostridia, which evade antimicrobials by sporulation [6]. Among gram-negative pathogens *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are increasingly seen in many ICUs, often from patients who have received multiple previous antibiotics [7, 8]. *A baumannii* is often resistant to all drugs besides carbapenems

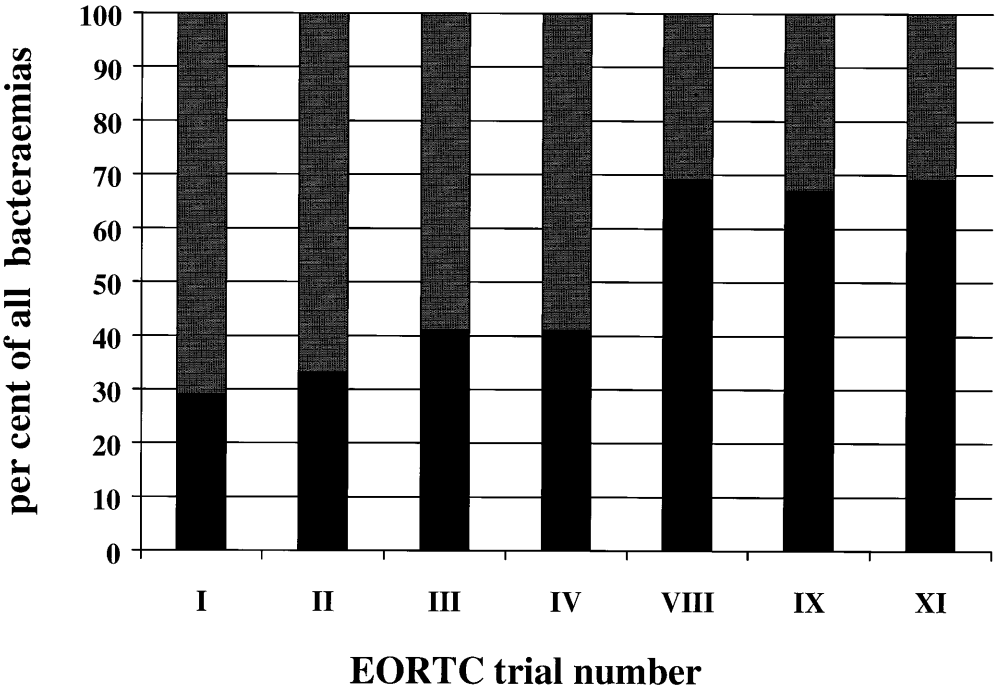

Fig. 2 Proportions of gram-positive (black) and -negative (grey) organisms from bacteraemias in neutropaenic patients entered into antibiotic trails run by the European Organisation for the Research and Treatment of Cancer. Trial I, 1973–76; II, 1977–80; III, 1980–83; IV, 1986–87; VIII, 1988–90; IX, 1991–92; and XI, 1993–94

and *Stenotrophomonas maltophilia* is inherently resistant to all except co-trimoxazole and ticarcillin/clavulanate. Once again, resistance is a likely factor in the emergence of these pathogens. As a final example, *Enterobacter* and *Serratia* species have proved better able to develop mutational cephalosporin resistance than *Escherichia coli,* and their increasing role as opportunist nosocomial pathogens in the seriously-ill may reflect the heavy use of these antibiotics [9].

Sceptics will point to the many "may have's" in the preceding paragraphs, and it is doubtful whether changing antimicrobial use has been the sole factor in the changing epidemiology of infections. Other critical factors include the patients and the severity of their underlying diseases; changing anti-cancer regimens (whose interactions with bacteria and antimicrobials deserves greater study); increased use of indwelling catheters, which predispose to infection with coagulase-negative staphylococci; increased mucositis, which may favour α-haemolytic streptococci, and changing attitudes to whether some bacterial species are viewed as colonists or pathogens [5, 10]. Despite these caveats, it would be an extraordinary coincidence if the increasing role of inherently resistant pathogens was unrelated to the use of those antibiotics to which they are resistant.

Mutational and acquired resistance

Resistance in previously sensitive species can arise through mutations, which are random spontaneous genetic changes. Antibiotics do not 'cause' these mutations (a mutagenic antibiotic would not receive a product license). Rather, they select pre-existing resistant mutants from susceptible populations. Such mutants doubtless have arisen throughout microbial history but enjoyed no competitive advantage before the antibiotic era. Hitherto-susceptible species can also acquire resistance determinants from other organisms, whether as plasmids – discrete self-transmissible loops of DNA – or as chromosomal inserts. Chromosomal inserts include transposons – sticky ended sections of DNA able to 'jump' from one genome to another – and genes transferred by bacteriophage (viruses that infect bacteria). A few species directly accept and incorporate fragments of naked DNA released by dead cells of related organisms. These fragments enter the corresponding site in the recipient's chromosome, and the resulting 'mosaic' genes yield products with reduced affinity for antibiotics. This is the basis of penicillin resistance in pneumococci and meningococci and of β-lactamase-independent ampicillin resistance in haemophili and gonococci [11]. The origins of most plasmid mediated-resistances are obscure but some originated in antibiotic-producing streptomycetes (filamentous bacteria) [12] and some plasmid-mediated β-lactamases are genetic escapes from the chromosomes of other species [13].

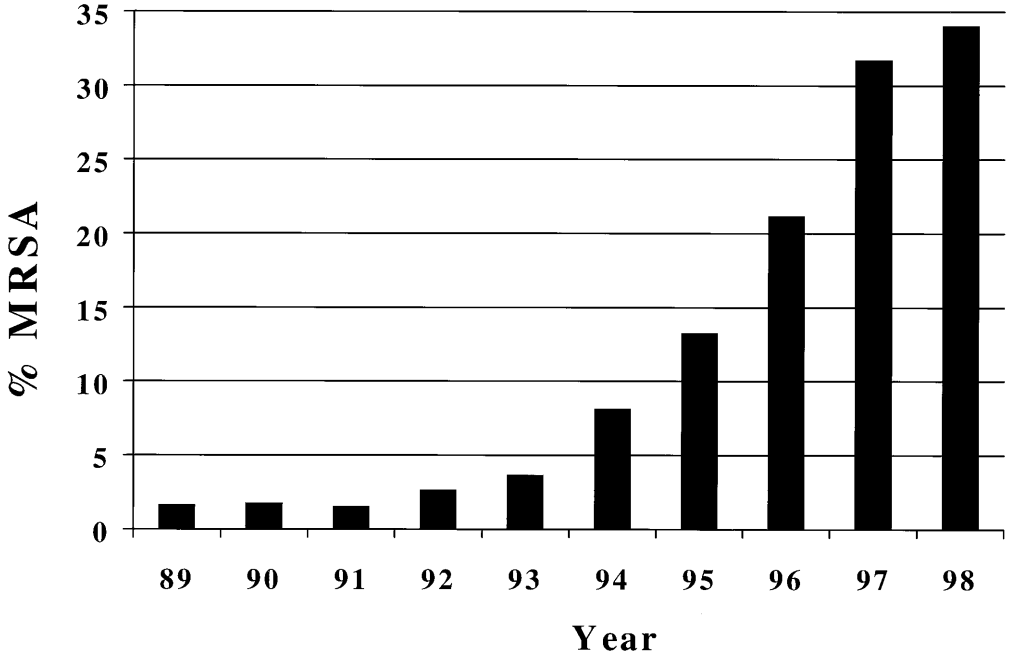

Fig 3 Proportion of MRSA among all *S. aureus* from bacteraemias in England and Wales. Data as reported to the PHLS. Based on references [1] and [28]

Epidemiology of mutational resistance

According to the particular combination of bacterium and drug, resistant mutants arise more or less readily. If mutational resistance emerges at high frequency without deleterious side-effects, it can swiftly undermine an antibiotic's utility by repeatedly allowing the selection of resistance in patients receiving therapy. A high risk of mutational resistance applies to several of antimicrobial groups introduced in the 1980s. Fluoroquinolones initially were perceived as active against methicillin-resistant *Staphylococcus aureus* (MRSA) but staphylococci have an endogenous efflux pump and resistance arises if this is up-regulated by mutation at *nor*A [14]. The result is that ciprofloxacin has had disappointing efficacy against staphylococcal infections, with most MRSA now resistant. Why *S. aureus* should have an efflux pump for synthetic antimicrobials is less clear, but efflux systems may have a primary role of cleaning *any* amphipathic foreign substance from biological membranes [15]. Another example of rapid mutational resistance concerns the oxyimino-aminothiazolyl 'third-generation' cephalosporins. These initially were thought active against *Enterobacter*, *Citrobacter* and *Serratia* spp., but it quickly became apparent that their activity critically depends on failure to induce the chromosomal AmpC β-lactamases of these species. The result is that the cephalosporins select for 'derepressed' mutants, which hyperproduce these β-lactamases without induction [13]. Such mutants arise at high frequency (one cell per million), and the risk of their selection, and of contingent clinical failure, is put at ca. 20% during cephalosporin therapy of *Enterobacter* bacteraemia [16]; the risk in *Enterobacter* pneumonia is probably higher [9] whereas the risk is minimal in urinary tract infections, where the cephalosporins achieve concentrations above the MICs for derepressed mutants.

Once selected in one patient, resistant mutants may spread to others, giving strain epidemics (see below). Moreover, and critically, if resistance demands only a single point mutation (as in AmpC derepressed *Enterobacter* spp. and ciprofloxacin-resistant MRSA), identical mutants may be selected afresh in further patients, giving an easy potential for swift multifocal accumulation. Over 30% of Enterobacter isolates from bacteraemias in the UK now are resistant to oxyimino-aminothiazolyl cephalosporins through derepression of AmpC enzymes, and this resistance is widely seen among different strains (PHLS data on file).

Not all mutational resistances arise in a single step. Cephalosporin use has also selected for extended-spectrum β-lactamase (ESBL) mutants of the plasmid-mediated TEM and SHV β-lactamases (see also below). These have one to six amino acid substitutions compared with the parent enzymes, giving a more accommodating active site and allowing hydrolytic attack on oxyimino aminothiazolyl 'third-generation' compounds [13]. Since multiple mutations are involved, the initial evolution of ESBLs should be infrequent, and their emergence in individual patients during therapy certainly is not a significant risk. Nevertheless ESBLs have accumulated rapidly in klebsiellae, where a recent survey

found them in 23% of 966 isolates from 35 European ICUs [17]. This accumulation is partly explicable by strain spread (see below) but this is not the whole story, as it does not account for the multiplicity of different TEM and SHV ESBL mutants now recorded – more than 60 at the present count. Moreover the same ESBLs have evolved independently at different times and places [18]. The inescapable conclusion is that the evolution of ESBLs is *not* uncommon, and two points may be critical to this deduction. First, the parent TEM and SHV β-lactamase genes are often encoded by multi-copy plasmids, or by plasmids carrying multiple gene copies, thereby multiplying the number of genomes available for mutation [19]. Secondly, β-lactamase genes are not under selection pressure for most of a host cell's existence, meaning that they are free to accumulate random mutations some of which may yield progenitor types for potent ESBLs.

Epidemiology of transferable resistance: gene epidemics

Mosaic gene formation and bacteriophage-mediated transfer of resistance genes appear to be rare genetic events, and their importance lies in the fact that strains which initially acquire their resistance by these mechanisms may spread among patients (see below). Plasmids, by contrast, often are freely transmissible, as are some chromosomally-inserting transposons. Dissemination of these elements can give 'gene epidemics,' with the same determinants becoming established among diverse organisms. Plasmids encoding the TEM-1 plasmid-mediated β-lactamases were first recognised in 1965 but have since spread to 20–60% of clinical isolates of *Enterobacteriaceae*; to a few strains of *P. aeruginosa* and, according to the country, to anywhere between 1 and 50% of *Haemophilus influenzae* and *Neisseria gonorrhoeae* [9, 13]. By any standards, this is a phenomenal evolutionary success; on a more practical basis the dissemination of these enzymes has dealt the death-knell for the use of pencilling without the protection of β-lactamase inhibitors in serious infections involving gram-negative bacteria.

There have been many instances where single plasmids have disseminated among multiple host strains in the course of on outbreaks. Plasmids coding one of the first ESBLs recorded – TEM-3 – disseminated among klebsiellae, *E. coli* and *Serratia* spp. in the hospitals around Clermont-Ferrand in 1985 to 1987 [20] and a single plasmid coding another ESBL – TEM-26 – disseminated in multiple Klebsiella and *E. coli* strains in several hospitals and nursing homes in and around Chicago in the mid-1990s [21]. A few gene epidemics have spread between gram-positive and negative bacteria. Thus, tetracycline resistance contingent on *tet*M now occurs in streptococci and staphylococci, *Ureoplasma ureolyticum* and *N. gonorrhoeae* [22].

The extent of plasmid epidemics partly depends upon the transmissibility of individual plasmids or conjugative transposons, and on the ability of transposon-encoded genes to transfer among plasmids, some of which have broad host ranges than others. A further key factor is the location some resistance genes in integrons, which may themselves be located within transposons. Integrons can be crudely described as natural recombination systems, with a propensity to accumulate separate resistance genes behind a single promoter [24]. The *aad*3 gene, encoding a streptomycin nucleotidyl transferase, ANT3"-1, is integron-determined, and its consequently frequent linkage to other resistance determinants may explain its continued frequency in the absence of significant streptomycin usage [25].

The factors that determine whether a mobile gene will spread widely remain imperfectly understood. TEM-2 β-lactamase differs from TEM-1 by just one amino-acid, confers similar resistance and can be coded by similarly promiscuous plasmids. Both enzymes have been known from diverse strains for over 30 years, and there is no obvious reason why one should have been a greater evolutionary success than the other. Nevertheless, surveys in every populated continent have found TEM-1 enzyme to be 10–20-fold more prevalent than TEM-2 [9, 13]. Likewise, integron-borne genes with a greater potential to cause clinically-significant resistance than *aad*3 have failed to disseminated widely: examples include the genes for many OXA and PSE β-lactamases [13]. A final consideration is that many epidemic plasmids carry multiple resistances and remain under active evolution, with determinants being gained, altered or lost. Amplification of resistance genes can occur within plasmids, increasing product expression and the level of resistance[19, 25].

It is sometimes suggested that plasmid spread should be self-limiting, as the need to replicate additional DNA should burden the host strain in the absence of selection pressure. Evidence for this view is scanty, and it seems more likely that host strains evolve to minimise the burden exerted by the plasmid, which achieves symbiosis with its host strain [26]. What is beyond dispute is that many plasmid-mediated resistances have become extremely widespread and that some e.g., streptomycin resistance in *E. coli*,have remained prevalent despite long absence of selection pressure [25]. These observations argue that plasmid carriage exerts little real burden.

Dissemination of resistant strains

The final key factor behind the increasing resistance problem is patient-to-patient transfer of resistant strains. Nosocomial spread of resistant infection is a frequent clinical problem and, with hindsight, it is usually possible to identify some deficiency in hygiene that facilitated the process. Common vectors are the hands of staff, non-sterile devices or procedures and, as a source of initial gut colonisation, hospital food [27].

A few resistant strains establish themselves widely. Figure 3 shows the rising proportion of MRSA from bacteraemias in England and Wales since 1990. Until 1993 this proportion was steady at 1–2% but thereafter it rose 50% year-on-year until 1997, when the overall rate reached 32 [1, 28]. This explosive increase was largely attributable to the dissemination of just two strains, EMRSA15 and 16. Earlier 'epidemic' MRSA proved far less successful at spreading among patients or, if able to spread, were less able to give significant infections [29].

Success of individual multi-resistant strains is apparent also with *Burkholderia cepacia*, where one ribotype dominates in cystic fibrosis patients in the UK [30], and in *Klebsiella pneumoniae,* where a serotype K25 strain with SHV-4 β-lactamase and cross-resistance to amikacin and ciprofloxacin has disseminated widely in France, and a serotype K41 strain with SHV-5 β-lactamase is widespread in southern England [26, 31]. Finally, the increasing problems of penicillin-resistant pneumococci hugely reflects the international dissemination of just a few clones belonging to serotypes 6, 9, 14, 19 and 23 [32].

The reasons for the epidemic success of some resistant strains remain obscure: many similarly-resistant strains remain sporadic or confined to single hospitals. In the case of MRSA it is possible (though unprovable) that the increase illustrated in Fig. 3 reflected changing hospital practice and that earlier MRSA strains might have been equally successful as EMRSA15 and 16 had they been given opportunities provided by greater patient throughput. However, it is equally plausible that EMRSA15 and 16 differ from earlier MRSA strains in having a greater ability to colonise and, having colonised, to cause infection. Many factors potentially contribute to the spread and persistence of individual clones, including: (i) increased adherence to host cells or prosthetic materials, (ii) tolerance to desiccation and (iii) resistance to the disinfectants used to clean hospitals. Both EMRSA15 and 16 strains are quinolone resistant [29, 33] and may have been advantaged by use of these drugs. This, however, is a tenuous argument, allowing that mutational quinolone resistance can arise readily in any *S. aureus* strain (see above). The reasons for the success of those multi-resistant *Klebsiella* strains that have spread between units and cities are equally unclear. The serotype K25 strain has SHV-4 β-lactamase, which gives no greater resistance to oxyimino-aminothiazolyl cephalosporins than many other ESBL types [25]. What is perhaps more relevant is that the strain was among the first klebsiellae to become quinolone resistant, and this may have facilitated its early spread. If so, the strain's dominance is likely to decline as quinolone resistance becomes more widespread. In addition, one report suggests that the strain has a plasmid-mediated fimbial antigen which aids adherence to gut mucosa – which is often a first step in the infective process [34].

Countering resistance epidemics

The accumulation of resistance is causing increasing concern, exacerbated by the lack of new antimicrobial classes since the 1960s [1]. Several strategies are appropriate in confronting this problem [1, 10]. Developing new antimicrobials is vital, as is extending the life of old antibiotics, e.g. by combining penicillins with β-lactamase inhibitors [35], or (in principle) by combining effluxed drugs with efflux inhibitors. Ways also are needed to minimise the emergence and spread of resistance, by less and better use of existing antibiotics and by stopping the spread of resistant strains among patients and of plasmids among bacteria.

Minimising antibiotic usage is easier said than done, especially in ICU patients. Nevertheless it is critical to distinguish infection, which requires therapy, from colonisation, which does not. When antibiotics are given, it is desirable to minimise the duration of therapy, thus reducing the selection pressure of the body microflora, which is often the source of future opportunist infections. Better tailoring the therapy depends -ideally- on early recognition of the causative pathogen and its resistances [36]. In the future gene-chip technology may enable precise bedside recognition of pathogens and their resistances without the need for two days of microbiological culture and sensitivity testing. In the meantime, much therapy remains empirical, and should be based on good local knowledge of the likely pathogens and their resistances. Such information must be communicated effectively to prescribing physicians and surgeons [1].

Some contend that the oldest antibiotics should always be used first, with newer agents reserved for resistant strains, but this argument seems based more on prejudice than logic. Older agents that prove inactive exert selection pressure for no gain, and the inevitable change to an active drug then leads to a further round of selection pressure. In a few cases, newer antibiotics have allowed displacement of resistance to older agents. Betts et al. [37] displaced gentamicin resistance by formulary substitution of gentamicin with amikacin, al-

though others who followed this strategy were less successful; Rahal et al. [38] displaced ESBL-positive klebsiellae by restricting cephalosporin use and Bradley et al. [39] displaced vancomycin-resistant enterococci from a haematology unit by switching from ceftazidime to piperacillin/tazobactam. What thus seems more vital than a preference for old agents is a greater appreciation, by all physicians, of the likely ecological and public health consequences of the use of particular antimicrobial classes as well as of antimicrobials in general.

References

1. Standing Medical Advisory Committee (1998) The path of least resistance. Department of Health, London
2. Chen HY, Yuan M, Ibrahim-Elmogboul IB, Livermore DM (l995) National survey of susceptibility to antimicrobials amongst clinical isolates of *Pseudomonas aeruginosa*. J Antimicrob Chemother 35: 521–534
3. Archibald L, Phillips L, Monnet D et al. (1997) Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis 24: 211–215
4. Woodford N, Johnson AP, Morrison D, Speller DC (1995) Current perspectives on glycopeptide resistance. Clin Microbiol Rev 8: 585–615
5. Oppenheim BA (l998) The changing pattern of infection in neutropenic patients. J Antimicrob Chemother 41 Suppl D:7–11
6. Spencer RC (1997) The role of antimicrobial agents in the aetiology of *Clostridium difficile*-associated disease. J Antimicrob Chemother 41 [Suppl C]:21–27
7. Humphreys H, Towner KJ (1997) Impact of *Acinetobacter* spp. in intensive care units in Great Britain and Ireland. J Hosp Inf 37: 281–286
8. Muder RR, Yu VL, Dummer JS et al (l987) Infections caused by *Pseudomonas maltophilia*: expanding clinical spectrum. Arch Intern Med 147: 1672–1674
9. Sanders CC, Sanders WE (1992) β-Lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin Infect Dis 15: 824–839
10. Spratt BG (1994) Resistance to antibiotics mediated by target alterations. Science 264: 388–393
11. Klastersky J, Zinner SH, Calandra T et al. (1988) Empiric antimicrobial therapy for febrile, granulocytopenic cancer patients: lessons from four EORTC trials. Euro J Cancer Clin Oncol 24 [Suppl 1]:S35–S45
12. Davies JE (1997) Origins, acquisition and dissemination of antibiotic resistance determinants. In: Chadwick DJ, Goode J (eds) Antibiotic resistance: origins, evolution, selection and spread. Wiley, Chichester
13. Livermore DM. (1995) β-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev 8: 557–584
14. Piddock LJ (l995) Mechanisms of resistance to fluoroquinolones: state-of-the-art 1992–1994. Drugs 49 [Suppl 2]:29–35
15. Nikaido H (1996) Multidrug efflux pumps of gram-negative bacteria. J Bacteriol 178: 5853–5859
16. Chow JW, Fine MJ, Shlaes DM et al. (1991) Enterobacter bacteraemia: clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med 115: 585–590
17. Livermore DM, Yuan M (1996) Antibiotic resistance and production of extended-spectrum β-lactamases amongst *Klebsiella* spp. from intensive care units in Europe. J Antimicrob Chemother 38: 409–424
18. Hibbert-Rogers LC, Heritage J, Todd N, Hawkey PM (1994) Convergent evolution of TEM-26, a β-lactamase with extended-spectrum activity. J Antimicrob Chemother 33: 707–720
19. Xiang X, Shannon K, French G (1997) Mechanism and stability of hyperproduction of the extended-spectrum β-lactamase SHV-5 in *Klebsiella pneumoniae*. J Antimicrob Chemother 40: 525–532
20. Petit A, Gerbaud G, Sirot D et al. (1990) Molecular epidemiology of TEM-3 (CTX-1) β-lactamase. Antimicrob Agents Chemother 34: 219–224
21. Schiappa DA, Hayden MK, Matushek MG et al (1996) Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. J Infect Dis 174: 529–536
22. Roberts MC (1997) Genetic mobility and distribution of tetracycline resistance determinants. Ciba Foundation Symposium 207: 206–218
23. Hall RM, Collis CM (l995) Mobile genes and integrons: capture and spread of genes by site-specific recombination. Mol Microbiol 15: 593–600
24. Chiew YF, Yeo SF, Hall LMC, Livermore DM (1998) Can susceptibility to an antimicrobial be restored by halting its use? The case of streptomycin versus Enterobacteriacae. J Antimicrob Chemother 41: 247–251
25. Yuan M, Aucken H, Hall LMC et al (1998) Epidemiological typing of klebsiellae with extended-spectrum β-lactamases from European intensive care units. J Antimicrob Chemother 41: 527–539
26. Bouma JE, Lenski RE (1998) Evolution of a bacteria/plasmid association. Nature 335: 351–352
27. Department of Health and Social Security (l988) Guidance on the control of infection in hospitals prepared by the joint DHSS/PHLS Hospital Infection Working Group ("The Cooke Report"). Department of Health and Social Security, London
28. Anon (1999) Methicillin resistance in *Staphylococcus aureus* isolated from blood in England and Wales: 1994 to 1998. CDR Weekly 9: 65–68
29. Cookson B, Morrison D, Marples R (1997) Antibiotic resistance. Nosocomial gram-positive infection. J Med Microbiol 46: 439–442
30. Pitt TL, Kaufmann ME, Patel PS et al (1996) Characterization and antibiotic susceptibility of *Burkholderia (Pseudomonas) cepacia* isolates from patients with cystic fibrosis in the UK and Ireland. J Med Microbiol 44: 203–210
31. French GL, Shannon KP, Simmons N (1996) Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and β-lactam-β-lactamase inhibitor combinations by hyperproduction of SHV-5 β-lactamase. J Clin Microbiol 34: 358–363
32. Doern GV, Brueggemann AB, Blocker M et al (l998) Clonal relationships among high-level penicillin-resistant *Streptococcus pneumoniae* in the United States. Clin Infect Dis 27: 757–761
33. Working Party (1998) Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. J Hosp Infect 38: 235–290

34. di Martino P, Livrelli V, Sirot D et al (1996) A new fimbrial antigen harboured by CAZ-5/SHV-4-producing *Klebsiella pneumoniae* strains involved in nosocomial infections. Infect Immun 64: 2266–2273
35. Livermore DM (1998) β-Lactamase-mediated resistance and opportunities for its control. J Antimicrob Chemother 41 [Suppl D]:25–41
36. Trenholme GM, Kaplan RL, Karakuis PH et al (1989) Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. J Clin Microbiol 27: 1342–1345
37. Betts RF, Valenti WM, Chapman SW et al (1984) Five-year surveillance of aminoglycoside usage in a university hospital. Ann Intern Med 100: 219–222
38. Rahal JJ, Urban C, Horn D et al (1998) Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. JAMA 280: 1233–1237
39. Bradley SJ, Wilson ALT, Allen MC et al (1999) The control of hyperendemic glycopeptide-resistant *Enterococcus* spp. on a haematology unit by changing antibiotic usage. J Antimicrob Chemother 43: 261–266