International Journal of Antimicrobial Agents 39 (2012) 283–294

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Review

Fourteen years in resistance

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ARTICLE INFO

Keywords:
MRSA
Streptococcus pneumoniae
ESBL
Carbapenemase
Neisseria gonorrhoeae
Antibiotic development

ABSTRACT

Resistance trends have changed greatly over the 14 years (1997–2011) whilst I was Director of the UK Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL). Meticillin-resistant *Staphylococcus aureus* (MRSA) first rose, then fell with improved infection control, although with the decline of one major clone beginning before these improvements. Resistant pneumococci too have declined following conjugate vaccine deployment. If the situation against Gram-positive pathogens has improved, that against Gram-negatives has worsened, with the spread of (i) quinolone- and cephalosporin-resistant Enterobacteriaceae, (ii) *Acinetobacter* with OXA carbapenemases, (iii) Enterobacteriaceae with biochemically diverse carbapenemases and (iv) gonococci resistant to fluoroquinolones and, latterly, cefixime. Laboratory, clinical and commercial aspects have also changed. Susceptibility testing is more standardised, with pharmacodynamic breakpoints. Treatments regimens are more driven by guidelines. The industry has fewer big profitable companies and more small companies without sales income. There is good and bad here. The quality of routine susceptibility testing has improved, but its speed has not. Pharmacodynamics adds science, but over-optimism has led to poor dose selection in several trials. Guidelines discourage poor therapy but concentrate selection onto a diminishing range of antibiotics, threatening their utility. Small companies are more nimble, but less resilient. Last, more than anything, the world has changed, with the rise of India and China, which account for 33% of the world's population and increasingly provide sophisticated health care, but also have huge resistance problems. These shifts present huge challenges for the future of chemotherapy and for the edifice of modern medicine that depends upon it.

1. Introduction

In September 2011 I ceased, after 14 years tenure, to be Director of the UK Health Protection Agency's (HPA) Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) moving to a split role between the University of East Anglia and the HPA. By coincidence, my time at ARMRL matched, to within a year, the span of the Blair–Brown Government in the UK (1997–2010), which began in much optimism, rode a credit boom, increased both public spending and regulation in many areas, and ended in considerable debt. It spent particularly on the National Health Service (NHS)—£54 billion in 1997, rising to £118 billion in 2010 [1]—but faced many challenges there, including from microorganisms. Critical reports [2] and press coverage led to the introduction of national targets for the reduction of meticillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. To the surprise of many microbiologists, myself included, these were largely met [3,4]. Nevertheless, evolution has continued, with new challenges emerging, mostly from Gram-negative pathogens more diverse and complex than MRSA. These must now be confronted by a country where the money to respond has become dramatically tighter since 2008.

There have been other big shifts. Clinical medicine and laboratory performance have become ever more regulated, and susceptibility testing more standardised. Moves to molecular testing in diagnostic microbiology laboratories have been slower, and much diagnostic laboratory practice still moves at the speed it did in Fleming's time: 1 day from specimen to culture and another from culture to identification and susceptibility data. In the antibiotics industry there are now far fewer large profitable companies than a decade and half ago, but there are more small ones, often without the stability of sales income. Beyond the world of antibiotics, wealth and productive capacity have moved decisively eastward to India and China—hugely populous countries with growing provision of sophisticated health care and largely unregulated pharmaceutical use. The purpose of this article is to review this broad sweep of changes, to highlight the many unknowns and to ask what the future may hold.

2. Changing resistance in the UK

When I joined the then Public Health Laboratory Service (PHLS) in 1997, the proportion of MRSA amongst *S. aureus* from

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doi:10.1016/j.ijantimicag.2011.12.012

bacteraemias was rising sharply [5]; extended-spectrum β-lactamases (ESBLs) were TEM and SHV mutants largely seen in nosocomial *Klebsiella*, and acquired carbapenemases were IMP types, recorded only from a sprinkling of *Pseudomonas aeruginosa* and Enterobacteriaceae in the Far East, principally Japan [6–8]. Gonococci were universally susceptible to cephalosporins and, in the UK, also to ciprofloxacin, although resistant strains were circulating in the Far East and had been associated with clinical failure [9]. Penicillin-resistant pneumococci were a growing issue [10], although they were less prevalent in the UK than in the USA or southern Europe; most were serotype 6B, 9V, 14, 19F or 23F organisms with low-level resistance that could be overcome by increased dosages of penicillin and ampicillin. Amongst the bacteria submitted to ARMRL for investigation, Gram-positives outnumbered Gram-negatives three to one, and mostly comprised penicillin-resistant pneumococci, vancomycin-resistant enterococci and borderline MRSA. All this has since changed.

2.1. The rise and fall of MRSA

The proportion of MRSA amongst *S. aureus* from bacteraemias in the UK continued to grow in my early years at the HPA, exceeding 40% by the year 2000 and becoming an issue in the 2003 General Election. This led to the national targets, which sought a 60% reduction over 3 years from fiscal 2003/04 (the British financial year runs from April to March). A major reduction in the incidence of MRSA bacteraemias (from 7790 in 2003/04 to 1481 in 2010/11) has indeed been achieved, along with a reduction in the proportion of MRSA amongst *S. aureus* bacteraemias (Fig. 1) [3]. What is less clear is what has been the most important factor in this achievement, as hospitals adopted a care-bundle approach [11,12]. Perhaps the proliferation of alcoholic hand-rubs, along with better infection control? Perhaps better management of intravenous (i.v.) lines, which are the entry portal for many MRSA bacteraemias? Perhaps screening of elective admissions, along with decolonisation of carriers? Or maybe the 30–40% reduction in hospital use of quinolones, to which the UK-predominant EMRSA-15 [sequence type (ST) 22] and EMRSA-16 (ST36) strains are resistant? Or the combination of all these? Similar reductions in MRSA prevalence have been seen in several European countries as well as many US and some Far Eastern hospitals, despite different mixtures of actions [12]. For example, decolonisation with mupirocin is rarely attempted in the USA. The fact that MRSA bacteraemias have declined disproportionately to meticillin-susceptible *S. aureus* (MSSA) bacteraemias [13] suggests that measures aimed at hospital transmission of MRSA, or MRSA decolonisation, have played the major role, but we know too little about the epidemiology and strain structure of MSSA bacteraemias to be sure.

What is more, we cannot exclude the possibility that biological factors also contributed to the fall in MRSA. One of the two predominant strains, EMRSA-16, began to decline from around the year 2000, 3 years before the first big national efforts began (Fig. 1), with this fall initially masked by the continued rise of EMRSA-15 [14]. Why? 'Burn out' or bacteriophage attack are both plausible, but unproven, explanations. By the same token, we cannot be sure that *some* of the subsequent decline in EMRSA-15 since 2004–2005 did not reflect biological factors rather than human effort.

2.2. Streptococcus pneumoniae

The other big success—of reducing resistant pneumococci—definitely does reflect human efforts.

The 7-valent conjugate vaccine (Prevenar) was added to the UK childhood immunisation schedule in September 2006 and protects (*inter alia*) against the pneumococcal serotypes in which resistance was most frequent (6B, 9V, 14, 19F and 23F). Its deployment was associated with (i) a reduction in invasive pneumococcal infection [15], (ii) a marked decline in macrolide-resistant isolates, from 11–14% of bloodstream pneumococci until 2005 to ca. 5% by 2009 [16] and (iii) a greater fall in macrolide resistance amongst isolates from children aged from 2 months to 2 years, down from 20–30% to 3% [16]. This fall is seemingly because the vaccine has prevented infections due to a resistant serotype 14 lineage, previously in wide circulation [17]. Vaccine effects on penicillin resistance have been less significant, but only because this trait was never prevalent in the UK. As elsewhere, there has been some emergence of 19A organisms with penicillin minimum inhibitory concentrations (MICs) of 2–4 mg/L, now regularly sent to ARMRL [15]. But these seem unlikely to gain traction, being covered by the 13-valent vaccine that is now replacing the 7-valent vaccine [18,19]. Whether or not resistance will proliferate in further serotypes remains uncertain. ARMRL sees regularly penicillin-non-susceptible serotype 35B isolates, for example, but they remain uncommon.

2.3. Extended-spectrum β-lactamases

In contrast to these positive shifts for MRSA and pneumococci, the resistance changes amongst Gram-negative bacteria have been for the worse and, from 2003, these organisms came to dominate ARMRL's workload, now by a ratio of approximately two to one over Gram-positive pathogens.

The first big change, from 2003, was a sharp rise in the prevalence of CTX-M ESBLs [20], which had been sporadically recorded from 2001 [21,22]. Unlike TEM and SHV ESBLs, CTX-M types did not remain confined to *Klebsiella* spp. but have proliferated in *Escherichia coli* (Fig. 2). Moreover, they have spread amongst community patients, although mostly those who were elderly and had repeated hospital contact [20].

Approximately 90% of the ESBL-producing *E. coli* that were referred in 2003–2004 as this big shift began proved to have CTX-M-15 β-lactamase, although minorities had group 9 CTX-M enzymes, principally CTX-M-9 and -14. The isolates with group 9 enzymes were clonally diverse, but those with CTX-M-15 principally included five major pulsed-field gel electrophoresis (PFGE) types (A–E), all of them variants of the international *E. coli* ST131 lineage [23,24] that has proved adept at acquiring ESBL plasmids, particularly IncF variants encoding CTX-M-15 [25]. The reasons for the continuing success of ST131 *E. coli* are uncertain but plausibly reflect a particular ability to colonise and infect the urinary tract, along with a facility to recruit resistance [26].

The reasons for the relative success of CTX-M enzymes compared with other ESBL types are also uncertain. Classical TEM penicillinase is produced by many gut *E. coli* and must have been exposed to massive amounts of ceftriaxone over the past quarter century, for this drug was long the world's best-selling injectable antibiotic and has substantial biliary excretion. Nevertheless, TEM-mutant ESBLs have not proliferated in *E. coli*. Why? One hypothesis is that, as enzymes related to penicillin-binding proteins, β-lactamases have some collateral effect on peptidoglycan assembly and that, depending on the enzyme type and species, this may be more or less of a burden. This might be investigated by comparing the peptidoglycan structures of β-lactamase-producers and non-producers, but such studies remain to be done.

What also remains uncertain is the stability of the present ESBL epidemiology. There has been some recent dip in ESBL prevalence amongst *E. coli* and *Klebsiella* in the UK since around 2005–2006 (Fig. 2), perhaps now levelling out. This effect, not replicated elsewhere in Europe, may reflect massive prescribing changes in UK hospitals, outlined below, with a move away from cephalosporins and quinolones and towards β-lactamase inhibitor combinations. In order to monitor shifts in ST131 and other strain types, the HPA and the British Society for Antimicrobial Chemotherapy

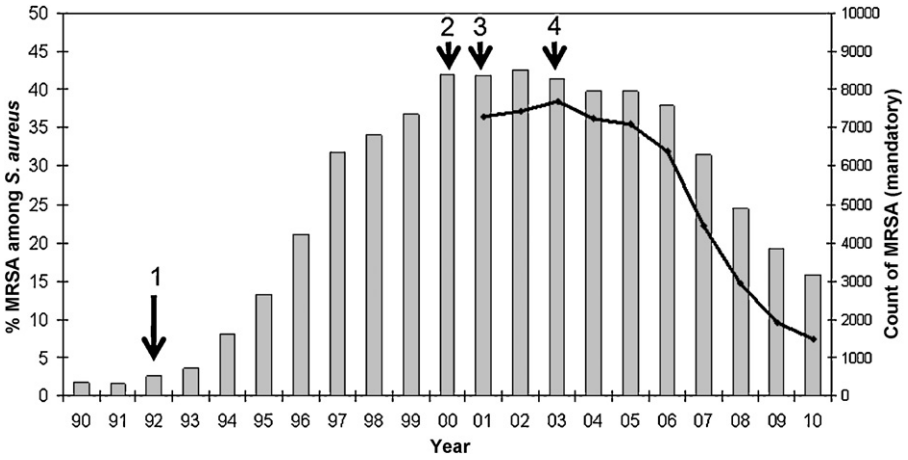

Fig. 1. Rise and fall of meticillin-resistant *Staphylococcus aureus* (MRSA) bacteraemias in the UK. The black line shows the number of cases in England (only) reported under the mandatory scheme initiated in 2001, and the grey bars show percent MRSA amongst all *S. aureus* bacteraemias reported under the Health Protection Agency's voluntary surveillance, covering >90% of hospitals in England, Wales and Northern Ireland. Salient events: 1, first recognition of EMRSA-15 and -16; 2, initiation of mandatory surveillance of MRSA bacteraemias; 3, beginning of decline of EMRSA-16; and 4, issue of Department of Health targets for MRSA reduction.

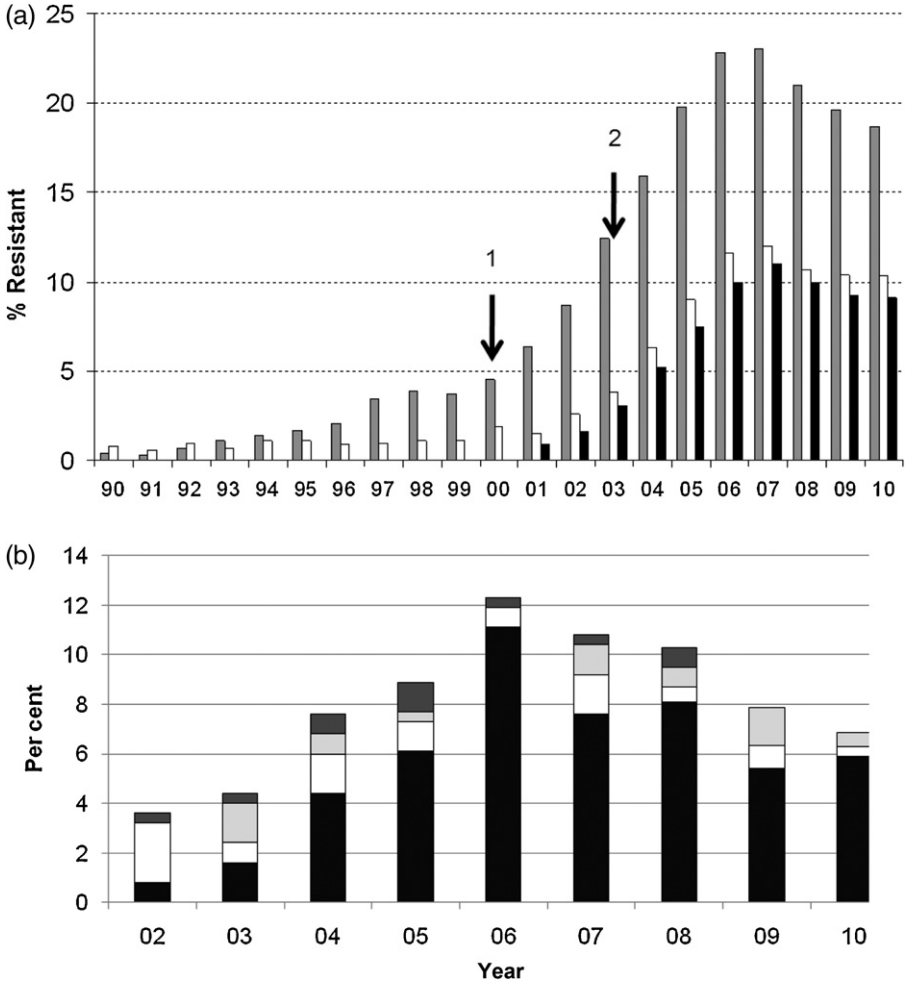

Fig. 2. Rise of cephalosporin resistance, multiresistance and extended-spectrum β-lactamases (ESBLs) in *Escherichia coli*. (a) Resistance amongst bacteraemia isolates reported under the Health Protection Agency's (HPA) voluntary scheme, estimated to capture antibiogram data on ca. 70% of bacteraemias in England, Wales and Northern Ireland: ▒, fluoroquinolones; □, cephalosporins; ■, both fluoroquinolones and cephalosporins. Salient events: 1, first recognition of CTX-M ESBLs in the UK; and 2, multiple calls and isolate referral to HPA indicating that UK diagnostic laboratories were 'Seeing ESBL *E. coli* from community patients'. (b) Distribution of mechanisms amongst cephalosporin-resistant *E. coli* collected under the British Society for Antimicrobial Chemotherapy (BSAC) Bacteraemia Programme (http://www.bsacsurv.org), showing that the rise in cephalosporin resistance was largely attributable to isolates with CTX-M ESBLs: ■, CTX-M ESBLs; □, other ESBLs; ▒, AmpC; ▒, other.

(BSAC) are now sequence typing over 2000 *E. coli* from the BSAC bacteraemia collection (http://www.bsacsurv.org), collected from 2001 onwards.

A final big uncertainty, difficult to probe for ethical reasons, is the prevalence of ESBL-producing *E. coli* in the gut flora of different groups of the healthy population. Snapshot surveys suggest a low prevalence of ESBL-producers in community stools routinely passing though clinical laboratories [27], but a much higher prevalence in those from returning travellers, particularly from East and South Asia [28], and a 40% prevalence in care-home residents in Belfast [29]. Nevertheless, these data are fragmentary at best. Data on food carriage are also limited, but show (i) that ESBL-producing *E. coli* can readily be recovered from supermarket chickens in the UK, but that these are non-ST131 strains, mostly with CTX-M-2 and -8 enzymes [30,31] and (ii) that *E. coli* with CTX-M ESBLs, mostly CTX-M-14 or -15, occur increasingly in UK beef cattle but, again, are non-ST131 types [32,33]. This lack of relatedness to human strains does not, however, prove the absence of risk. In The Netherlands, the same ESBL-producing *E. coli* strains and ESBL-encoding plasmids, determining CTX-M-1 enzyme, are circulating both in chickens and humans [34]. And, one of the lessons that I learnt at the HPA is that when you try to put across these subtleties about food and resistance to the press, you always lose out to those with a vested interest and a simple sound-bite that: 'ESBL *E. coli* from humans and food are different, so food's not implicated' or 'ESBL *E. coli* cause infections in humans and they are found in mass-produced meat. . .' Both these statements are true, but each hides as much as it says.

2.4. Carbapenemases

The growing prevalence of ESBLs has increased dependence on carbapenems, often the last 'good' antibiotics against multiresistant ESBL-producers. Carbapenem resistance was slow to emerge, particularly in Enterobacteriaceae, and the first organisms where carbapenemases presented a concern to ARMRL were non-fermenters.

An ARMRL survey in 2000 found <2% imipenem resistance in *Acinetobacter* spp. [35], but problems began soon afterwards with the sequential proliferation of two carbapenemase-producing *Acinetobacter baumannii* strains: there was the 'SE clone', with insertion sequence IS*Aba1*-upregulated expression of the chromosomal OXA-51-like β-lactamase (an inherent enzyme present even in the *A. baumannii* type strain, dating from the 1950s); whilst the second, OXA-23 clone 1, has OXA-23 carbapenemase [36–38], which *A. baumannii* originally acquired from *Acinetobacter radioresistens* [38]. By 2003–2004, these two strains were widespread in southern England and remain so, accounting for much of the 30% imipenem resistance now seen amongst *A. baumannii* (Fig. 3).

Metallo-β-lactamases (MBLs)—mostly VIM types—have been recorded in UK pseudomonads since 2001, with low-grade multiyear outbreaks by *P. aeruginosa* with VIM carbapenemases at two hospitals, as well as in many overseas sites. Nevertheless, non-fermenters with MBLs remain rare: amongst referrals to ARMRL, *Acinetobacter* spp. with these enzymes (specifically *A. baumannii* with NDM-1 enzymes or non-*baumannii Acinetobacter* with IMP types) are outnumbered ca. 100-fold by those with OXA carbapenemases, and *P. aeruginosa* with metallo-carbapenemases are outnumbered heavily by those with impermeability- and efflux-determined resistance.

The emergence of carbapenemases in Enterobacteriaceae is now the bigger concern, both because these are more frequent pathogens than non-fermenters and because of the diversity of emerging enzyme types (Table 1), which complicates recognition, treatment and response.

Until 2007, ARMRL received only two or three carbapenemase-producing Enterobacteriaceae per year for investigation, despite

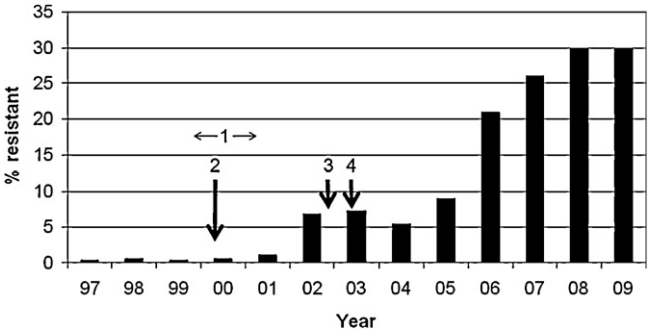

Fig. 3. Rise of carbapenem resistance amongst *Acinetobacter baumannii* isolated from bacteraemias, based on reporting to the Health Protection Agency's (HPA) voluntary system. Salient events: 1, Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) survey [35] showing <2% carbapenem resistance in collected isolates; 2, first recognition of carbapenem-resistant variants of SE clone; 3, recognition of OXA-23 clone 1; and 4, recognition of OXA-23 clone 2.

encouraging submission. This changed from 2008 when the submission of *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli* with KPC, IMP, NDM, OXA-48 and VIM enzymes began to grow dramatically (Fig. 4). The laboratory now confirms 10 or more producers in most weeks, some from infections, but many from faecal screening in affected units.

Three crucial facts distinguish this emerging carbapenemase problem from the earlier rise of ESBLs. First, and most obviously, there is no obvious next line of antibiotics to use against carbapenemase-producers [39] as there was (with carbapenems) against ESBL-producers. Rather, the few treatment options that remain—colistin, tigecycline and fosfomycin—have significant drawbacks in terms of toxicity, efficacy or potential for resistance. Second, the rise of the problem has been slower: ESBL-producing *E. coli* went from minimal reference submissions to 20–30 per week over a matter of weeks in 2003, whereas carbapenemases have still only reached half this level. Third, the carbapenemase-producers are considerably more diverse in terms of enzymes, species and epidemiology, complicating the development of inhibitor-based responses by the pharmaceutical industry [40].

As highlighted by much press coverage in the summer of 2010, many isolates with NDM β-lactamases are from patients who have

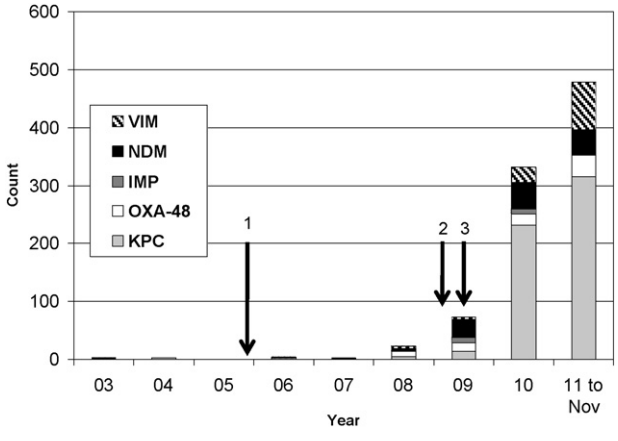

Fig. 4. Carbapenemase-producing Enterobacteriaceae referred to the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) (three isolates with IMI enzyme are omitted). Salient events: 1, issue of National Resistance Alert on carbapenem resistance due to combinations of AmpC/extended-spectrum β-lactamase (ESBL) and impermeability; 2, issue of National Resistance Alert on carbapenem resistance due to carbapenemases, highlighting import of VIM enzymes from Greece and KPC from Israel and the USA; and 3, issue of National Resistance Alert on carbapenem resistance due to NDM carbapenemases, epidemiologically linked to India and Pakistan.

Table 1
Emerging carbapenemases amongst Enterobacteriaceae in the UK and internationally.

	UK and international distribution	Molecular epidemiology
NDM	Widespread in Enterobacteriaceae (especially *Klebsiella pneumoniae* and *Escherichia coli*) in India and Pakistan and often imported to the UK (and elsewhere) via patients with travel and hospitalisation/dialysis there. UK cases are scattered geographically, with little clustering; only isolated examples of UK cross-infection [41]	Commonly encoded by labile and promiscuous plasmids belonging to diverse incompatibility types, although IncA/C types predominate; bla_{NDM} has spread to a wide diversity of species [41]
VIM	Globally scattered, mostly in *K. pneumoniae*. Endemic but being replaced by KPC in Greece and sometimes imported to the UK via patients previously hospitalised there [42]. Clusters of *K. pneumoniae* with this enzyme are now occurring in Cheshire; these are partly clonal but with some plasmid spread amongst strains [43]	In general, plasmid spread amongst strains is more important than clonal spread of strains
IMP	Scattered internationally, with little clear association to locale	Mostly spread amongst strains by plasmids; clonal outbreaks are rare
KPC	Recorded in the USA since 1997. Also now prevalent in Israel and Greece and rapidly expanding in Italy, with outbreaks elsewhere in Europe. Some UK cases imported via patient transfers, but significant local dissemination around Manchester	International dissemination greatly reflects the spread of epidemic *K. pneumoniae* ST258 with KPC-2 or -3 enzymes [45]. In the UK, the greater problem is the spread of bla_{KPC}-encoding plasmids amongst strains of *K. pneumoniae*
OXA-48	Widespread in *K. pneumoniae* in Turkey (where it originated), Levant and Maghreb. Some imports to the UK from these regions, but also unlinked cases and an outbreak in one London renal unit in 2008–2009	Mixture of plasmid and clonal spread; often encoded by ca. 70 kb plasmids with Tn*1999* or Tn*1999.2* [41a]

travelled or been hospitalised in the Indian subcontinents where this enzyme is widespread [41], and some OXA-48 are epidemiologically linked to Turkey and North Africa where this enzyme is prevalent [41a]. Some early isolates with KPC and VIM enzymes were imports too [42], but the greater UK problems now are (i) the spread of plasmids encoding KPC enzymes amongst non-clonal *K. pneumoniae* around Manchester (ARMRL data on file) and (ii) the clonal spread of *K. pneumoniae* with VIM carbapenemases around Cheshire [43]. It is this proliferation that explains the numerical dominance of KPC enzymes in 2010–2011 in Fig. 4. If, instead, one counts hospitals, more sites have referred isolates with NDM carbapenemase.

Most carbapenemase-producers have multiple mechanisms. Those with NDM, in particular, usually also have plasmid AmpC β-lactamases and ESBLs, together with the ArmA and/or RmtC methylases that modify the 16S ribosomal rRNA so as to block the binding of most aminoglycosides [44]. Similarly, although OXA-48 enzyme lacks activity against oxyimino-cephalosporins, many strains with this enzyme are resistant owing to co-production of ESBLs. An exception to these generalisations is the situation around Manchester, where the plasmids encoding KPC carbapenemases often reside in *Klebsiella* spp. strains that remain sensitive to multiple aminoglycosides and ciprofloxacin. By international standards this situation is unusual; in Greece, Israel and, latterly, Italy the major factor in the accumulation of KPC carbapenemases is their carriage by a successful ST258 clone [45] usually susceptible only to colistin, gentamicin and, marginally, to tigecycline. It has been introduced to the UK on several occasions but, so far, has not caused major outbreaks.

2.5. Neisseria gonorrhoeae

Neisseria gonorrhoeae only briefly fell into ARMRL's remit in 2002–2003, before transferring to the HPA's Sexually-Transmitted Bacterial Reference Laboratory (STBRL). Nevertheless, the organism deserves highlighting, for major changes have occurred. As recently as the year 2000, just 2% of *N. gonorrhoeae* collected in the annual Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) were resistant to ciprofloxacin, mostly at low level (MICs of 1–2 mg/L). By 2002 this proportion had increased to 10%, with high-level resistance proliferating (MICs of 16–64 mg/L) [46]. Subsequently, and despite ciprofloxacin therapy being largely abandoned, this proportion has grown to 35%, implying that the resistant strains are fit and competitive [47].

The practical consequence is a massive switch to cephalosporin therapy, used in just 5% of UK gonorrhoea patients in 2001 but in 87% by 2007. This change, recapitulated globally, has altered the selection pressure, leading to an upward creep of cephalosporin MICs, far more convincing than any for vancomycin and MRSA [48]. In 2005 no cefixime MICs > 0.06 mg/L were recorded in the GRASP surveillance but, by 2009, 12% of collected isolates 'had' MICs of 0.12–0.25 mg/L, entering a range where pharmacodynamics predict failure [48] and where clinical failures are seen [49,50], although probably limited by frequent co-administration of azithromycin.

Based upon these shifts, the British Association for Sexual Health and HIV (BASHH) 2011 gonorrhoea guideline advocates a move away from oral 400 mg cefixime to 500 mg ceftriaxone intramuscular (i.m.) plus 1 g azithromycin orally (p.o.) irrespective of chlamydial co-infection, with test-of-cure follow-up [51]. Even this looks vulnerable to events, for the Japanese have described a gonococcus with ceftriaxone and cefixime MICs of 2–4 mg/L and 8–16 mg/L, respectively [52]. Although the organism, from the throat of a female sex worker in Kyoto, disappeared after two courses of ceftriaxone, this 'cure' may reflect spontaneous eradication from a suboptimal infection site. It remained susceptible to spectinomycin (MIC = 16 mg/L, but ineffective in pharyngeal infections), borderline to azithromycin (MIC = 1 mg/L) and inhibited by ertapenem (0.06 mg/L) and piperacillin/tazobactam (0.25 mg/L). To forearm against similar strains in the UK (one has just been reported in France) [53], the STBRL and ARMRL are reviewing the activity of alternative drugs against isolates with reduced cefixime susceptibility.

2.6. And some resistances that haven't taken off

Other resistance deserves mention as not having proliferated in the past 14 years. Vancomycin resistance in enterococci, predominantly due to VanA, had been a big development in the years before I joined ARMRL and continues to be seen in 15–25% of *Enterococcus faecium* and 2–3% of *Enterococcus faecalis*—rates it reached in the early 1990s [54,55]. The 1997 ban on avoparcin, a glycopeptide growth promoter that was blamed for selecting vancomycin-resistant enterococci in food animals, has had no discernible effect, probably because most infections involve hospital-adapted strains often belonging to the *E. faecium* CC17 lineage [56].

Likewise, there has been little resistance trend, except a slow appearance of carbapenemases, in *P. aeruginosa*—with percentage resistance rates to key β-lactams, aminoglycosides and quinolones little higher than in the 1980s, and still contingent on mutation, not gene acquisition [57]. This is typical of northern Europe, but rates in southern Europe, the Middle East, Latin America and East Asia are

markedly higher, with acquired resistance genes more prevalent [58].

Despite fears spanning my entire period at ARMRL, there has been no emergence of resistance to vancomycin in *S. aureus*, except insofar as the definition of resistance has become stricter, with (i) the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints reduced (on good pharmacodynamic grounds) from 4 mg/L to 2 mg/L, (ii) growing concern about heterogeneous vancomycin-intermediate *S. aureus* (hetero-VISA) isolates [59] and (iii) accumulating evidence that MICs at the high end of the susceptible range (i.e. 1–2 mg/L) are associated with poorer outcomes in bacteraemia [60]. The UK has yet to see any *Staphylococcus* with an acquired Van determinant and, although those with VanA have been found in the USA (and, apparently, Iran), they remain extremely rare and unstable [61]. Likewise, ARMRL has yet to see any VISA with a stable MIC of 8 mg/L, as found (and confirmed by us) for the original Hiramatsu Mu50 strain [62]. More generally, and in contrast to findings in the USA, there is no convincing MIC creep with vancomycin [63] and it may be that this trait, where it does occur, reflects strain replacement, not selection of resistance within strains.

In its first year of licensure (2001/02) we received a steady trickle of linezolid-resistant enterococci, all with the G2576U mutation in their 23S rRNA (G2576T in the corresponding DNA), mostly from patients who had received prolonged linezolid therapy [64]. Such submissions dwindled as fears about linezolid's long-term toxicity grew. They are now rare submissions, and the UK has yet to see isolates with the plasmid-mediated *cfr*-type oxazolidinone resistance [65]. ARMRL is regularly, if infrequently, asked to examine *S. aureus* where daptomycin resistance has emerged during treatment of endocarditis, and generally finds the MIC raised from 0.25–0.5 mg/L to 2–4 mg/L, as also seen in cases in a Phase III trial [66]. However, we lack any good denominator and cannot comment on the incidence of this selection. We rarely see daptomycin-resistant *S. aureus* from other sources.

In the case of tigecycline, we have seen a few staphylococci with borderline resistance (MIC of 1–2 mg/L) and three enterococci with MICs of 4–8 mg/L; one of these latter was found by our collaborators to have upregulated expression of a chromosomal efflux pump [67]. On the other hand, we see many *K. pneumoniae* and *Enterobacter* spp.—referred mostly for other reasons—with tigecycline MICs of 4–16 mg/L. Where investigated, these prove to have upregulated RND (regulation–nodulation–division) efflux pumps [66,68].

2.7. And what we may have missed

ARMRL's investigation is predicated on reviewing phenotypes then, where appropriate, seeking mechanisms. We have concentrated on β-lactams as the most critical antibiotics against Gram-negative bacteria, and on emerging resistance to new anti-Gram-positive agents. This strategy addresses the major drugs of public health concern, but leaves big gaps. We know little on the prevalence of the ArmA and Rmt 16S rRNA methylases that confer broad aminoglycoside resistance, except that these enzymes—proliferating in the Far East [69]—are commonly associated with bla_{NDM} [44,70] and are present in *A. baumannii* OXA-23 clone 1. Otherwise, we fail to distinguish isolates with rRNA methylases from those with combinations of aminoglycoside-modifying enzymes. This is potentially important because the methylases, unlike modifying enzymes, confer resistance to ACHN-490, a novel aminoglycoside now entering Phase III trials. Likewise, we know little of the prevalence of the *qnr* and *aac(6′)-Ib-cr* determinants that contribute to fluoroquinolone resistance, except that *aac(6′)-Ib-cr* is often encoded by the same IncF plasmids that carry $bla_{CTX\text{-}M\text{-}15}$ [25]. An association between high-level quinolone resistance (chromosomal) and gentamicin resistance (plasmidic) in *E. faecalis* was noticed by chance whilst reviewing BSAC bacteraemia surveillance results and proved to reflect national dissemination of two strains [71]. How many other national strains are circulating with low-profile resistances?

And then there's the issue of why the MICs of imipenem and meropenem for referred carbapenem-resistant *P. aeruginosa* are higher than 10 years ago—commonly 32–64 mg/L rather than 8–16 mg/L—even in the absence of carbapenemases. Are the bacteria changing, or the media?

Some of these questions, although not the last, will be answered as ARMRL moves to gene chip technology. The intention is that this will allow most or all referred isolates to be screened for a wide battery of resistance genes, including those relevant to aminoglycosides and fluoroquinolones as well as β-lactams.

3. Changing the measurement and definition of resistance

In 1997, most susceptibility testing in UK clinical laboratories was still by Stokes' method, which was an excellent way for confirming that disks contained antibiotic, but one with arbitrary categorisations so that, irrespective of the antibiotic and disk content, a zone radius >3 mm smaller than the control scored as intermediate and an annular zone radius ≤2 mm as resistant [72]. This was gradually replaced by the BSAC's standardised disk method, initially calibrated against the BSAC's MIC breakpoints and, latterly, against those agreed under EUCAST harmonisation [73]. This BSAC method, with semi-confluent growth on Iso-Sensitest agar, may next be superseded by the EUCAST method on Mueller–Hinton agar (http://www.eucast.org). More quietly, a growing minority of laboratories have moved to automated systems, and I anticipate that more will prefer this as an alternative to making further changes in disk methodology. A consequence for ARMRL is a growing number of submissions with queries along the lines of 'The Vitek (or Phoenix or MicroScan) found that... but confirmatory tests by disk or Etest found the opposite... Which is right?' And, from experience, there's little consistent pattern—half the time we agree with the manual test, half with the machine.

The definition of breakpoints, by BSAC and then EUCAST, has become increasingly predicated on pharmacodynamics, a science that has done much to define the critical target parameters predicting success with different antibiotic classes [74,75]. Pharmacodynamic prediction, with Monte Carlo simulation to model population scatter, is undoubtedly more scientific than what went before, when the BSAC had a rather dubious breakpoint-setting formula that included relevant parameters and a 'fiddle factor' [76].

But despite the undoubted advance, one has to ask whether pharmacodynamic modelling has quite the precision sometimes claimed. In particular, (i) MICs are determined with a two-fold dilution scale, with a ±1 dilution tolerance, precluding fine discrimination, (ii) estimates of the necessary value for the critical parameter are quietly nudged upwards—β-lactams were long proposed to need to achieve free drug serum levels above the MIC (fT > MIC) for 40% of the dosage interval, but values of 50% are now increasingly cited for severely ill patients [77], implying that some breakpoints may have been set too high, and (iii) the distribution of drug levels achieved in particular patient groups may be more scattered than is modelled in Monte Carlo simulations. In particular, some Intensive Care Unit (ICU) patients have a radically increased volume of distribution and fluid output, lowering the critical parameters of maximum drug concentration:minimum inhibitory concentration ratio (C_{max}:MIC), area under the antibiotic concentration–time curve:minimum inhibitory concentration ratio (AUC:MIC) and T > MIC [78,79]. Even with gonorrhoea—about the simplest bacterial infection to model, being caught by

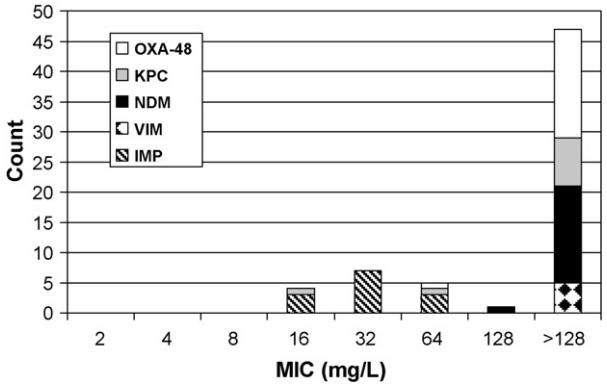

Fig. 5. Minimum inhibitory concentration (MIC) distributions of piperacillin/tazobactam for Enterobacteriaceae isolates with carbapenemases, determined by British Society for Antimicrobial Chemotherapy (BSAC) agar dilution.

otherwise healthy people unlikely to have aberrant pharmacokinetics, and being treated with a single antibiotic dose—we found a cephalosporin to need a *fT* > MIC of ca. 20 h compared with 7–10 h for benzyl penicillin, although the reasons for this differential remain uncertain [48].

In short, pharmacodynamic modelling has greatly extended our understanding, but we should not overestimate its precision. This issue becomes critical when such modelling is used to predict the dosages needed for developmental antibiotics. In this case, underestimates can lead to a useful drug being abandoned or to trials having to be repeated, with considerable cost and delay, and recent examples are discussed below.

4. Changing therapy

In 1997, cefuroxime and cefotaxime were the workhorse antibiotics of UK hospitals, used alone for urinary infections, with metronidazole for intra-abdominal infections and with macrolides for respiratory infections. This has changed in the past 5–6 years, predicated on evidence that cephalosporins—and (more debatably) quinolones—are the chief selectors for *C. difficile* infection [80], which, like MRSA, became a major political issue and the subject of national reduction targets. The result has been a 30–40% reduction in i.v. cephalosporin and fluoroquinolone use, with these reductions more than balanced by a 3–4-fold increased in the use of β-lactamase inhibitor combinations, principally piperacillin/tazobactam and amoxicillin/clavulanic acid. There is good evidence that piperacillin/tazobactam is less selective than cephalosporins for *C. difficile*, whilst the selectivity of amoxicillin/clavulanic acid is debated [81,82]. This aversion to cephalosporins is peculiar to the UK and is not replicated to anything like the same extent in continental Europe, where the detection of *C. difficile* infection (which may reflect testing as well as true incidence) is counterintuitive, being higher in the northwest than around the Mediterranean [83], whereas the weight of antibiotic use, including cephalosporins, and the general burden of antibiotic resistance, increases southward and eastward.

The view that piperacillin/tazobactam is ecologically benign—it is also less selective than cephalosporins for vancomycin-resistant enterococci [84] and for some ESBL-producers [85]—has driven its use, so far without detrimental consequences. Nevertheless, it is hard to believe that such heavy use can continue without new selection occurring. Potential mechanisms of piperacillin/tazobactam resistance include inhibitor-resistant penicillinases, whether TEM mutants, classical OXA-1 or any one of the carbapenemases, which all confer resistance (Fig. 5).

These points lead to a more general observation: that whilst guidelines, which increasingly determine treatment choices, discourage inappropriate antibiotic use and the use of high-selection-risk antibiotics, they also concentrate use and selection on a dwindling fraction of the armamentarium. Once resistance emerges it can spread without firebreaks, as illustrated by the case of gonorrhoea. In 1997, spectinomycin, ciprofloxacin and oral cephalosporins all retained near-universal activity in the UK, but ciprofloxacin was used in ca. 75% of gonorrhoea cases, reflecting its convenience and efficacy [48]. This continued until resistance rose above a 10% threshold in 2002; then ciprofloxacin was replaced by cefixime 400 mg p.o. [51], to which reduced susceptibility is now emerging, forcing a move to ceftriaxone 500 mg i.m. combined with azithromycin 1 g p.o. [51]. Spectinomycin, meanwhile, has been withdrawn owing to lack of sales.

Might it not have been better had there been more diversity in treatment at the end of the 1990s, with patients allocated (sequentially, by gender or date of birth) to treatment with cephalosporins, spectinomycin and ciprofloxacin, or if these treatments had somehow been rotated? Antibiotic cycling has proved disappointing in the resistance hothouse of the ICU [86] but might well have served better in the context of a transmissible community infection.

5. Changing industry

The loss of antibiotics that gain few sales is a problem when a new need for them arises. As well as spectinomycin, both fosfomycin and quinupristin/dalfopristin too have been 'lost' to the UK market during the 14 years and now need to be imported if indicated in a particular patient. Isepamicin, previously importable from Belgium, became completely unavailable once Schering-Plough, who made it, was taken over by Merck.

Several new anti-Gram-positive agents have been launched since 1997, including quinupristin/dalfopristin, linezolid, daptomycin and, on a very restricted licence, telavancin, but the only drugs to show improved anti-Gram-negative activity are doripenem, with a modest increase in antipseudomonal activity over meropenem, and tigecycline, which has enjoyed very mixed trial success beyond its original indications. The main reasons for the decline in antibiotic development have been well discussed elsewhere [87,88] and need only be briefly restated. First, it is technically difficult to find antibiotics that enter Gram-negative bacteria and evade endogenous efflux [89]. Genomics-based discovery, which was much in vogue in the late 1990s, failed to deliver, partly because it was too predicated on finding targets and not on finding drugs that could adequately reach these targets [90]. Second, the increasing and ever-changing burden of licensing regulation has increased the size, complexity and cost of clinical trials, creating a barrier to all but the biggest and richest companies [91]. Moreover, it is a common complaint that the patients included in trials are not representative of those in whom clinicians would actually wish to use the antibiotic, often because the trials exclude patients who have had prior therapy with other antibiotics. Third, as short-course, likely-to-be-restricted treatments for acute infections, antibiotics are less profitable than treatments for chronic diseases [92]. Fourth, takeovers and mergers have reduced the number of profitable companies engaged in antibiotic discovery and development, and discovery has increasingly migrated to start-up and spin-off companies, without sales income and dependent on venture capital, big-pharma collaboration or charity. This model delivered daptomycin (developed by Cubist alone) [93] and is bringing forward several other promising developments, including Anacor's boron-based antibiotics [94,95] (jointly with GSK), Novexel's β-lactamase inhibitor avibactam [95] (after a takeover of Novexel by AstraZeneca), Tetraphase's new tetracycline

analogues [96] (funded by venture capital), Achaogen's aminoglycosides [97] (US Biodefense) and Rib-X's novel-action protein synthesis inhibitors (joint with Sanofi-Aventis). But it is a business model that kills rather than wounds its casualties: Arpida, Targanta and Microcide all collapsed after the failure of their lead compounds (iclaprim, oritavancin and RWJ-54428, respectively). It is hard to believe that this is a good way of retaining expertise and compound flow.

Perhaps, though, one more factor has contributed to several failures—overoptimistic dose selection? Whereas previously the pattern was to select a dose that 'looked reasonable' for the drug class, or was the highest tolerated, the pattern now is to review the critical parameter in relation to the MIC distribution ($T > MIC_{90}$, AUC:MIC_{90} or C_{max}:MIC_{90}), then, using Monte Carlo simulation, to predict the regimen attaining this target in ≥95% of patients [98]. However good the principle, the *actualité* is a string of disappointments, including antibiotics that (i) have failed in clinical trials, with a suspicion that they would have done better had they been dosed at a higher level [e.g. ceftobiprole 500 mg three times a day (tds) and tigecycline 50 mg twice a day (bds) in ventilator-associated pneumonia] [99], (ii) have had dosages adjusted upwards once on the market (e.g. daptomycin, from 4 mg/kg/day to 10 mg/kg/day) [100] or (iii) have had potential advantage eroded (e.g. doripenem, which is more active than meropenem against *P. aeruginosa* but which was assigned lower breakpoints based on a 500 mg tds regimen when meropenem can be administered at 2 g tds). Most recently, the US Food and Drug Administration's (FDA) breakpoint for ceftaroline (susceptible ≤1 mg/L; resistant >1 mg/L), based on a 600 mg bds regimen, cuts the tail of the MIC distribution for MRSA, which extends to 2 mg/L [101]. Perhaps, given this series, it is as well to temper pharmacodynamic modelling with Ehrlich's '*Frapper fort et frapper vite*' (hit hard and hit fast) when choosing dosage regimens, and I am encouraged that AstraZeneca, now trialling ceftazidime/avibactam, are using 2/0.5 g tds.

The last shift is the renaissance in vaccines, long a backwater. Interest here is spurred by the fact that they, unlike antibiotics, are hard to copy even once patents expire; moreover, unlike with antibiotics, genomics, in the form of reverse vaccinology, has delivered [102,103]. The success of antipneumococcal conjugate vaccines has been mentioned already and, whilst two anti-MRSA vaccines (Nabi and Merck) have failed, other developers remain active in the field, and an anti-MRSA vaccine given before elective surgery would need only to give brief protection [104]. Most uropathogenic *E. coli*, including the ST131 clone, belong to phylogenetic group B2, which provides another attractive vaccine target, as do gonococci, but, more generally, it is hard to see vaccines as a practicable weapon against the wide array of hospital opportunists that often affect patients whose underlying disease impairs their immune response.

6. A changing world

The two great geopolitical shifts of the past 14 years have been the rise of China and India and the growth of a globalised market for goods and services, with increasing migration of people within and between countries. All this impacts upon infection and resistance.

Based on UN data, China's 1997 gross domestic product (GDP) was $812 billion (at current values) and India's $427 billion. By 2011, those figures had grown to $3769 billion and $1075 billion, respectively [105]. Both have growing middle classes with increasing access to sophisticated medical services and, in both, these developments are outstripping infection control and antibiotic regulation, leading to a massive accumulation of resistance. Even in 1997–1998, ESBLs were present in 13–35% of *E. coli* from Chinese centres participating in the SENTRY surveillance [106], and the scanty data available for India suggest ESBL rates up to 60% [107]. These rates have since grown to ca. 50–80% for *E. coli* and *K. pneumoniae* in both countries [108]. More recently, India and Pakistan have become the epicentre of NDM carbapenemase, which was found in 2–10% of Enterobacteriaceae at multiple hospitals in India [109,110] and in bacteria from 27.1% of inpatients and 13.8% of outpatients at a military hospital in Rawalpindi, Pakistan [111].

It is common for resistance to proliferate in newly prosperous countries, and similar patterns apply in much of Southeast Asia and Latin America. But several points distinguish India and China. The first and most obvious is their sheer size, collectively accounting for one-third of the world's population. Second, there is the fact that China, with its one child policy, has a swiftly ageing population, which guarantees that the next half century will deliver a huge population of the vulnerable elderly, likely to need collectivised care. Third, in China at least, there is heavy agricultural use of antibiotics and frequent contamination of foodstuffs with multiresistant bacteria [112,113]. Last, turning to India, the striking issue is that excellent, sophisticated hospitals offering complex procedures both to locals and 'medical tourists' are balanced on a weak public health structure where more than half the population lack access to a flush toilet and where 18% of tap water samples in New Delhi show evidence of faecal contamination [114]. Never before has a country built first-world medical services, for a proportion of its population, balanced on a third-world sewage infrastructure, prone to recycle resistant gut bacteria around the population. A small sampling of surface seepage waters in New Delhi indicated widespread contamination by multiple Enterobacteriaceae, aeromonads and non-fermenters carrying bla_{NDM} [115], whilst river sediment sampling downstream of antibiotic plants found high burdens of antibiotic-resistant bacteria along with ciprofloxacin concentrations above the blood levels in a treated patient [116]! It is hard to believe that any hospital, however good its surgeons and physicians, can protect itself from the resistance circulating in the outer milieu that supplies its patients, food and staff.

This resistance does not stay confined. bla_{NDM} was first recognised in bacteria isolated from patients transferred from India to Sweden and the UK, and around one-half the first 29 patients affected in the UK had a history of prior hospitalisation in India or Pakistan, whether as (i) medical tourists, (ii) accident victims or (iii) people who, for family reasons, divided their time between countries. Similarly, a Swedish study showed that 7/8 visitors to India experienced gut colonisation by ESBL-producers, as did 10/34 visiting the Far East [116a]. Adding fuel is the fact that medical tourism to India is expanding [117], with a predicted turnover of $2.3 billion per annum (p.a.) in 2012, growing at 20% p.a. [118].

7. The future

An Arab proverb asserts that 'He who claims to predict the future lies, even when he gets it right' although what can be safely predicted is that new resistance challenges will continue and that, for the next decade, the biggest threats will come from Gram-negative bacteria. It also seems reasonable to predict that, whatever the exact future trajectory of the financial crisis, money will be tighter than for many years past, and that this will impact on health care, including microbiology, infection control and antibiotic development. Last year (2011) saw regime change in three North African countries, with a likelihood of further instability, potentially leading to migrations into Europe. Will this lead to major introductions of OXA-48, a difficult-to-detect carbapenemase, already disseminated in these countries [41a,119–120] and causing a major outbreak in The Netherlands? Introduction of OXA-48 to Slovenia from Libya has already been reported [121] and we are aware of

several cases to the UK (ARMRL, unpublished data). And then there is the issue of whether China, India and other newly prosperous countries can be persuaded to do more to combat resistance. At this writing, China is considering restricting over-the-counter antibiotic availability, whilst India is back-pedalling on a proposal to do so, concerned that it will restrict antibiotic availability in rural areas where there currently are few doctors. As with global warming, it is in these highly populous, rapidly growing, new powers that key cards will be played. . .

There are positive developments too, and not just the investigational antibiotics already mentioned. One tantalising possibility, slow to reach fruition, is to use fast molecular microbiology to identify pathogens and their resistances in primary clinical specimens, without culture. If this can be achieved it will be possible to personalise treatments to patient's infections whilst avoiding antibiotics most likely to cause collateral damage. Such strategies have the potential to reconcile the conflicts that arise when, as now, most treatment is empirical: that the individual patient may benefit from early use of the most powerful antibiotics but that the community suffers if the widespread use of these antibiotics accelerates selection of resistance. The challenge of improving diagnostic tests is huge, though, particularly with specimens from non-sterile sites where it is hard (i) to identify the responsible pathogen in a mixture of extracted DNA, some of it coming from accompanying commensals and (ii) to determine whether any resistance genes detected are in this pathogen not in one of these commensals. Because the success of this strategy remains uncertain and because resistance is presently still increasing, there remains a great need for a renaissance in antibiotic discovery. This is only likely if the area again becomes attractive for investment.

There are now encouraging attempts to bring this issue into the political arena, with the EU:US 10:20 initiative [122], the GAIN Bill in the USA [123] and the Antibiotic Action initiative (http://antibiotic-action.com/) in the UK. Various mechanisms have been proposed, including patent extensions or accepting higher prices, as for cancer medicines, which commonly do far less to prolong life than antibiotics. But these, to my mind, leave the biggest issue: the extent to which the regulatory process acts as a massive barrier to entry [91,92]. Phase III trials now commonly cost $100 000 per patient entered and are done in indications where it is perceived as 'possible to get a license' rather than where there is a clinical need. For example, both ceftazidime/avibactam and CXA-201 are being evaluated in intra-abdominal infection ahead of hospital-acquired pneumonia, yet CXA-201 in particular is most notable for activity against *P. aeruginosa*, which is most important as a pathogen of hospital-acquired pneumonia.

Which returns us to the broader issue of regulation, which has grown so much in so many areas in these 14 years: *that it so often concerns itself with the detail and not with central and existential risk*. At a small local level, what strikes me about the many visits that ARMRL has enjoyed from Clinical Pathology Accreditation and other regulators is the extent to which these have concerned themselves with process ('Is there a standard operating procedure?', 'Does the laboratory have monthly meetings?', 'Do the minutes have action points?', 'Is there a completion date for these actions', 'Does the laboratory have records of staff leave', etc.).

Never once has any inspector taken a handful of our reports along with the raw MIC data used to generate them and asked the seminal question: 'Do these reports, and their interpretations of resistance mechanisms, represent a valid interpretation of the results?' This worries me. On a bigger scale, and away from microbiology, the Financial Services Authority fined banks that allowed old ladies to open savings accounts without the required money laundering checks, but missed the fact these same banks faced existential risk as a result of reckless lending and trading in securities based on pooled mortgages of doubtful (but disguised) quality. Am I alone in seeing a similarity to the regulatory process where perfection is sought in the details of antibiotic trials and their interpretation, but at the hazard that we will run out of antibiotics against critical pathogens?

And, herein, it is critical to achieve a public and political understanding of the extent to which the edifice of modern medicine depends on the ability to treat infection. If that ability is lost, through resistance, the edifice - from transplants to immunosuppressive cancer treatments to much of intensive care medicine - becomes unstable and unsustainable.

Acknowledgment

I wish to thank all who have worked or collaborated with me at ARMRL. The views expressed here are my own; all the data depend on their efforts, and I am indebted.

Funding: No funding sources.

Competing interests: DML has shareholdings in AstraZeneca, Dechra, Eco Animal Health, GlaxoSmithKline, Pfizer and Merck, within diversified portfolios, has accepted grants, speaking invitations and conference invitations from GSK, Merck, Pfizer, Novartis and AstraZeneca, has recent or ongoing consultancy with Achaogen, Astellas, AstraZeneca, Basilea, Bayer, Cubist, Kalidex and Tetraphase, and has ongoing contract work for Achaogen, Basilea, Cubist, Meiji and Merck.

Ethical approval: Not required.

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