REVIEW ARTICLE

Antibiotic resistance

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Introduction

In 1991, a meeting on Antibiotic Resistance was held at Addenbrooke's Hospital, Cambridge, with the Proceedings published subsequently in the *Journal of Medical Microbiology*. This highly successful event attracted much attention. However, in the last 6 years there have been many changes in bacterial resistance to antibiotics, particularly in bacteria associated with nosocomial infection. The original meeting sponsors, Bayer (UK) plc, kindly agreed to arrange a subsequent symposium, held at the University of Southampton on 12 July 1996 as a satellite to the Summer Meeting of the Pathological Society of Great Britain and Ireland. This latest symposium comprised papers on nosocomial pathogens, the epidemiology of resistance in particular infections, reviews of the mechanisms of resistance to specific antibiotic groups, and a final presentation that examined the philosophy of antibiotic resistance. This composite review provides a detailed summary of the papers presented at this symposium.

Nosocomial gram-negative infection

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Introduction

Streptococci and staphylococci were predominant pathogens early this century [1], but the introduction of antibiotics led to their decline and gram-negative infections became prominent [2]. Although a resur-

gence of gram-positive infections followed, gram-negative bacteria continued to cause much nosocomial sepsis. Both *Pseudomonas* spp. and non-pseudomonas gram-negative non-fermenting pathogens were recognised as increasingly important nosocomial pathogens [3, 4]. Among gram-negative bacilli, acquired resistance to aminoglycosides, β -lactam agents and quinolones has evolved [5]. Therefore, in many hospitals, infections now occur not only with resistant strains of traditionally susceptible bacteria, such as *Escherichia coli* and *klebsiellae*, but also with more inherently resistant gram-negative organisms such as *Enterobacter*, *Serratia*, *Pseudomonas* and *Acinetobacter* spp. [5, 6]. Furthermore, gram-negative organisms producing extended spectrum β -lactamases (ESBLs), particularly *Klebsiella pneumoniae* in hospital patients, have often caused outbreaks [7, 8]. Finally, there are infections with newly recognised pathogens, such as *Stenotrophomonas maltophilia*, that are inherently resistant to multiple antibiotics.

Despite increasing reports of multi-drug-resistant gram-negative nosocomial infections, it was not our impression that these organisms were a major problem at Guy's Hospital, although we have many high risk patients. The aim of this study was to define the range of nosocomial gram-negative infections and to assess current susceptibility patterns by analysis of data derived from the laboratory's own computing system and a database of those patients with clinically significant positive blood cultures.

Materials and methods

Patients. Guy's Hospital is a teaching hospital, part of the Guy's and St Thomas' Hospital Trust, which serves the local population in south-east London and tertiary referrals. Services include general medicine, renal medicine, general, cardiothoracic and transplant surgery, haematology and oncology, adult and paediatric intensive care.

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Data sources. Data were obtained from the microbiology laboratory's computer system for all samples from hospital inpatients in 1995. A computer program generated a list of all results from samples for which antimicrobial susceptibility tests were performed. The susceptibility profiles were analysed after exclusion of repeat isolates with the same susceptibility pattern from individual patients within a period of 1 month [9]. Medical microbiologists kept daily prospective records of clinical and microbiological data on all patients with clinically significant blood cultures [10]. The focus of infection was determined on the basis of supporting microbiological evidence of a culture from the site, or on clinical grounds if cultures were not available.

Microbiology. Organisms were identified by standard methods, with antimicrobial disk susceptibility testing used for routine isolates and a broth micro-dilution technique for blood-culture isolates. The following breakpoints (mg/L) were used in the interpretation of MIC results: co-amoxiclav 8, cefuroxime 4, ceftazidime 2 for Enterobacteriaceae and 8 for *Pseudomonas* spp., ciprofloxacin 1 for Enterobacteriaceae and 4 for *Pseudomonas* spp., gentamicin 4 and imipenem 4. A confidence interval of 95% was used to assess the significance of differences in the percentages of strains resistant to individual antibiotics.

Results

Routine isolates. During 1995, 47 878 samples from patients in Guy's Hospital underwent culture in the Department of Microbiology. The most frequent organisms isolated are shown in Table 1, together with their susceptibility to first-line antibiotics. The two most common organisms (from the total of 3018 isolates excluding duplicates) were *E. coli* (1192 patient isolates) and *Pseudomonas aeruginosa* (572), followed by *Klebsiella*, *Enterobacter* and *Acinetobacter* spp. The prevalence of amoxycillin resistance was high, found in one-half of all *E. coli* and almost one-

fifth of *Proteus mirabilis* isolates. Co-amoxiclav was considerably more active, but almost 7% of the *E. coli* hospital isolates were resistant *in vitro* to the combination. Resistance to cefuroxime was low for *E. coli* but present in almost 10% of klebsiellae. For the most frequently isolated Enterobacteriaceae, ciprofloxacin resistance was unusual, but was observed in *Serratia marcescens* (40.5%), *Acinetobacter* spp. (15.5%) and *Citrobacter freundii* (12.5%). Gentamicin resistance was detected in 11.4% of *Acinetobacter* spp., 9.3% of *C. freundii* and 7.9% of *Klebsiella* spp., but was otherwise uncommon among the Enterobacteriaceae. Comparison of *P. aeruginosa*, *Pseudomonas* spp. and *St. maltophilia* demonstrated increasing resistance to azlocillin, imipenem, ceftazidime, ciprofloxacin and gentamicin – for each of these compounds, *P. aeruginosa* was the most susceptible of these three organisms and *St. maltophilia* the least, with other *Pseudomonas* spp. intermediate between the two.

Some isolates were selected, generally because of resistance to appropriate first-line agents, for further susceptibility testing. Nine of 39 *Klebsiella* spp. tested were resistant to ceftazidime and two to amikacin, but none to imipenem. Among 59 *E. coli* isolates, three were resistant to ceftazidime, but none to either amikacin or imipenem. The corresponding figures for 42 *Enterobacter cloacae* isolates were nine resistant to ceftazidime, none to amikacin and one to imipenem. *Acinetobacter* spp. were rather less susceptible, with 18 of 42 isolates resistant to ceftazidime, three of 39 to amikacin and two of 39 to imipenem.

Significant blood culture isolates. There were 524 episodes of clinically significant bacteraemia. Hospital-acquired isolates accounted for 390 (74%) episodes and, of these, 150 (38%) were caused by gram-negative organisms. The foci of infection associated with the 150 gram-negative blood culture isolates were, in order of frequency, intravascular access devices (44), urinary tract (24), lower respiratory tract (14), alimentary tract

Table 1. Antibiotic resistance patterns amongst organisms isolated from general patients – Guy's Hospital 1995

Species	n	Percentage of isolates resistant to				
		Amoxycillin	Co-amoxiclav	Cefuroxime	Ciprofloxacin	Gentamicin
<i>E. coli</i>	1191	49.5	6.9	2.6	1.8	0.8
<i>Ent. cloacae</i>	237	98.7	98.7	40.2	2.1	0.4
<i>Ent. aerogenes</i>	28	96.4	89.3	32.1	0	0
<i>Klebsiella</i> spp.	306	99.3	10.9	9.5	3.9	7.9
<i>Pr. mirabilis</i>	199	19.3	0	0	0.5	0.5
<i>Pr. vulgaris</i>	27	96.3	7.4	88.9	0	0
<i>Acinetobacter</i> spp.	114	48.1	11.0	47.7	15.5	11.4
<i>C. freundii</i>	43	98.0	95.0	55.0	12.5	9.3
<i>C. koseri</i>	30	100	6.7	6.9	0	0
<i>Ser. marcescens</i>	74	100	100	100	40.5	0
<i>Morganella morganii</i>	32	100	100	87.5	0	0
<i>P. aeruginosa</i>	572	8.1*	4.1 [†]	3.7 [‡]	9.8	7.2
<i>Pseudomonas</i> spp.	92	13.6*	10.3 [†]	12.5 [‡]	24.2	15.4
<i>St. maltophilia</i>	73	18.1*	100 [†]	29.2 [‡]	70.4	76.7

*Percentage resistant to azlocillin.

[†]Percentage resistant to imipenem.

[‡]Percentage resistant to ceftazidime.

(11), surgical wound (7), biliary tract (7) and others (4), while in 39 cases no focus was identified.

Susceptibility data were available for 132 of the gram-negative blood culture isolates. Table 2 lists the results for the 120 most frequent isolates; the others were: *Proteus* spp. (4), *Agrobacterium radiobacter* (2), *C. freundii* (1), *C. koseri* (1), *Commamonas acidovorans* (1), *Erwinia herbicola* (1), *Morganella morganii* (1) and *Yersinia enterocolitica* (1).

Comparison of the distribution of bacteria from blood with those from all samples showed that the following were relatively under-represented among blood culture isolates: *E. coli* (22.0% versus 38.2%, respectively) and *Proteus* spp. (3.0% versus 8.0%). In contrast, other genera were relatively more common among blood culture isolates: *Klebsiella* spp. (16.7% versus 10.0%), *Enterobacter* spp. (19.7% versus 9.2%), *Ser. marcescens* (10.6% versus 2.6%) and *St. maltophilia* (7.6% versus 2.3%). However, *P. aeruginosa* and *Acinetobacter* spp. were similarly represented in the two groups (18.9% versus 18.4% and 5.2% versus 3.6%, respectively).

MIC data suggested that only two episodes of bacteraemia were associated with klebsiellae producing ESBLs. They occurred in the same patient and the organism was acquired in another hospital before transfer to Guy's Hospital paediatric intensive care unit. The percentages of *E. coli*, *Klebsiella* spp. and *Acinetobacter* spp. susceptible to ciprofloxacin were significantly higher in blood isolates compared with others, and the same differences were noted for *Acinetobacter* spp. with gentamicin and *P. aeruginosa* with ceftazidime. In contrast, blood isolates of *E. coli*, klebsiellae and *Ent. cloacae* were resistant to cefuroxime significantly more often at a breakpoint of 4 mg/L, although at 16 mg/L there was no significant difference.

Multiresistant strains. Ten (7.6%) blood culture isolates were resistant to three or more of gentamicin, amikacin, ciprofloxacin, imipenem and ceftazidime. They included eight *St. maltophilia*, one *Ag. radiobacter* and one *K. pneumoniae*. In nine cases, the focus of infection was an intravascular access site, while the

last focus was not identified. All were resistant to gentamicin and amikacin, eight to imipenem, three to ceftazidime, and one to ciprofloxacin. All 10 patients survived the episode.

Discussion

Computer analysis of all isolates from the diagnostic laboratory should provide a ready supply of antimicrobial resistance data to define the nature and extent of nosocomial infection with resistant organisms, but there are several disadvantages. Repeated samples should be excluded and it is difficult to distinguish contaminating from infecting organisms. The technique employed in this study did not permit the detection of multiresistant isolates. More detailed surveillance is required for an accurate assessment. The optimum method remains unclear [11] and it has been suggested that monitoring infections at specific body sites may lead to earlier detection of changes in antimicrobial resistance compared with surveillance of blood cultures [12]. Although the number of gram-negative septicaemias was relatively low in the present study, the results suggest that the organisms found in blood cultures tend to be more susceptible to certain antibiotics. A recent Swedish study noted that blood isolates were generally more sensitive to cephalosporins than urine and wound isolates collected at the same time [13]. McGowan considered that nosocomial blood stream infection was a useful indicator of overall changes in hospital-acquired infection [3]. Moller [14] found that there was good concordance between the distribution of isolates in blood and other sites although, as in the present study, there were relatively few *E. coli* isolates from blood.

Analysis of the blood culture data may give a broad indication of the sites of gram-negative infections, although there is a large bias towards intravascular access-associated sepsis which is much more likely to lead to a positive blood culture than infection at other sites of the body. Although the most recent results published by the National Survey of Nosocomial Infection in the United Kingdom [15] do not distinguish between gram-positive and gram-negative infections, it is likely that the urinary tract, respiratory tract and surgical wounds remain the most common

Table 2. Antibiotic resistance patterns amongst organisms isolated from blood cultures – Guy's Hospital 1995

Organisms isolated	n	Percentage of isolates resistant to				
		Co-amoxiclav	Cefuroxime	Ceftazidime	Ciprofloxacin	Gentamicin
<i>Enterobacter</i> spp.	27	96.2	85.1	25.9	0	0
<i>E. coli</i>	24	12.5	25.0	8.3	0	4.2
<i>Klebsiella</i> spp.	20	10.0	30.0	10.0	5.0	5.0
<i>Ser. marcescens</i>	10	100	100	0	20.0	0
<i>Acinetobacter</i> spp.	6	16.7	66.7	0	0	0
<i>P. aeruginosa</i>	23	4.3*	4.3†	0	4.3	13.0
<i>St. maltophilia</i>	10	10.0*	100†	10.0	10.0	80.0

*Percentage resistant to azlocillin.

†Percentage resistant to imipenem.

sources. These, together with intestinal foci, have been reported [16, 17] to be the main sites of nosocomial infection. Caution must always be observed before interpreting blood culture data as representative of general infection patterns.

The overall susceptibility data in the present study were for a limited range of antimicrobial agents – those considered to be ‘first-line’ agents. Although ‘second-line’ antibiotics were also tested, they were not included in the analysis as they would have reflected the susceptibility of a selected group of more resistant bacteria. The more detailed analysis of MIC results from blood culture isolates avoids this bias, as they were all tested routinely against a full range of agents. However, the number of isolates included is inevitably much smaller. Larger numbers could be accrued by a study of longer duration or by enrolling several centres simultaneously. The latter approach has been used in the observational surveys of the Public Health Laboratory Service (PHLS) [18].

In general, the antibiotic resistance patterns accord with the ranges reported in a recent survey of European intensive care and oncology units [19]. Aminoglycoside resistance at Guy’s Hospital remains uncommon and other centres have reported similar results [4] despite continued, although generally cautious, use of the agents. In particular, resistance to amikacin (a restricted drug at Guy’s Hospital) appears to be exceptional, apart from in *St. maltophilia*. Among Enterobacteriaceae, resistance to ciprofloxacin was more prevalent than to gentamicin. Higher levels of quinolone resistance have been found elsewhere [20], notably in strains of *E. coli* from patients with cancer and neutropenia [21, 22], a finding attributed in part to the use of these agents for prophylaxis against gram-negative sepsis. However, this has not been a universal finding [23].

Only tentative conclusions can be drawn from the data presented here concerning the classes of β -lactamase likely to be present in isolates. While there are well recognised relationships between β -lactamases and antibiograms, the latter should, ideally, be used to screen for the presence of particular β -lactamases [24]. From the blood culture data there is strong evidence for the presence of ESBLs in *Klebsiella* spp. One centre in the UK has reported a large and prolonged outbreak of resistant *Klebsiella* spp. leading to endemicity with a high carriage rate [8]. Mayer [25] has noted that klebsiellae are often the index species for the dissemination of nosocomial plasmids. In one centre in France, 40% of *K. pneumoniae* isolates are resistant to ceftazidime and aztreonam; most of these are also resistant to quinolones and aminoglycosides [26].

Only 38% of nosocomial bacteraemias were associated with gram-negative bacilli. Resistance did not present

great difficulty; while 7.5% of gram-negative isolates from blood cultures were multiresistant, the majority of these were *St. maltophilia* infections associated with intravascular access sites and caused no mortality. Multiresistance is not as great in Guy’s Hospital as might be predicted from some reports [27, 28] and we are fortunate not to have observed, so far, the problems with resistant strains encountered elsewhere and the prospect of transferable carbapenem resistance [29]. It is unclear why the problem varies in geographic areas [30], but we believe control has been successful at Guy’s Hospital because of a firm antibiotic policy, close involvement of medical microbiologists in the prevention and management of infection in vulnerable groups, and good infection control practice.

Nosocomial gram-positive infection

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Introduction

Nosocomial gram-positive infection has a high profile in the 1990s. There are two major reasons for this. Firstly, hospital infection is now regarded as a potential quality indicator of the whole health care organisation [31]; gram-positive organisms comprise at least one-third of the organisms causing hospital infection. Secondly, antibiotic resistance is emerging in many parts of the world in gram-positive nosocomial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and enterococci resistant to glycopeptides such as vancomycin (VRE). There is also the threat of vancomycin resistance transferring to MRSA. The media are heralding the end of the antibiotic era. A recent WHO report expressed concern about the threat of antibiotic-resistant gram-positive and gram-negative pathogens [32]. The problem of another gram-positive bacterium, *Clostridium difficile*, which is emerging as a potential marker for quality of antibiotic prescribing practice, is not discussed in this contribution. Guidelines for control and prevention were described recently [33].

Approximately one-half of the infections occurring in hospitals originate from the community; patients are admitted with or incubating these infections which can then present opportunities for cross-infection to others. The other half are acquired in hospitals, mainly from the patients’ own normal microbiota or from other patients, staff and the environment. Antibiotic resistance has, ironically, provided more readily identifiable strain markers to enable greater understanding of the more complex dynamics of hospital infection. It is known from studies of MRSA over the last two decades and, more recently, VRE that policies for

control have to consider inter-hospital transfers of staff and patients and re-admissions to hospitals. There are also additional interactions with other institutions such as elderly care and nursing homes.

In certain respects, history is repeating itself. In the 1950s, virulent *S. aureus* (phage type 80/81) strains were the major driving force to improve standards of infection control in the UK and many other countries.

Changing patterns of nosocomial gram-positive infections

The first PHLS survey in 1960 [34] described the organisms causing surgical site infection (SSI) in 3276 wounds in 21 hospitals in England and Wales. There was a sepsis rate of 9.7%, but there were few data on the organisms causing these infections. *S. aureus* was the commonest organism isolated, followed by *E. coli*. The investigators were particularly concerned about the phage types of *S. aureus* causing infections. Nasal carriage of the 'virulent' type 80 strain was a significant risk factor for sepsis but, as with the current epidemic MRSA (EMRSA), there was a geographical association for this strain in that it was not found in three hospitals in the North of England. Nasal staphylococcal carriage did not, overall, increase the risk of sepsis, which was related to age, length of pre-operative stay, length of incision (> 6 cm), duration of operation and use of a drainage tube.

Some interesting data on changing trends in gram-positive infection come from the National Nosocomial Infection Surveillance System (NNIS) established in the USA in 1970 [35]. However, these and other studies discussed here must be interpreted in the light of dramatic changes in surgical and invasive procedures (e.g., day and fibre-optic surgery, increasing use of catheters, implanted prostheses and other devices), and changes in patient populations and treatments (e.g., age, immunosuppression and transplantation). Hospitals participating or studied have changed with time but, more importantly, the surveillance process has not been modified to compensate for the decreasing lengths of hospital stay. This makes it essential to consider post-discharge surveillance. However, this is often omitted or performed with non-standardised or unvalidated methods [36]. Hospital infection is thus being displaced into the community. Lack of clinical sampling in hospitals threatens the alert organism system and skews the data recorded in the hospital to the most ill patients, or perhaps certain specialties. New strategies for outbreak control must also take this into account; routine discharge screening from wards suspected or known to be affected with MRSA or other resistant organisms must be considered [37].

Risk factor data are available and could assist interpretation of trends, e.g., for SSIs and duration and type of device usage [38]. However, it would also

be important for analyses of antibiotic resistance trends to access data on total antibiotic usage, audit of compliance with antibiotic policies, and the extent and correct use of antibiotic prophylaxis.

The USA NNIS data suggest changing patterns in organisms causing hospital infection between 1980 and 1986–89 [39]. There were decreases in certain gram-negative pathogens (*E. coli* by 20% and *Klebsiella* spp. by 2%), whereas there was a doubling of coagulase-negative staphylococcal infections, associated with an increased number of blood stream infections, from 4% to 9%. There was a slight increase (by 1–2%) in infections caused by enterococci and *S. aureus*, but also in infections caused by *P. aeruginosa* and *Enterobacter* spp. Currently, *Enterococcus* spp. are the second, *S. aureus* the fourth, and coagulase-negative staphylococci (CNS) the fifth most common pathogens if the non-*E. coli* members of the Enterobacteriaceae are disregarded.

Overall, the incidence of MRSA has risen in the USA from 2.4% of all isolates in 1975 to 29% in 1991. Hospitals with > 500 beds have the highest incidence (38.3%), but increases are being seen in hospitals of all sizes, albeit with temporal differences [40]. Methicillin resistance in CNS increased 300% over this period and, like MRSA, increases are occurring in hospitals of all sizes, although the highest incidence is seen in the larger hospitals.

In the 35 years following the first 1960 PHLS survey, there have been two repeats of National UK Studies. The results of a 19-centre PHLS audit study are about to be published and there are many other initiatives including a PHLS Nosocomial Infection National Surveillance Scheme for England. The preliminary results of the second UK National Prevalence Survey organism data indicate increases in gram-positive infections from 30% to 39.7% compared with the previous study [41]. Enterococci increased from 4% to 7%, and CNS from 3% to 7.7%, but *S. aureus* decreased from 17.6% to 14.7%. However, there was an increase in MRSA from 0% to 5%, and MRSA accounted for 14 of the 228 SSIs (15% of all *S. aureus* SSIs.) (B. Cookson and colleagues, unpublished observations). Clearly these data will need to be stratified further by site of infection. For SSI they should be further stratified by class of wound. Several studies have shown that *S. aureus* is a commoner cause of infection in clean wounds, with gram-negative bacilli and enterococci associated with the more contaminated or dirty wounds [42]. There are many differences between the two UK prevalence studies and comparisons will be extremely difficult.

MRSA

MRSA re-emerged as a significant cause of hospital infection in the early 1980s, soon after the last

Prevalence Survey. Epidemic MRSA (EMRSA) causing infection in two or more patients in at least two hospitals were characterised [43]. However, some MRSA strains spread much more widely than others and might be termed 'Super' EMRSA (SEMRSA) or re-defined as genuine EMRSA. In the 1980s, these EMRSA were largely (but not exclusively) causing outbreaks in a particular UK region.

The revised UK guidelines for MRSA control [44] recommended that contact be made with the reference laboratory and that the measures taken would be influenced by whether the strain was a known EMRSA. The continuing problems with MRSA in the 1990s are resulting in a re-appraisal of control methods, including addressing infection control precautions in nursing and elderly care homes. One study has shown that EMRSA-16 has affected three of eight nursing, and three of seven residential, homes [45]. Fourteen of 350 patients and one of 72 staff were colonised. In three instances, spread occurred between patients sharing rooms and to 11 patients not sharing rooms. Similar results have been seen in two other homes affected by EMRSA-3 and EMRSA-1 (B. Cookson, unpublished observations). There also seems to be an emerging realisation of the possibility of MRSA acquisition within the family, and, rarely, even pets may constitute a reservoir.

EMRSA-15 emerged in the West Midlands, but has now spread. A recent audit suggested that 40% of colonised patients became infected (I. Blair, personal communication). EMRSA-16 caused a large outbreak in the hospital in Northamptonshire affected originally. Eighty-three (21%) of the 400 patients involved over a 20-month period were infected. This comprised 4% of the hospital's *S. aureus* infections, and the strain caused significantly more chest infections and deaths from septicaemia [46]. EMRSA-16 spread locally, then to West and South London, and thence to the South-East of England. Hospitals differ in their experience; some are able to control the organism, whilst others are suffering from significant outbreaks. Spread is now occurring further afield and the Reference Laboratory is reporting on the situation regularly and feeding this information to the newly convened MRSA working party formed by the Hospital Infection Society, Infection Control Nurses Association and the British Society for Antimicrobial Chemotherapy. Typing loads at the Central Public Health Laboratory are immense and criteria for submission of isolates for typing are having little effect. A possible reason for this is that hospitals are increasingly affected by more than one EMRSA and typing is perhaps of more use than ever before.

One hospital reporting to the Reference Laboratory has shown that one-half of wounds colonised with EMRSA-16 have become infected. Orthopaedic wards continue to be affected by EMRSA, and joint

replacement infections are often mentioned. The debate over the measures required to control UK MRSA, or indeed the necessity, continue. It is very difficult to determine the relative importance of the many outbreak control measures that have been advocated; they include isolation, effective therapy of cases, increased education, and institution of hand-washing agents and antibiotic policies.

Clinical microbiologists are convinced that these newer EMRSA (types 15 and 16) are causing significant infections, and this is supported by two other sources of information. The preliminary univariate analysis of the Second Prevalence Survey (M. Griffin, personal communication) has shown that MRSA colonisation had the highest relative risk (5.09) for hospital-acquired infection. The data from the Communicable Disease Surveillance Centre (CDSC) bacteraemia reporting system also indicate dramatic increases in MRSA infections (D. Speller, personal communication). MRSA infection had remained approximately static (< 2%) until the 'take-off' in EMRSA types 15 and 16, with an increase to 8.1% in 1994 and a worrying 13.6% for the first 6 months of 1995. EMRSA-15 became ciprofloxacin-resistant fairly early on, followed by EMRSA-3; EMRSA-16 was quinolone-resistant *ab initio*. That the increases are caused by these strains is perhaps supported by the increasing percentage of ciprofloxacin-resistance in the data. However, these hypotheses will have to be investigated further by comparing these data with those in the Reference Laboratory database of referred isolates. Sample migration of reported bacteraemia isolates is a possible (but unlikely) explanation.

Several groups of workers have used mupirocin to eradicate MRSA from the nose and wounds. This should reduce MRSA transmission to staff and then to other patients [47]. High-level mupirocin-resistant MRSA (MIC $\geq$ 256 mg/L) were reported in 105 patients from 34 UK centres (14 with EMRSA-16) in the first 6 months of 1994 [48]. The significance of low-level resistance is more difficult to interpret, but it is even more widespread (865 patients from 115 centres; 638 patients from 51 centres with EMRSA-16). However, we are concerned that the agent is becoming abused in that it is used repeatedly on the same patient for prolonged periods, or prophylactically, in situations where it is impossible to prevent MRSA re-colonisation, and 'blanket use' after initial MRSA screening of all patients and staff. Sometimes it has been used on staff without establishing if the carriage is transient. At the other extreme, the full extent of carriage has not been established and there has been no consideration of combination with systemic agents. Judicious and careful control of its use is imperative and the use of alternative agents (old and new) is being explored for these strains.

Enterococci

These organisms have become labelled as the nosocomial pathogens of the 1990s [49]. Some of the data should be viewed with caution in that enterococci are often isolated in mixed culture from many infected sites, especially wounds and perhaps urine. However, stratifying the USA NNIS data by site of isolation, they are still the third commonest pathogen from blood. Strains of VRE are often also resistant to high levels of gentamicin, thus compromising the standard therapies for these organisms. In the USA, centres are anecdotally describing resistance to vancomycin in up to 50% of their isolates. The Centers for Disease Control report an increase in resistance from 0.3% to 7.9% between 1989 and 1993, with an even more dramatic increase from 0.4% to 13.6% amongst ITU isolates [50].

In the UK, the first incidence of VRE occurred in a London hospital renal unit, resulting in a large outbreak involving 41 patients. A number of different strains, mainly *Enterococcus faecium* but also *En. faecalis*, were involved and eradication proved difficult [51]. These features are typical of other UK outbreaks. Strains appear to spread rapidly between patients, and heavy environmental contamination is often observed. Contamination of surfaces, such as dripstand hand grips, monitoring devices [52] and computer terminals, and evidence that these organisms survive for up to 30 min on fingertips [53], suggests that hospital personnel may be involved in transmission.

Since 1987 the spread of enterococcal isolates with acquired vancomycin resistance in UK hospitals has been monitored by the Reference Laboratory. Between 1987 and 1995, 720 patients from 71 hospitals were affected. The number of hospitals with VRE continues to increase each year, with 18, 27 and 47 hospitals affected in 1993, 1994 and 1995, respectively [54]. The proportion of *En. faecium* (86%) to *En. faecalis* (11%) is the reverse of that seen when all clinical specimens are considered [55], and most (80%) isolates are of the *vanA* phenotype. Pulsed-field gel electrophoresis (PFGE) typing performed on a large number of vancomycin-resistant *En. faecium* (VREM) isolates suggests that, nationally, a large number of heterogeneous strains are involved. In general, each hospital appears to have its own strain or set of strains. However, five epidemic strains have also been observed. These strains, designated epidemic vancomycin resistant *En. faecium* (EVREM), have been isolated from two or more patients in at least two hospitals. In two instances, the transfer of a patient from a unit at one hospital with a known VRE problem resulted in an outbreak at the receiving unit. One of these epidemic strains, EVREM-3, is particularly worrying as it appears to have a remarkable ability for intra- and inter-hospital spread. Over a 4-year period it has been isolated in 13 hospitals in the London area and, more recently, it has caused an

outbreak via known patient transfer at a hospital on the South coast. Such epidemic strains have also been reported in the USA. One strain was isolated at seven hospitals in Seattle during a 4-year period [56]. These facts suggest that hospitals should consider screening new patients for VRE, as has been suggested elsewhere [57].

The origin of VRE is an area of intense debate with two theories prevailing. The first suggests that they emerged in the hospital environment following heavy use of vancomycin, possibly acquiring the vancomycin resistance gene from a hitherto uncultured bacterium [58], while the second suggests a link with the use of avoparcin (a glycopeptide growth promoter) in the farming industry [59]. The European Union have banned the use of avoparcin, but more work is clearly required in this area.

Epidemiology of resistance – respiratory tract infections

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Introduction

Increasing levels of resistance to antimicrobial agents are leading to a dwindling resource of treatment options for bacterial infections. Treatment of community-acquired respiratory infections is an area of particular concern, as treatment often has to begin empirically before the causative organism is identified. Several bacterial species are responsible for respiratory tract infections, the three most common being *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. In order to assess critically the threat of increasing antimicrobial resistance, it is necessary to examine, first, trends in the levels of resistance observed and, second, the mechanisms of resistance. This article reviews the incidences and mechanisms of antibiotic resistance in these three organisms.

Haemophilus influenzae

Ampicillin resistance was first detected in *H. influenzae* in the 1970s and has risen steadily to the present level of 10–30% reported by most centres. Current levels of resistance in the UK are detailed in Table 3 [60]. The major mechanism of resistance to β -lactam drugs in *H. influenzae* is the production of β -lactamases, and the prevalence of β -lactamase-producing isolates has increased over the last 20 years. The extent of non- β -lactamase-mediated resistance has always been difficult to assess and it is possible that β -lactamase production may be missed by routine testing [61, 62]. β -lactam resistance in strains apparently negative for β -lactamase

Table 3. Current levels of antibiotic resistance amongst lower respiratory tract isolates from England and Wales

Antibiotic	<i>H. influenzae</i>		<i>M. catarrhalis</i>	
	Breakpoint (mg/L)	Resistant isolates (%)	Breakpoint (mg/L)	Resistant isolates (%)
Amoxycillin	1.0	16.9	1.0	33.0
Co-amoxiclav	1.0	4.5	1.0	4.0
Cefuroxime	1.0	5.0	1.0	4.7
Ciprofloxacin	1.0	0	1.0	2.0
Erythromycin	0.5	97.2	0.5	6.0
Tetracycline	1.0	4.1	1.0	2.7
Trimethoprim	1.0	9.6	1.0	100

Isolates obtained in 1995–1996 from 13 centres in England and Wales (Brown, Amyes and Thomson [60] and unpublished results).

production has been attributed to changes in penicillin-binding proteins [63].

The most important β -lactamase in *H. influenzae* is TEM-1, which is found in c. 80% of β -lactamase-positive isolates [61, 62, 64]. This accounts for the present susceptibility of these organisms to β -lactam- β -lactamase inhibitor combinations and cephalosporins. A second β -lactamase, ROB-1, has also been detected in *H. influenzae*. Although less numerous than TEM-1, ROB-1 has been found in up to 8% of isolates in some studies [61, 65]. Although not closely related, DNA sequence analysis has demonstrated that both ROB-1 and TEM-1 belong to Bush group 2b, Ambler class A enzymes [66]. Both TEM-1 and ROB-1 are plasmid-mediated and transferable. Until recently, these were the only β -lactamases that had been described in *H. influenzae*; however, recent evidence suggests that the situation may be more complex. First, DNA sequence analysis of TEM-1 genes in *H. influenzae* suggests that the *bla*_{TEM-1} gene in this species may differ from its *E. coli* counterpart, and that silent amino-acid substitutions in TEM-1 have occurred [67]. The impact of these changes on the ability of TEM-variant β -lactamases to evolve in *H. influenzae* is not clear at present. Second, a completely novel β -lactamase, VAT-1, has also been identified recently in *H. influenzae* [68]. The significance of this enzyme is not clear at present, but it has been detected in isolates from different geographical centres in Scotland [62, 67]. The gene encoding VAT-1 has not been sequenced, but biochemical data suggest that it is a Bush class 1, Ambler class C β -lactamase [66]. VAT-1 exhibits cephalosporinase activity and resistance to the β -lactamase inhibitors clavulanic acid, sulbactam and tazobactam [68].

Erythromycin has little activity against *H. influenzae* (Table 3). However, data demonstrating azithromycin activity is currently being accumulated [69]. Resistance to chloramphenicol and tetracycline has been detected, but is currently < 5% for each (Table 3). Resistance to chloramphenicol results either from the production of chloramphenicol acetyl transferase or impermeability [70, 71], while tetracycline resistance is related to energy-dependent efflux of tetracycline, although other mechanisms also exist [72]. The genes

encoding chloramphenicol and tetracycline resistance are plasmid-mediated and associated with transposons [73]. Decreased susceptibility to trimethoprim results from alterations in chromosomal dihydrofolate reductase production [74], with a current trimethoprim resistance level of 9.6% in the UK. Although there are a number of isolated reports of the emergence of quinolone resistance in *H. influenzae* [75], this does not appear to be a widespread phenomenon and resistance to ciprofloxacin and other quinolones remains low.

Moraxella catarrhalis

M. catarrhalis was regarded originally as a harmless commensal of the upper respiratory tract, but its role as a human pathogen has now been recognised. At present, *M. catarrhalis* remains susceptible to most antibiotics, with the exceptions of ampicillin and trimethoprim. β -Lactamase production in *M. catarrhalis* is now an almost universal phenomenon, with > 80% of isolates being β -lactamase-positive. Earlier reports indicated a much lower incidence of β -lactamase production; indeed, β -lactamases were not detected in *M. catarrhalis* until 1977. It is not clear if this apparent dramatic increase in the incidence of β -lactamase production represents the rapid dissemination of β -lactamase genes or reflects more sensitive detection methods. The presence of two β -lactamases (BRO-1 and BRO-2) in *M. catarrhalis* is well established [76]. Unlike most β -lactamases, the BRO enzymes form a number of different bands following isoelectric focusing, and these vary depending on the extraction method employed. Although these discrete bands have identical biochemical properties, suggesting that they are different forms of the same enzyme, interpretation of isoelectric focusing gels is difficult [76, 77]. Whilst the differences between BRO-1 and BRO-2 are defined, the occurrence of a third related β -lactamase, BRO-3, as a discrete enzyme and not a precursor of BRO-1 and BRO-2 is not confirmed [76, 78]. The BRO enzymes confer resistance to ampicillin, but remain susceptible to β -lactamase inhibitors [76]. Although the genes encoding the BRO enzymes are chromosomally located, transfer of BRO β -lactamases has been demonstrated, indicating that the genes may reside within conjugative transposons [79]. Resistance

to trimethoprim in *M. catarrhalis* is almost universal although the mechanism is unknown. At present, resistance in the UK to other agents remains low (Table 3).

Streptococcus pneumoniae

Perhaps nowhere has the spectre of the post-antibiotic era been more clearly illustrated than with the development of penicillin resistance in *Str. pneumoniae*. Initial reports of penicillin resistance appeared in the USA in the 1960s as isolated cases, but resistant isolates are now widespread. Reported levels of penicillin resistance in *Str. pneumoniae* differ widely. In Western Europe, Spain has a particular problem [80], but high levels of resistance in Eastern Europe are also of concern, as typified by Hungary with resistance levels of > 50% [81]. South Africa also represents a hot spot with high levels of resistance [82]. Changing patterns in levels of resistance are best established by follow-up studies within the same area and, whilst the levels of resistance vary with country, within most areas there has been a continued increase in the level of resistance over the last 20 years. Local factors influence the levels of resistance observed greatly and, in addition, resistance levels within the same centre vary depending on the source of the isolates (Fig. 1) [80]. The emergence of penicillin resistance can be correlated with local use of penicillin; however, clonal spread of resistant strains also occurs and export of penicillin-resistant strains from one country to another has been demonstrated [83, 84].

Penicillin resistance in *Str. pneumoniae* results from alterations in penicillin-binding proteins (PBPs). *Str. pneumoniae* produces five high molecular mass PBPs (1A, 1B, 2A, 2B and 2X) and one of low molecular mass (PBP3) [85]. Alterations in the high molecular mass proteins are associated with decreased susceptibility to penicillin whereas, in contrast, changes in PBP3 appear to have no role in the development of

resistance. The alterations found in resistant strains appear not to have arisen by a process of mutation and selection, but through homologous recombination. Analysis of the sequences of genes encoding PBPs in sensitive strains demonstrates that they are uniform. However, in contrast, the genes found in resistant isolates have mosaic structures containing blocks of divergent DNA. *Str. pneumoniae* is naturally transformable and it is likely that the evolution of resistant PBP genes has occurred through natural homologous recombination with other species [85].

Resistance in *Str. pneumoniae* is not limited to penicillin, and resistance to other classes of antibiotics – particularly chloramphenicol, tetracycline and the macrolides – has also been rising steadily. Furthermore, the emergence of multiresistant strains has caused therapeutic dilemmas. Multiresistance in *Str. pneumoniae* – defined as resistance to at least three different classes of antibiotics – was first detected in South Africa in 1977. At present the level of multiresistant strains varies from 13.6% of isolates in Spain to 2.2% of isolates in the USA [86]. The mechanisms of resistance in *Str. pneumoniae* are well understood. Resistance to chloramphenicol results from the production of chloramphenicol acetyl transferase [87] and tetracycline resistance usually results from ribosomal protection as a result of the production of the TetM protein [88]. Reduced susceptibility to the macrolides results from methylation of ribosomal RNA [89]. Co-trimoxazole and trimethoprim resistance has been detected and probably results from the production of a resistant dihydrofolate reductase (as in *S. aureus*), but this has not been clarified. Although plasmids have been detected in *Str. pneumoniae*, it is thought that the dissemination of resistance genes among *Str. pneumoniae* may occur on conjugative transposons.

Finally, concern has also been raised over the development of resistance to other β -lactam agents,

Fig. 1. Antibiotic resistance of *Str. pneumoniae* isolates from different sources. Data from Linares *et al.* [80].

especially the cephalosporins. In contrast to high-level resistance to penicillins, which requires modifications in four PBPs, high-level ceftriaxone resistance has been shown to result from changes in only PBPs 1A and 2X. The remaining PBPs have a natural low affinity for the cephalosporins and do not require to be modified [85].

Conclusion

Antibiotic resistance in respiratory pathogens continues to increase and is a worldwide problem. At present, a variety of options still exist for *H. influenzae* and *M. catarrhalis*. Of greater concern is the development of penicillin resistance, and indeed multiresistance, in *Str. pneumoniae* where there are fewer remaining options in our antibiotic armamentarium.

Epidemiology of antibiotic resistance: blood and cerebrospinal fluid (CSF)

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Introduction

A significant feature of medical microbiology in the 1990s has been increasing concern about antibiotic resistance. This has led to a heightened awareness of the importance of surveillance of resistance rates locally, nationally and internationally. In terms of national surveillance of antimicrobial resistance, one approach is for bacteria isolated in laboratories in different parts of the country to be referred to one centre where susceptibility testing may be undertaken. Such surveillance may involve testing of isolates submitted to reference laboratories for typing or other specialised investigation [90], or the testing of isolates collected specifically for surveillance purposes [18]. The former approach will usually give biased results (e.g., isolates may be referred preferentially for confirmation of particular resistance phenotypes) and may not be a reliable source of data for assessment of prevalence rates. Nonetheless, such an approach may give useful information about trends in resistance, and may be particularly valuable as an early warning system for detecting emergence of novel resistance traits. The latter approach of collecting isolates specifically for surveillance purposes may allow a more accurate assessment of prevalence rates, but the performance of such studies on a regular basis is often limited by availability of resources and staff. An alternative approach to national surveillance involves the centralised collection and analysis of susceptibility testing results from diagnostic laboratories around the country [91]. This paper reports on surveillance of antibiotic resistance in bacteria causing bacteraemia or

meningitis in England and Wales, as reported to the PHLS CDSC.

Collection of data

Since 1974, diagnostic microbiology laboratories in the PHLS network, and increasing numbers of National Health Service and private hospitals in England and Wales, have reported to CDSC, on a voluntary basis, isolation of bacteria from blood or CSF. From the beginning of 1989, laboratories have also been requested to report the results of their susceptibility testing of these isolates. Results, which are reported as 'S' (susceptible), 'R' (resistant) or 'I' (intermediate), are commonly submitted as written entries on the Communicable Disease Report (CDR) Form 2 although, during the 1990s, variable numbers of laboratories have submitted results electronically. Laboratories contributing results to this surveillance scheme do not necessarily test the same antibiotics, although certain agents are commonly tested against particular pathogens (e.g., pneumococci are commonly tested for susceptibility to penicillin). In this report, data on four pathogens (*Str. pneumoniae*, *S. aureus*, *E. coli* and *K. pneumoniae*) commonly reported to CDSC as causing bacteraemia or meningitis will be reviewed. The data presented cover the 6-year period from 1989 to 1994.

Str. pneumoniae

During the period 1989–1994, there was a virtually consistent annual increase in the prevalence of resistance to penicillin among isolates of *Str. pneumoniae* (expressed as a percentage of the total number of isolates tested against this antibiotic), from 0.3% in 1989 to 2.5% in 1994 (Table 4) [92, 93]. The number of isolates tested for penicillin resistance each year ranged from 2667 to 3265. For the purpose of this analysis, the term 'resistant' includes both intermediate resistance and full resistance, as the proportion of laboratories attempting to differentiate between the two levels of resistance was not known. However, in a study carried out in 1995 in which 583 pneumococci (isolated in 52 Public Health Laboratories) were collected and studied, and in which the prevalence rate for any degree of penicillin resistance was 4.9%, intermediate and fully resistant organisms occurred in about equal proportions [18].

Table 4. Reported resistance to penicillin and erythromycin amongst blood and CSF isolates of *Str. pneumoniae* in England and Wales

Antibiotic	Percentage of resistant isolates in					
	1989	1990	1991	1992	1993	1994
Penicillin*	0.3	0.5	0.7	1.9	1.7	2.5
Erythromycin	3.3	5.1	6.4	8.6	10.8	11.2

*Includes both intermediate and full resistance.

Of potentially greater interest was the observation that not only did resistance to erythromycin also increase between 1989 and 1994, but its prevalence was much higher than that of resistance to penicillin, rising from 3.3% in 1989 to 11.2% in 1992 (Table 4).

S. aureus

In recent years, there has been a striking increase in the occurrence of colonisation or infection of hospital patients caused by *S. aureus* strains resistant to methicillin and other antibiotics [94, 95]. In the past, the incidence of bacteraemia caused by MRSA was low, but in recent years, the proportion of *S. aureus* isolates obtained from blood or CSF that are resistant to methicillin has increased. Between 1989 and 1991, the proportion of *S. aureus* isolates obtained from blood or CSF that were resistant to methicillin remained fairly stable at *c.* 1.5%, but increased thereafter to 8.1% by 1994. Analysis of over 2000 isolates obtained during the first half of 1995 showed a prevalence rate for methicillin resistance of 13.6% (D. C. E. Speller, unpublished observations). Interestingly, by way of contrast, the prevalence of resistance to benzyl penicillin among blood and CSF cultures of *S. aureus* remained relatively stable over this time period, fluctuating within the range 85.3–87.4%, and resistance to fusidic acid also stayed relatively unchanged, varying between 1.8% and 2.4%.

There has also been an increase in the prevalence of resistance to other antimicrobial agents, including erythromycin (which increased from 7.5% in 1989 to 13.3% in 1994) and ciprofloxacin (which increased from 2.9% to 16% over the same period). Both resistances appeared to be highly associated with methicillin resistance, with 90% of MRSA obtained from blood or CSF in 1995 showing these resistance phenotypes, in contrast to methicillin-sensitive *S. aureus* isolates, of which < 10% were resistant. These findings may reflect that, from 1993 onwards, the majority of MRSA isolates occurring in England and Wales comprised two epidemic strains, designated EMRSA-15 and EMRSA-16 [96], both of which are frequently resistant to erythromycin and ciprofloxacin (D. C. E. Speller and colleagues, unpublished observations).

E. coli

Analysis of the bacteraemia and meningitis database also shows ciprofloxacin resistance to be increasing in *E. coli*, albeit at a much lower rate than that seen in *S. aureus*. In 1989, 0.5% of *E. coli* isolates from blood or CSF were reported as being resistant to ciprofloxacin. For the next 5 years, there was a gradual but consistent annual increase in resistance such that, by 1994, the prevalence of ciprofloxacin resistance was 1.7%. The large number of isolates for which data are available (3296 in 1989, rising to 5186 in 1994) allows meaningful

statistical analysis to be performed, which shows the increase to be highly statistically significant ($p < 0.001$; χ^2 for trends). Although the impact on clinical practice of this increase may be minimal at present (as > 98% of isolates remain susceptible to ciprofloxacin), the detection of a trend towards increasing resistance to such an important antibacterial agent means that it will be crucially important to continue to monitor resistance for any acceleration in the rate at which resistance is increasing. In contrast to the situation seen with ciprofloxacin, resistance to some other antibiotics did not appear to change significantly. For example, during the 6-year period, resistance to gentamicin fluctuated between 1.5 and 1.7%, while the prevalence of resistance to cefotaxime was 1% in 1989 and 0.8% in 1994 (with the prevalence rates in the intervening years varying within the range 0.5–1.2%). Resistance to ampicillin or amoxycillin was high throughout, with 54.7% of 6078 isolates and 55.8% of 6611 isolates being resistant in 1989 and 1994, respectively.

K. pneumoniae

A similar trend in patterns of antimicrobial resistance was noted in *K. pneumoniae*. Rates of resistance to ciprofloxacin increased annually, rising from 2.9% in 1989 to 6.5% in 1994 ($p < 0.001$). The numbers of isolates reported as being tested against ciprofloxacin showed a continuous annual increase from 756 in 1989 to 1158 in 1994. As noted with *E. coli*, there was no significant change in the prevalence of resistance to gentamicin (4.1% of 1236 isolates in 1989; 3.8% of 1378 isolates in 1994). There was variation in the prevalence of resistance to cephalosporins from year to year. The reported prevalence of resistance to cefotaxime was 3.9% (of 618 isolates) in 1989 and 4.3% (of 670 isolates) in 1994, with the overall prevalence rates falling within the range 2.7–4.3%. For ceftazidime, the rates of resistance in 1989 and 1994 were 2.7% (of 525 isolates) and 5.9% (of 819 isolates), respectively, with the overall rates falling within the range 2.7%–5.9%.

Conclusions

Certain of the data presented here give cause for concern. With pneumococci, the high prevalence of resistance to erythromycin (11.2% in 1994) is of probable clinical significance, as erythromycin is used to treat pneumococcal infections in patients who are allergic to penicillin or who are infected with penicillin-resistant organisms. Although the prevalence rate of 2.5% for penicillin resistance (both intermediate and full) in pneumococci in 1994 is lower than the prevalence rates of 11–36% seen in other countries [97], the upward trend described here raises the possibility that similar levels of resistance might be seen in this country in years to come.

Another area of concern is the increase in the proportion of *S. aureus* isolates that are resistant to

methicillin. This may have implications for the empirical treatment of sepsis, as it has been shown that patients with bacteraemia caused by MRSA have a poorer outcome than patients with bacteraemia caused by methicillin-sensitive *S. aureus*, particularly if the former group do not receive appropriate therapy with an agent such as vancomycin [98]. The increasing prevalence of resistance to ciprofloxacin among staphylococci (particularly MRSA), *E. coli* and *K. pneumoniae* is also worrying. On the other hand, the continuing susceptibility of most isolates (> 98.2%) of *E. coli* to gentamicin shows that the picture is not entirely gloomy.

The examples presented here show the value of surveillance of antibiotic resistance based on analysis of the results of routine diagnostic testing. Such surveillance allows not only the quantitative assessment of prevalence rates for specific types of resistance, but if carried out over a prolonged period, allows changing trends in resistance to be monitored. In view of the trends towards increasing antimicrobial resistance among a number of important pathogens, it is important that surveillance of resistance continues in the future.

Epidemiology of resistance in intensive therapy units (ITUs)

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Infection in ITUs

Infections in ITUs represent 20% of nosocomial infections. Units affected most commonly include burns units, neonatal units and units dealing with immunocompromised patients. The main risk factors for ITU-associated infection are invasive procedures (particularly endotracheal intubation), venous cannulation, length of stay on the ITU and the presence of renal impairment.

The results of the European Prevalence of Infection in Intensive Care (EPIC) study were reported recently [99]. In total, 4501 patients were investigated, with an overall incidence of infection of 44.8%. Of the patients studied, 20.6% acquired their infection on the ITU. The main identifiable risk factors were: a length of stay of > 48 h; a requirement for mechanical ventilation; antecedent trauma; the presence of intravenous cannulae or urinary catheter; and the use of H₂ antagonists for stress ulcer prophylaxis.

Antibiotic usage in ITUs

At the City Hospital, Birmingham, the use of antimicrobial agents on the ITU has been monitored.

Although the unit is small, having only seven beds, antimicrobial usage is substantial. During Oct.–Dec. 1995, 38 patient days of ciprofloxacin (at a dose of 800 mg b.d.) and 144 patient days of ceftazidime (at 1 g t.d.s.) were prescribed. This corresponds with an annual usage of 122 g of ciprofloxacin and 1.73 kg of ceftazidime. The financial implications of such large scale usage are considerable. In 1995, the hospital spent £54 715 on antimicrobial use in the ITU. Although the ITU only accounts for just over 1% of the in-patient population, this antibiotic bill is 13% of the hospital's annual antibiotic budget. Main items included £8659 for ceftazidime and £20 760 for ciprofloxacin.

Antibiotic resistance in ITUs

Given this substantial use of antibiotics, it is not surprising that ITUs are the hospital areas where most antimicrobial resistance is encountered. The EPIC study [99] identified a total of 528 ITU-acquired *S. aureus* infections, of which 272 (51%) were caused by MRSA. Of all laboratory confirmed *S. aureus* bacteraemias, 72.4% were MRSA. Similarly, of 504 ITU-acquired *P. aeruginosa* infections, 267 (65.1%) were resistant to one or more of the following antibiotics: gentamicin, imipenem, ceftazidime, ciprofloxacin and piperacillin. In addition, 70.1% of the 335 ITU-acquired coagulase-negative staphylococcal infections were caused by methicillin-resistant strains, 3.5% were vancomycin-resistant, and 9.3% were teicoplanin-resistant.

Resistance in ITUs versus general hospital

Although antimicrobial resistance is a problem in all areas of the hospital, as well as in the community, it is the ITU that is affected most severely by this problem. Isolates from the Birmingham City Hospital ITU show a much higher prevalence of resistant organisms than the rest of the hospital. Most noticeably, the prevalence of MRSA is nearly twice that in the rest of the hospital, and the proportion of gram-negative organisms resistant to ceftazidime, ciprofloxacin and gentamicin is also much higher than in general wards.

Enterococci

Several organisms known to be intrinsically resistant to a variety of antimicrobials are particular problems on ITUs and appear to be increasing in importance. Enterococci were previously thought of as harmless commensals, yet the National Nosocomial Infection Survey (NNIS) demonstrated that, between 1986 and 1989, enterococci were the second commonest nosocomial pathogen in the USA [100]. A study of enterococcal bacteraemia [101] identified several factors that are associated with increased mortality. These included: age > 56 y; severe underlying disease; nosocomial acquisition; trauma; previous antimicrobial

therapy; vascular or urinary catheterisation; and the presence of a surgical wound.

VRE

Vancomycin resistance in enterococci was first reported from London [51], but the scale of the problem in the UK has escalated so that *c.* 40 centres are now affected. The difficulty in treating infections caused by VRE has been exacerbated by the concurrent development of resistance to penicillin and high levels of gentamicin. This renders these organisms untreatable with commonly available drugs. An outbreak of infection caused by an *En. faecium* isolate that was resistant to vancomycin, ampicillin and high-levels of gentamicin was reported from Houston in 1990 [102]. These strains also cause infection in some London hospitals, and data from the UK and the USA suggest that the number of centres affected by resistant strains, as well as the number of isolates is increasing.

MRSA

MRSA strains have caused cross-infection problems in various countries in the world. One of the first countries to experience large outbreaks with this organism was Australia, although it is also now endemic in hospitals in the USA and Europe. It is generally thought that the main mode of MRSA transmission is by hand contact, and the Hospital Infection Society has published guidelines for the control of MRSA that concentrate on procedures likely to limit the risk of hand-to-hand spread [44]. MRSA is a major problem on ITUs and this may be related to the intensity of contact with medical, nursing and paramedical staff, as the number of contacts with health care personnel has a dramatic impact on the potential for an organism to spread. Airborne spread is not generally thought to be a significant factor in the spread of MRSA, although an outbreak in the City Hospital was probably related to the proximity of an air extract from an isolation cubicle to an open window in the main ITU [103].

Acinetobacter spp.

Acinetobacter spp. are being recognised increasingly as nosocomial pathogens causing infections on ITUs. Strains are becoming more resistant to antibiotics and have been isolated from ventilators and hand wash basins. As many as 45% of tracheostomy sites may be colonised with these organisms. Risk factors for acinetobacter infection include intravenous cannulation, antimicrobial use and admission to ITU.

Induction and derepressed mutants

Certain gram-negative organisms, such as *Enterobacter*, *Serratia*, *Citrobacter* and *Pseudomonas* spp., have the propensity to produce chromosomal β -lactamases that are inducible and active against a wide

range of penicillins and cephalosporins. These β -lactamases are often termed AmpC type β -lactamases are usually chromosomal, and are inducible. Plasmid-mediated enzymes with similar activity have also been described. This inducible property is reversible and can cause problems with in-vitro detection of resistance unless appropriate methods such as a disk approximation technique are used. One important aspect of these strains is that the presence of an inducible AmpC type β -lactamase is a risk factor for mutation to the derepressed state. In this state the organism produces large amounts of β -lactamase, resulting in resistance to most β -lactam antibiotics. This state tends to be irreversible.

Cephalosporin use and resistance in gram-negative bacteria

The large scale use of cephalosporins has been associated with outbreaks caused by organisms that produce cephalosporinases. A prospective case controlled study [104] examined 386 isolates from 340 patients and showed that 18.1% were resistant to extended spectrum cephalosporins (ESCs). *Enterobacter* spp. were a particular problem, with 31.1% exhibiting resistance to ESCs. Furthermore, there was a statistically significant association between cefotaxime, ceftazidime and piperacillin usage and resistance to ESCs ($p = 0.008, 0.004, 0.001$, respectively). Amino-glycoside usage seemed to protect against this effect.

Quinolones and resistance

Most quinolone resistance is caused by mutations in the *gyrA* gene (mainly at codon 83). Although there have been reports of plasmid-mediated resistance, these reports have not been substantiated. Several workers have demonstrated that resistance can develop following treatment, but outbreaks resulting from large-scale use seem to be rare. This may be because quinolones have been shown to have plasmid curing properties and also seem to inhibit plasmid conjugation.

What can we do?

Given the scale of the resistance problems in ITUs, and the consequent difficulty in treating infections on these units, what can be done to control the problem? It is becoming clear that the pharmaceutical industry cannot be relied upon to produce ever more powerful agents. An alternative strategy is the use of selective decontamination of the digestive tract (SDD) to reduce the risk of infection. Unfortunately, published regimens for SDD frequently include a parenteral cephalosporin and, although resistance has not been encountered in units using SDD, it has to be appreciated that extensive use of SDD may eventually worsen the problem of resistance on ITUs. Most medical microbiologists therefore recommend restrictive antibiotic policies, but the pertinent question remains: do they work?

Do antibiotic policies work?

A study from the Fillmore Hospital, Buffalo, NY, was conducted following detection of an increased number of multiresistant strains of *Ent. cloacae* in the ITU between 1988 and 1990 [105]. Ceftazidime usage was restricted and the preferred antibiotic combination for infections caused by *Pseudomonas* spp. was piperacillin plus gentamicin. As a result, the proportion of *Ent. cloacae* isolates resistant to ceftazidime dropped from 46% to 25%. A similar study at the Truman Medical Centre, Kansas City [106] introduced a restricted prescribing policy that required the signatures of the attending physician and an infectious disease physician. In addition, susceptibility testing was performed for certain agents only if requested by the attending physician. This resulted in a reduction in the number of ceftazidime-resistant strains of *P. aeruginosa* from 27% to 20%.

ITUs are some of the most problematic areas in hospitals with respect to antibiotic resistance. It is likely that problems with multiresistant and intrinsically resistant organisms will increase and it is important that antibiotic prescribing is controlled to prevent an unnecessary escalation of this problem.

Vancomycin resistance

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Introduction

Although the glycopeptide antibiotic vancomycin was licensed in 1958, it has been used extensively in hospital practice only for the last 20 years. This increased use has been in response to the emergence of multiresistant gram-positive pathogens, particularly enterococci and coagulase-negative staphylococci. The use of oral vancomycin as first-line treatment of *Clostridium difficile* colitis has also become established practice.

Glycopeptide resistance in enterococci was first reported in the late 1980s in England and France

[51, 107]. Since these early reports, vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens in many countries, particularly the USA [108]. These data are mirrored in the UK; in 1987 a single hospital reported the isolation of VRE, increasing to 27 hospitals in 1994 and 47 hospitals in 1995 (B. Cookson, personal communication). Glycopeptide resistance in staphylococci has been reported in *S. haemolyticus* and *S. epidermidis*. Coagulase-negative staphylococci, like enterococci, are important nosocomial pathogens causing infections, particularly infections associated with foreign bodies such as intravascular catheters, prosthetic joints and peritoneal dialysis catheters.

Glycopeptide resistance in enterococci

Resistance to glycopeptides in enterococci is phenotypically and genetically heterogeneous (Table 5). Three genotypes have been described – *vanA*, *vanB* and *vanC*. Strains of the *vanA* genotype show high-level resistance to both vancomycin and teicoplanin; resistance is inducible and is mediated by a transposon, Tn/546. Studies on the mechanism and transmission of glycopeptide resistance in enterococci have concentrated on the *vanA* genotype and form the basis for much of the work described below.

Epidemiology

VRE have affected principally larger hospitals, particularly tertiary referral centres and teaching hospitals [110]. In the UK, most reports of VRE outbreaks have centred on renal, oncology and haematology wards, reflecting the high levels of vancomycin usage on these units. Case-control studies have identified a number of patient risk factors for acquiring VRE; these include severe illness with prolonged hospital stay, multiple antibiotic usage (particularly that involving cephalosporins), proximity to a colonised or infected patient, and previous treatment with vancomycin [111, 112]. The use of oral vancomycin has been associated with the isolation of both VRE and vancomycin-dependent enterococci (see below).

Epidemiological studies by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA have provided

Table 5. Relationship between genotype and phenotype for vancomycin-resistant *Enterococcus* spp.

Property	Genotype and associated phenotypic characteristics		
	<i>vanA</i>	<i>vanB</i>	<i>vanC</i>
Vancomycin susceptibility	High-level resistance	High- or low-level resistance	Low-level resistance
Teicoplanin susceptibility	Resistant	Sensitive	Sensitive
Expression of resistance	Inducible	Inducible	Constitutive
Resistance gene location	Plasmid	Chromosome*	Chromosome
Species showing each genotype	<i>En. faecium</i> <i>En. faecalis</i> <i>En. avium</i> <i>En. durans</i>	<i>En. faecium</i> <i>En. faecalis</i>	<i>En. gallinarum</i> <i>En. casseliflavus</i> <i>En. flavescentis</i>

*Plasmid-mediated transferable *vanB* resistance has been described recently [109].

valuable information on the epidemiology and spread of VRE in hospitals [113]. Outbreaks have involved multiple strains, indicating horizontal transfer *via* either plasmids or transposons. However inter- and intra-hospital spread of VRE strains with identical PFGE profiles have also been observed, thereby indicating clonal transmission [109, 113]. A small initial outbreak involving a single strain of *En. faecium* of *vanB* genotype was experienced at the Queen Elizabeth Hospital, followed 12 months later by a much larger outbreak involving multiple strains, all carrying the *vanB* resistance gene [109]. There is some evidence for food and animal sources of VRE, but the principal problem probably now lies in hospitals with endemic VRE [114].

Environmental contamination with VRE is found frequently in units during outbreaks. The relative resistance of enterococci to drying and decontamination regimens increases the problems of controlling spread of this organism within the hospital environment. In response to the increasing problem of VRE in the USA, the Hospital Infection Control Practices Advisory Committee has provided guidelines for the control of VRE [115]. These include advice on surveillance methods and on prevention of spread by various control of infection methods.

Biochemical basis of glycopeptide resistance

Glycopeptide antibiotics inhibit cell-wall synthesis by complexing with the D-alanine:D-alanine (d-Ala:d-Ala) residues of the pentapeptide side chain of peptidoglycan precursors. This prevents the transfer of the precursors into the peptidoglycan molecule by transglycosidases. The conservation of the d-Ala terminal sequence in peptidoglycan from many bacterial species led to the conclusion that glycopeptide resistance relating to changes in target site would be unlikely to arise.

vanA genotype. Tn1546 was detected originally on a plasmid from a strain of *En. faecium* [116]. It was shown to carry the genes responsible for glycopeptide resistance – *vanR* and *vanS* are important in regulation of vancomycin resistant genes; *vanH* and *vanA* are responsible for the synthesis of the peptide d-Ala:d-lactate (see below); *vanX* and *vanY* are responsible for hydrolysis of peptidoglycan precursors; *vanY* and *vanZ* (function unknown) are not essential for expression of glycopeptide resistance [117].

Glycopeptide-resistant enterococci synthesise peptidoglycan precursors with pentapeptide side chains terminating in d-Ala:d-Lac rather than d-Ala:d-Ala [118]. Vancomycin and teicoplanin bind poorly to d-Ala:d-Lac terminal sequences. The *vanA* gene codes for an enzyme VanA, a ligase similar to the d-Ala ligase of glycopeptide-sensitive strains but which catalyses the synthesis of d-Ala:d-Lac depsipeptide [117]. The latter then replaces d-Ala:d-Ala in the

pentapeptide precursors of peptidoglycan. *vanH* encodes a dehydrogenase that synthesises d-Lac by the reduction of pyruvate. *vanX* codes for a dipeptidase that hydrolyses peptidoglycan precursors terminating in d-Ala, thus allowing the preferential incorporation of d-Ala:d-Lac into peptidoglycan. *vanY* also encodes for a peptidase, but this is not essential for the expression of glycopeptide resistance.

vanB genotype. Enterococci with *vanB* genes show various levels of inducible resistance to vancomycin, but remain sensitive to teicoplanin. In-vitro studies show that vancomycin can induce resistance to teicoplanin in *vanB*⁺ strains, suggesting that susceptibility to teicoplanin is related to a lack of induction of the resistance genes by this antibiotic [119]. Although the detailed mechanism of glycopeptide resistance in *vanB*⁺ strains has yet to be confirmed, it is similar to the resistance mechanism described for *vanA*⁺ strains; a ligase with 76% amino-acid homology to the *vanA* ligase is present, and this also synthesises the depsipeptide d-Ala:d-Lac [120]. Genes analogous to the *vanY*, *vanX* and *vanZ* genes of the *vanA* genotype are probably present. *vanB* resistance appears to be transferable by conjugation, and is associated with a large (90–250-kb) element [121]. Recently, plasmid-mediated transferable *vanB* resistance has been described [116].

vanC genotype. Three enterococcal species demonstrate intrinsic resistance to vancomycin – *En. gallinarum*, *En. casseliflavus* and *En. flavescentis*. These species show low level resistance to vancomycin, but remain sensitive to teicoplanin. In *En. gallinarum*, the *vanC* gene has been shown to code for a ligase that synthesises the dipeptide d-Ala:d-Ser as the terminal residue of the pentapeptide precursor [122].

Vancomycin-dependent enterococci (VDE)

Recently, strains of enterococci that will grow *only* around vancomycin-containing disks, or on media supplemented with vancomycin, have been isolated in the UK and USA [123, 124]. VDE have been isolated from blood, urine and faeces, and appear to have pathogenic potential. VDE seem to arise in patients already colonised with VRE, and their emergence has been associated with the use of oral vancomycin [112]. The mechanism of dependence is not understood clearly, but is probably caused by a defect in the normal d-Ala:d-Ala ligase of VRE. Such strains would only be able to synthesise a peptidoglycan *via* the inducible d-Ala:d-Lac ligase, and would therefore be dependent on the presence of vancomycin. This theory is supported by the ability of such vancomycin-dependent strains to grow around disks containing d-Ala:d-Ala. The clinical significance of VDE is unknown, but their dependence on the presence of vancomycin for growth raises problems for diagnostic laboratories.

Glycopeptide resistance in staphylococci

Although less common, glycopeptide resistance in staphylococci probably represents a greater threat than that seen in enterococci. As with enterococci, there appear to be a number of different phenotypes and these are seen more often in *S. epidermidis* than in *S. haemolyticus*. Most strains remain sensitive to vancomycin (MIC ≤ 4 mg/L), but show high level resistance to teicoplanin (MIC 8 or < 128 mg/L). The mechanism of glycopeptide resistance in these strains has not been elucidated clearly. Teicoplanin-resistant isolates have been shown to have a thicker cell wall, and often have an altered colonial morphology [125–127]. In some isolates, a 39-kDa protein has been isolated, but this shows no homology with the *vanA* ligase of glycopeptide-resistant enterococci [126]. Furthermore, the pentapeptide peptidoglycan precursors of teicoplanin-resistant strains terminate in d-Ala:d-Ala residues [128].

Glycopeptide resistance in *S. aureus* has not been described, apart from a single report of a teicoplanin-resistant strain (MIC 8 mg/L) which emerged during long-term treatment with teicoplanin for bacterial endocarditis [129]. However, transfer by conjugation of the *vanA* gene complex from enterococci to *S. aureus* has been successful *in vitro*, although this resistance was lost in the absence of selective pressure [130]. This raises the spectre of in-vivo transfer of glycopeptide resistance from enterococci to staphylococci; the possibility of this occurring is supported by the homology observed between some enterococcal and staphylococcal genes, including those coding for β -lactamase and high-level resistance to gentamicin.

Resistance to carbapenems

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β -Lactam antibiotics are amongst the oldest classes of antimicrobial agents and are the most widely used. However, resistance by various mechanisms has dogged their use since the first dramatic demonstrations of the clinical use of penicillin in 1940 [131]. It was in response to the proliferation of both plasmid- and chromosomally mediated β -lactamases that the carbapenem series of antibiotics was developed. They are differentiated from conventional penicillins by the lack of a sulphur atom in the 5-membered ring and a double bond between positions 2 and 3. They have a broad spectrum of activity that encompasses gram-positive, gram-negative and anaerobic bacteria. They have extremely good stability to all of the commonly encountered plasmid-mediated β -lactamases (e.g., TEM, SHV, OXA, PSE), gram-positive β -lactamases and the AmpC-type enzymes from bacteria such as *Enterobacter*, *Citrobacter* and *Pseudomonas* spp.

Although some agents, such as imipenem, are potent inducers of this last group of enzymes, their stability ensures that they retain clinically useful activity. In Europe, imipenem is the most familiar carbapenem, although meropenem, with its improved stability to the mammalian enzyme dehydropeptidase I which degrades imipenem and requires the co-administration of cilastatin, is used increasingly. In other countries, such as Japan, panipenem and biapenem are also in clinical use. When considering the resistance profiles of these different agents, there is considerable cross-resistance between them.

When introduced, there was a great hope that a class of antimicrobial agents had been developed that would be immune from major resistance problems. Thus it was stated in 1986: 'This review will consider the reasons why imipenem appears to be more successful than other β -lactams in overcoming resistance in gram-negative rods' [132].

Resistance to carbapenems can arise from any one, or more, of three mechanisms: (i) insensitivity of the target site, penicillin-binding proteins (PBPs) to inhibition; (ii) impermeability of the cell to carbapenems and (iii) hydrolytic enzymes that degrade carbapenems (carbapenemases).

Insensitivity of the target PBPs to carbapenems is probably the most frequent mechanism of resistance encountered in *Acinetobacter* spp. [133] and has also been reported in *P. aeruginosa*. There is a complex interplay between permeability and PBP mutations which has not been well worked out in *Acinetobacter* spp. as yet [134]. Recently, two strains of *Pr. mirabilis*, isolated from blood cultures of two patients who were treatment failures with imipenem, were shown to have decreased amounts of PBP1A, with one strain also showing decreased affinity of imipenem for PBP2 [135]. Alterations in PBPs have also been ascribed as the cause of imipenem resistance in *Rhodococcus equi* [136].

One of the best known examples of impermeability conferring resistance to imipenem is the loss of the D2 porin by mutants of *P. aeruginosa*, thereby preventing the uptake of imipenem. Shortly after the start of the widespread clinical use of imipenem, reports began to appear of *P. aeruginosa* isolates with a raised MIC in up to 25% of patients treated with imipenem [137]. Subsequent investigations revealed that a marked decrease in amounts of a specific outer membrane protein (OMP) — OprD2 (now known as OprD) — was associated with resistance [138]. OprD facilitates the transport of basic amino acids into the bacterial cell and, as carbapenems have a positively charged group in the side chain attached to the penem nucleus, the structural similarity explains the mechanism of resistance [139]. The induction or derepression of the chromosomal β -lactamase, although weak, is

also as important as the mutation in the porin gene in conferring clinically significant resistance to imipenem [140]. Further evidence for the role of these β -lactamases in contributing to imipenem resistance is provided by the four-fold increase in susceptibility of *P. aeruginosa* strains treated with BRL42715, a powerful inhibitor of these β -lactamases [141]. Resistance to meropenem in *P. aeruginosa* may be associated more closely with 'intrinsic' resistance to β -lactams (some form of combination of impermeability and efflux) rather than OprD loss [142]. Differences in permeability were thought to account for the widely differing susceptibilities of three strains of *Bacteroides fragilis* (TAL3636, QMCN3 and QMCN4) known to produce three closely related carbapenemases. However, it appears that the susceptibility differences are caused by variations in enzyme expression [143]. There are, however, also reports of reduced susceptibility to imipenem in *B. fragilis* in the absence of β -lactamase activity [144].

The occurrence of β -lactamases capable of hydrolysing carbapenems is a comparatively new and increasingly significant phenomenon. Until recently, it was thought that only metallo- β -lactamases were capable of hydrolysing carbapenems and that no serine activity site carbapenemases existed [145]. Reference to the latest version of the 'Bush' functional classification of β -lactamases reveals the group 2f enzymes which are Ambler Class-A serine β -lactamases [146]. These are chromosomal β -lactamases (IMI-1, SME-1 and NMC-A) found rarely in isolates of *Ser. marcescens* and *Ent. cloacae* [147–149]. They are inhibited by clavulanate and, interestingly, SME-1 was isolated in 1982 from the UK before the widespread use of imipenem [147]. SME-1 was initially thought to be a metallo-enzyme because of its apparent inhibition by EDTA; however, this proved to be incorrect after further study [148]. The DNA sequence is closest to NMC-A. A transferable Class-A carbapenemase has been reported from *A. baumannii* strain 6B92, isolated in 1985 from the blood culture of a patient in the Edinburgh Royal Infirmary [29]. The enzyme was designated ARI-1, with a pI value of 6.65, and was encoded on a plasmid of c. 45 kb, but this plasmid transferred only to *A. junii* at 25°C.

By far the largest group of carbapenemases belong to the zinc-dependent metallo-enzymes (Ambler Class-B), reviewed in detail by Payne [150]. The chromosomal enzyme found in *Bacillus cereus* is the only such enzyme to undergo X-ray crystallography. This enzyme is capable of binding two zinc ions and is surrounded by two histidine residues, which are probably the true ligands, together with a cysteine residue [151]. The zinc ion is thought to bind a water molecule, which then results in the hydrolysis of the carbonyl group of the β -lactam ring. The zinc-dependent carbapenemase, L1, found in *St. maltophilia* is probably the enzyme encountered most fre-

quently in clinical microbiology. The level of resistance in this bacterium to imipenem is greatly influenced by the Zn^{++} content of sensitivity testing media [152]. The metallo- β -lactamases capable of hydrolysing carbapenemases have recently been grouped into the three functional sub-groups shown in Tables 6 and 7.

These groups will be considered in reverse order as the most clinically relevant carbapenemases fall into group 3a. Group 3c, characterised by a very high rate of cephalosporin hydrolysis, has a sole representative which was the only example of *Legionella* spp. examined (*L. gormanii*), although other *Legionella* spp. probably carry similar enzymes [153]. As carbapenems are not drugs used for the treatment of infections caused by *Legionella* spp., these enzymes are of largely academic interest.

Group 3b contains β -lactamases where the preferred substrate is carbapenems and there is much less activity against penicillins and virtually none against cephalosporins. Therefore, Bush has termed these enzymes 'true carbapenemases'. It seems that a range of enzymes can be produced by isolates and there is a need to clarify the taxonomy of these enzymes when more molecular information becomes available. At the moment, all of the examples are found in various species of *Aeromonas*. The most widely studied species has been *A. hydrophila* in which the CphA enzyme, also known as the A2 enzyme, has been looked at by several groups [154–156]. With a DNA probe derived from the *cphA* gene, it has been shown to be present in some other closely related species of *Aeromonas*, but only 83% of probe-positive strains express β -lactamase activity [157]. Indeed, the expression of these carbapenemases appears to be controlled by a range of mechanisms. An elegant study has demonstrated co-inducibility of the ImiS carbapenemase with two other chromosomal β -lactamases, indicating co-ordinate control of the genes [158]. It is also clear that the techniques used to identify carbapenemases are critical. Hayes and colleagues identified the enzyme ASA-I in *A. salmonicida* although this enzyme is not detected by nitrocefin as it does not hydrolyse nitrocefin as a substrate [159]. The same group has examined *A. hydrophila* by the same techniques (overlaying gels with imipenem and a pH indicator) and have found a carbapenemase in *A.*

Table 6. Functional subgroups of metallo- β -lactamases*

• Group 3a
Imipenem hydrolysis < penicillins
Cephalosporins < penicillin
• Group 3b
Imipenem hydrolysis > penicillin
Very low cephalosporin hydrolysis
True 'carbapenemases'
• Group 3c
Very high cephalosporin hydrolysis

*According to K. Bush (personal communication).

Table 7. Metallo- β -lactamases – examples of strains expressing different subtypes*

Original host	Enzyme	Relative hydrolysis of				
		IMP	MER	PEN	LOR	ATM
Group 3a						
<i>Bac. cereus</i>	II	100	1200	380	16	< 0.1
<i>B. fragilis</i> QMCN3	CcrA3	100	ND	130	99	ND
<i>B. fragilis</i> QMCN4	CcrA4	100	ND	69	26	ND
<i>B. fragilis</i> TAL3636	CcrA	100	56	95	21	< 0.01
<i>P. aeruginosa</i> GN17203		100	23	430	60	< 0.6
<i>Bur. cepacia</i> 5QIV	PCM-1	100	18	7.7	24	< 0.6
<i>Ser. marcescens</i> TN9106	IMP-1	100	14	440	62	< 2
<i>St. maltophilia</i> ULA-511c	L1	100	60	1700	43	< 0.01
Group 3b						
<i>A. hydrophila</i> AE036	CphA	100	38	2.1	0.07	< 0.01
<i>A. hydrophila</i> AER19	A2h	100	ND	250	ND	4.7
<i>A. hydrophila</i> 872		100	ND	1.1	14	ND
<i>A. salmonicida</i> subsp. <i>achromogenes</i> ASA111	ASA-1	100	ND	< 1	< 1	< 1
<i>A. sobria</i> 163a	ImiS	100	6900	0.33	ND	ND
<i>A. sobria</i> 14M	AsbM1	100	310	1.8	< 0.06	< 0.01
Group 3c						
<i>L. gormanii</i> ATCC 33297		100	ND	140	1900	< 20

IMP, imipenem; MER, meropenem; PEN, penicillin; LOR, lorfloxacin; ATM, azthreomycin.

*According to K. Bush (personal communication).

hydrophila that also does not hydrolyse nitrocefin [160]. *Aeromonas* spp. may act as an environmental reservoir for carbapenemases to spread to *A. hydrophila* and other gram-negative pathogens under the selective pressures of carbapenem use. The use of carbapenem monotherapy in immunocompromised patients – the largest group of patients suffering these infections – needs very careful microbiological monitoring.

The 3a group of enzymes are a very diverse group both functionally and probably genetically, but are characterised by marked penicillinase activity with a lower cephalosporinase activity. The enzyme from *Bac. cereus* has been described already. The L1 enzyme from *St. maltophilia* has been the subject of some interest and is probably found in almost all isolates of this species, although variants with very low-level expression have been described [161]. This is a chromosomal enzyme and no evidence of mobility has emerged. The nucleotide sequence has been determined and it is most closely related to other metallo- β -lactamases, but only at a very low level. It appears to be a tetrameric protein with a pI of 6.8 and is often expressed along with other β -lactamases [162]. By using a clever technique of pre-incubating with EDTA before isoelectric focusing and then overlaying the gels with either zinc salts or BRL 42715, a potent inhibitor of serine β -lactamases, seven metallo- β -lactamases were identified by Payne *et al.* [163], including an enzyme with the same pI as L1 (6.8). The function and clinical significance of these enzymes is not yet clear, but requires much more investigation. An apparently species-specific and inducible chromosomal carbapenemase, designated PCM-1, has been reported from nine isolates of *Burkholderia cepacia* [164]. The clinical significance and distribution of this enzyme awaits further study.

Bacteroides spp., particularly *B. fragilis*, have long been recognised as producing β -lactamases, with the first carbapenemase being described in 1983 [165]. Ten metallo- β -lactamases have been reported from *Bacteroides* spp. [150]; however, a number of these enzymes may be identical. Bush has chosen three examples for her functional classification (Table 6). DNA sequencing has shown that *ccrA* and *cfiA* are identical [143, 166]. The *cfiA*-type gene has been identified in isolates of *Bacteroides* spp. from three different countries [150] and is probably the most common carbapenemase in the genus. Recently, it has become clear that the genes may, at times, be silent but can be expressed when a copy of IS1186 inserts upstream of the *cfiA* gene [167]. The level of imipenem resistance in *B. fragilis* has been shown to relate to the level of expression of the carbapenemase for the three enzymes shown in Table 2 [143]. There is evidence of transferability of carbapenemase genes in *B. fragilis* [168] and a survey in Japan, where carbapenem usage is high, showed a rise from 2.0% to 5.9% in carbapenemase-producing strains over a 4-year period [168]. This has clear implications for the use of carbapenems to treat anaerobic infections.

One of the most sinister developments in the story of carbapenem resistance has been the appearance in Japan of plasmid-mediated carbapenemases, initially in *P. aeruginosa* followed by a different gene appearing in *Ser. marcescens* with subsequent spread to *P. aeruginosa*. The first report of a plasmid-mediated carbapenemase (pI 9.0) was in a single isolate of *P. aeruginosa* (strain GN17203) on a 31-MDa plasmid which transferred only to *P. aeruginosa* and not to *E. coli* [169]. An isolate of *Ser. marcescens* (strain TN 9106) derived from a patient with a urinary tract infection in Okasaki in Japan in 1991 was found to produce a different β -lactamase which was named

IMP-1 [170]. This enzyme has considerable homology with the *B. fragilis* TAL 3636 enzyme and *Bac. cereus* II. A recently published analysis of *P. aeruginosa* isolates in Japan between 1988 and 1992 reports that a plasmid-mediated β -lactamase with the characteristics of IMP-1 has been identified by another group [171]. Ominously, this gene became disseminated amongst isolates of *Ser. marcescens* in Japan and is encoded on a large plasmid (120 kb) and can be transferred to *E. coli* at a frequency of 10^{-5} [172]. The situation deteriorated further with the report that *bla*_{IMP} is now disseminated amongst plasmids found in *P. aeruginosa* [173]. The molecular reason for this dissemination has become clear with the discovery that the *bla*_{IMP} gene is part of an integron [174]. Worse news still is that it has now been found in a strain of *K. pneumoniae* and isolates of *Serratia* and *Pseudomonas* spp. from throughout Japan [174]; spread to other countries seems inevitable.

In conclusion, carbapenems are extremely useful potent broad-spectrum β -lactam antibiotics with a number of indications for use in hospital practice. However, the very ease with which they can be used may be their downfall. Initially, it was thought that there would be very few resistance mechanisms in clinically significant bacteria. When the agents were used clinically, a wide range of genes capable of protecting bacteria from carbapenems have been selected in true Darwinian style. With the appearance of the *bla*_{IMP} gene we may be looking at the most effective retaliatory strike by bacteria against the ingenuity of the human mind that has produced such a useful class of antimicrobial agents as the carbapenems.

Resistance mediated by inhibitor-resistant and extended-spectrum TEM and SHV β -lactamases

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The TEM-1 β -lactamase is a Class A β -lactamase, with a serine residue as the active constituent in the catalysis that hydrolyses, and thus inactivates, penicillin. This serine residue lies at the back of the active site groove and is usually numbered 70 to fall in line with the conventional Class A β -lactamase numbering system [175]. Since it was first found in a plasmid from *E. coli* some 30 years ago, TEM-1 has become the most prevalent resistance gene in the world; it does not matter whether the survey is conducted in South Africa [176], India [177] or the UK [178]. No more than 10% of all the plasmid β -lactamases comprise the closely related TEM-2 β -lactamase [177, 178], which has identical biochemical properties and differs only by a substitution of lysine for glutamine at amino acid 39. Similarly, no more than 10% comprise the SHV-1 β -lactamase, which is also biochemically identical and

which, although also a Class A β -lactamase, differs in structure from TEM-1 by approximately 35%.

The reason why the TEM-1 β -lactamase is so widespread is not clear. It must be the β -lactamase molecule that can best bind and hydrolyse the most common β -lactams in clinical use, the aminopenicillins. Conventional assay techniques cannot distinguish between the hydrolysis of ampicillin by TEM-1, TEM-2 and SHV-1, but the TEM-1 enzyme may have a slight advantage. In the clinical situation, this would be translated into dominance of the gene. It is also true that many TEM-1 β -lactamase genes are located on the highly successful class II transposon Tn3, and this has promoted its spread. Both TEM-2 and SHV-1 β -lactamase genes have been found on efficient transposons, but our capability to distinguish differences in transposition rate is even less discriminatory than our ability to detect changes in biochemical efficiency, and genes are not distributed nearly so widely.

The TEM-1 β -lactamase is probably not derived from a single gene, but from different gene families that differ from one another by nucleotide differences that give silent mutations which are not shown up by amino-acid changes [179]. This conclusion has been reached from studies of its mutants as there have not been enough studies that have sequenced sufficient TEM-1 β -lactamase genes to demonstrate this directly. Certainly, it would be expected that a gene as widely disseminated as the TEM-1 β -lactamase gene would acquire some silent mutations, if not some amino-acid substitutions, that do not affect activity. Perhaps significantly, the *bla*_{TEM-1} gene has been transposed into *H. influenzae* and analysis of its sequence has shown that, in Scotland at least, the gene has mutated and two amino-acid substitutions have occurred sufficiently far from the active site to have no apparent effect on catalysis. These mutations may simply have been the price paid for carriage of the gene in *H. influenzae* [66].

The wide dissemination of the TEM-1 β -lactamase has dominated the development of β -lactam drugs, particularly the later-generation cephalosporins which were confidently considered to be totally incapable of being bound to the active β -lactamase site. The shape of the active site allows ampicillin and amoxycillin to bind easily and tightly; this is facilitated by a narrowing at the entrance by the presence of a small α -helix (α -7) [180, 181]. This α -helix is maintained by the two ionic bonds from the basic arginine residue at position 164 with the acidic residues glutamate-171 and aspartate-179. In 1982, ceftazidime was used extensively for the treatment of nosocomial infections, both for infections caused by *P. aeruginosa* (which it penetrates readily) and by the Enterobacteriaceae (which it penetrates far less readily). Therefore, when there was an outbreak of *K. oxytoca* in a neonatal unit

in Liverpool in 1982, ceftazidime was considered to be a viable treatment option. During treatment, the causative bacteria became ceftazidime-resistant and this resistance was transferable [182]. There had been a substitution of serine for arginine at position 164 [183]. This removed the ionic bonds so that the α -7 helix no longer hindered entry to the active site [180], thereby allowing the larger molecule of ceftazidime to bind and permitting limited hydrolysis. Fast penetrating cephalosporins, such as cefotaxime, could also now bind and were hydrolysed even more efficiently than ceftazidime, but the faster penetration of cefotaxime into the periplasmic space ensured that its improved binding to the active site was not translated into increased resistance [184, 185].

Perhaps the most significant feature was the fact that this mutation occurred in a *Klebsiella* sp. Examination of many TEM-based ESBLs suggest that this is the pivotal mutation. Its modest ability to raise the MIC of ceftazidime but not the MIC of cefotaxime, guarantees that most diagnostic laboratories fail to detect it; indeed, the presence of bacteria containing this mutation is likely to be demonstrated only by the double-disk synergy test [186] or a special E-test. It is the subsequent set of mutations that are so devastating. Usually these also occur in *Klebsiella* spp., most often in *K. pneumoniae*. Why this species should be so prominent in the emergence of ESBLs is unclear; perhaps the preferential codon usage in this species favours the mutations required, or it may simply be that *Klebsiella* spp. have an innate, albeit limited, insusceptibility to β -lactams and this may provide the 'breathing space' for these mutations to occur. In fact, remarkably few subsequent mutations occur, and they substitute amino acids at the side of the active site groove. This may improve the binding of ceftazidime, as a substitution of the basic lysine residue for the acidic glutamic acid residue at position 104 [187] or alterations of amino acids 237–240 may change the relative orientation of the β -3 pleated sheet on the opposite side of the active site so that it increases the size of the groove and further promotes the binding of the larger cephalosporins [180, 185]. Almost all studies that have examined these mutations have worked with cloned β -lactamase genes, but the hyper-production of the gene product in itself alters the phenotype. Therefore, to conclude what each of the mutations actually does, strains with levels of expression found in clinical bacteria should be

examined to demonstrate the possible effect of subsequent mutations. The mutations at positions 104 and 240 increase ceftazidime resistance; they also increase the hydrolysis of cefotaxime, but usually insufficiently to provide clinical resistance. Clinical resistance to cefotaxime is manifest by mutations at positions 237 and 238; the former has been found only in combination with other mutations, so it is not known whether this could be an effective cefotaxime resistance mechanism in its own right. In contrast, the substitution at position 238 is found on its own, but appears quite rare (Table 8).

The SHV ESBLs were first found in 1982 [187]. SHV-1 had undergone a single mutation that substituted serine for glycine at position 238 to form SHV-2. This is the pivotal mutation with this β -lactamase and the prevalence of SHV-2 [186] suggests that, in this enzyme, this substitution is not detrimental. The first stage in the development of extended-spectrum activity of the SHV-1 β -lactamase is the substitution of serine for glycine at this position [188]. Indeed, most of the SHV ESBLs possess this substitution; it appears essential for cefotaxime hydrolysis and is stable [189]. A more prevalent SHV-derivative appears to be SHV-5; this enzyme has an additional mutation that substitutes lysine for glutamic acid at position 240; this increases hydrolysis to ceftazidime considerably with the TEM molecule [189]. Enzymological data suggest that this is not so obvious in SHV, but epidemiological evidence suggests that the SHV-5 enzyme is much more prevalent than SHV-2 [190, 191], so either the enzymological data are not accurate enough or the enzyme may have a hitherto unrecognised activity; it may be better at conferring resistance to the penicillins than SHV-2. An interesting variation of this double mutation is SHV-7, which has a phenylalanine substitution for isoleucine at position 8, and serine for arginine at position 43 [192]. It is not clear what additional benefit these two extra substitutions confer over SHV-5, particularly as they are distant from the active site. Interestingly, unlike most other ESBLs, SHV-7 was found in *E. coli* not *Klebsiella* spp., and these mutations may promote the survival of the β -lactamase in this species. SHV can also undergo an arginine-to-leucine substitution at position 205, and this change appears to work *in concert* with the mutations at positions 238 and 240, with further increases in cefotaxime and ceftazidime resistance (Fig. 2).

Table 8. Effect of each mutation on the resistance function in the TEM β -lactamase

Position	Amino-acid change	Change in resistance function	Previous mutations
39	Glutamine to lysine	None	
104	Glutamic acid to lysine	High ceftazidime	
164	Arginine to serine	Low ceftazidime	After mutation at 164
238	Glycine to serine	High cefotaxime	
237	Alanine to threonine	High cefotaxime	After mutation at 240
240	Glutamic acid to lysine	High ceftazidime	After mutation at 164
265	Threonine to methionine	Little detectable change

Fig. 2. Inter-relationships of the SHV-derived β -lactamases. Each arrow indicates a single amino-acid change caused by a single nucleotide change in the β -lactamase gene. Numbering of the amino-acid residues is according to the recommendations of Ambler *et al.* [175]: E, glutamic acid; G, glycine; K, lysine; L, leucine; R, arginine; S, serine.

Du Bois, Marriott and Amyes [193] first demonstrated that cephalosporins may not be the only selection force for ESBLs and that co-amoxiclav could select for the reversal of some of the ESBL substitutions. As extended spectrum activity is achieved by progressive substitution around the active site, there is a concomitant decrease in the capability to hydrolyse ampicillin and amoxycillin, and an increasing susceptibility to inhibition by clavulanic acid [194]. TEM-5-producing bacteria challenged with co-amoxiclav undergo reverse mutations, first to TEM-10, which is more capable of binding amoxycillin and less able to bind clavulanic acid than TEM-5; thus the selective environment of both components of co-amoxiclav favours the emergence of TEM-10. Similar challenge of TEM-10 results in the emergence of TEM-12, and then TEM-1, for the same reasons [193].

In 1992, isolates of *E. coli* with reduced susceptibility to co-amoxiclav appeared first in Scotland [195] and then in France [196]. The TEM-1 β -lactamase had mutated again – under clinical conditions – and, as with the ESBLs, the mutations concentrated around a few substitutions. The mutant enzyme bound inhibitors less readily than the parent molecule and, surprisingly, this effect was less pronounced with the sulfone inhibitors, such as sulbactam and tazobactam, than with clavulanic acid. [195]. At least nine different inhibitor-resistant TEM β -lactamases have now been described [179], and there are probably more [65]. The most common alteration is the substitution of methionine at position 69, the amino acid adjacent to the active serine-70. This is usually substituted by an aliphatic residue, which can be isoleucine, leucine or valine. So it is the loss of methionine that is important, and this may affect the orientation of small inhibitors in the active site, making their binding less

secure [179]. Substitution of arginine-244 on the β -4 sheet is also important, but has been found in only two mutants (TEM-31 and TEM-32). The loss of the basic arginine residue at position 244 removes an ionic bond to the inhibitor and interferes with the inhibition process [179]. A more common mutation is the substitution of aspartate for asparagine at position 276 (found in five inhibitor-resistant TEM-type enzymes). As this mutation is never found alone, its contribution cannot be evaluated, but it is thought to alter an ionic bond with the inhibitor. Similarly, the role of the mutation at position 275 (arginine for leucine) is unknown. One of the most interesting mutations is the substitution of tryptophan-165 for arginine in the IRT-10 enzyme. Although these mutations do not confer high levels of resistance, they appear to give the strain a selective advantage in a co-amoxiclav environment, and this is supported by a recent study in a French hospital in which 8% of all urinary *E. coli* isolates were shown to possess these enzymes [197].

Are all these strains inter-related? In the inhibitor-resistant TEM-type genes found so far, there appears to be some variation, particularly in nucleotides 226, 346, 436 and 672. These cause silent mutations which, although they do not affect the amino-acid structure, reveal evolutionary diversity – the resultant enzymes are subgroups of TEM-1 (i.e., TEM-1a, TEM-1b) and TEM-2. Although the inhibitor-resistant TEM enzymes are derived from TEM-1-like enzymes, they are not all derived from the same gene. Indeed, there is evidence to suggest that different parent genes have undergone convergent evolution. Even in a single hospital, the diversity of inhibitor-resistant TEM enzymes cannot be explained by epidemic spread, which is often the case for the ESBLs. Instead, the inhibitor-resistant

TEM enzymes appear to have evolved by independent mutation under local antibiotic selection pressure [179].

Fluoroquinolone resistance: mechanisms and epidemiology

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Introduction

Since the introduction of nalidixic acid in the early 1960s, a further 10 or so quinolones or fluoroquinolones have been licensed. The early agents, such as nalidixic acid and oxolinic acid, were exclusively urinary tract antibacterial agents. Later compounds, such as ciprofloxacin and ofloxacin, have a much broader clinical utility in that they can also be used to treat respiratory, blood-borne, skin and soft tissue infections, and some of these compounds are available in both oral and parenteral formulations. As a result of this better profile, the usage of fluoroquinolones now constitutes c. 11% of global antibiotic prescriptions [198]. For comparison, amoxycillin and co-amoxycrav represent over 35% of the world's prescriptions [198]. Clearly, the use of fluoroquinolones is likely to increase as bacterial resistance to the β -lactam agents, macrolides and other traditional 'first-line' agents increases. It is the purpose of this brief review to outline how the quinolones work, how bacteria have developed mechanisms of resistance to these agents, and to report on recent epidemiological surveys of fluoroquinolone resistance.

Primarily, fluoroquinolones act by interfering with the DNA replication of the bacterial cell. This is achieved by blocking the activity of specific enzymes known as topoisomerases. These enzymes are essential to the bacterial cell as they catalyse the complex process of unwinding the DNA within the cell, thereby allowing transcription to take place, and then enable re-supercoiling of the two new helices, as recognised by Cairns in 1963 [199]. Subsequently, the topoisomerases have been categorised into two groups: type I topoisomerases, topoisomerases I and III; and type II topoisomerases, DNA gyrase (topoisomerase II) and topoisomerase IV. These two types of topoisomerase differ in their mode of action: type II enzymes cleave both strands of the double helix, while type I enzymes transiently break one strand of the double helix and pass through another single strand. In 1977, Gellert [200] and Sugino *et al.* [201] reported that quinolones act on topoisomerase II, also known as DNA gyrase. This enzyme is composed of two A subunits (GyrA) and two B subunits (GyrB). GyrA mediates DNA strand breakage and reunion with the tyrosine residue

at position 122 (Tyr-122), forming a transient phosphotyrosine linkage with a broken DNA strand. GyrB contains the ATP-binding site at the N-terminal end of the protein. The *gyrA* and *gyrB* genes from a number of bacterial species have been cloned and sequenced. The *gyrB* and *gyrA* genes of *Bac. subtilis*, *S. aureus* and *Mycoplasma pneumoniae* are contiguous, unlike those of *E. coli*. A more complete review of the roles of the topoisomerases in bacterial DNA replication has been published previously [202].

In addition to their direct action on DNA gyrase, the quinolones exert other effects on viable bacteria. These effects include: inhibition of DNA synthesis by decrease in negative supertwists, reduction of decatenation of interlocked DNA circles, induction of SOS DNA repair systems and some heat shock proteins; antagonism of RNA and protein synthesis; cellular filamentation; rapid cell death.

An interesting finding has shown that alterations of the cell peptidoglycan composition occur following cell lysis by a quinolone [203]. It has been suggested that the final stages of cell death may involve newly formed murein hydrolases or autolysins. These enzymes may be involved in cell death caused by β -lactam agents [204]; indeed, the *hipQ* mutant showed reduced killing by both quinolones and β -lactam agents [205]. To some extent, this may explain why inhibitors of RNA and protein synthesis reduce quinolone killing by virtue of the need for autolysins for quinolone-induced cell lysis.

Development of resistance to the quinolones

As always in our battle with the bacterial adversary, various means have evolved that ensure resistance to new classes of antibacterial agents. The quinolones are no different and, to date, there are two main ways in which resistance occurs. Alteration of the drug's main target site, DNA gyrase, is very common. Alternatively, a change may occur in the drug's opportunity to interact with bacterial gyrase because of reduced intracellular penetration or increasing efflux of the drug from the cell. To date, no 'quinolonase' has been reported; thus, the enzymic hydrolysis seen with other antimicrobial classes is not a problem. This may be related, in part, to the fact that quinolones are totally synthetic agents.

Each of these mechanisms seems to result from a chromosomal mutation and not from a plasmid or other genetic acquisition. To date, there have been several reports of alleged plasmid-mediated quinolone resistance in clinical isolates [206, 207]; however, none of these has been confirmed. The fact that quinolones inhibit plasmid conjugation and eliminate plasmids from host cells, combined with the dominance of susceptible wild-type *gyr*⁺ genes over resistant mutant *gyr* alleles, may mean that such

plasmid-mediated resistance would be difficult to initiate and sustain [208]. There have been some instances of laboratory engineered plasmids, but their viability has been poor [209].

Alteration of DNA gyrase (topoisomerase II) may occur by changes in either of the two subunits, i.e., GyrA or B. Mutation of only GyrA can lead to quinolone resistance; an observation made in a wide range of bacterial species. Alteration of GyrA has been shown to increase the ID₅₀ (concentration of quinolone inhibiting DNA synthesis by 50%) 2–500-fold in *E. coli*, *K. pneumoniae*, *Ent. cloacae*, *S. aureus* and *P. aeruginosa*. Genetic analysis has shown that single-point mutations in the structural genes of the A and B subunits lead to alterations in the gyrase and the concentration of quinolone required to inhibit growth (detected by an upward shift in the MIC) [202]. These alterations tend to be limited to the small region of the gene encoding amino acids 67–106 (subunit A) or 426–447 (subunit B). There are > 20 distinct *gyrA* mutations reported in *E. coli* alone; other point mutations have been seen in other gram-negative and gram-positive species [210]. These changes give rise to higher levels of resistance than other mechanisms. The most potent substitutions tend to be at codons 81 and 83; e.g., Leu or Trp for Ser-83 increases the ciprofloxacin MIC for *E. coli* from 0.0125 to 0.25 or 0.5 mg/L [211].

Changes in the B subunit were observed by Yamagishi *et al.* [212], who reported that *nalC* was a *gyrB* change at position 447, and that *nalD* conferred a shift at position 426. These two regions of the GyrB subunit are thought to be the ATP-binding site. The three-dimensional structure of DNA gyrase is still required in order to better understand the significance of the *gyrB* changes.

As with all antibiotics, quinolones must penetrate in order to exert their action. Many of the quinolone-resistant mutants have a decreased uptake mechanism; however, the hydrophobicity of each quinolone plays a role in this transportation [210]. Hydrophilic quinolones seem to traverse the outer membrane of gram-negative bacilli through aqueous-filled porin protein channels that allow direct access between the periplasmic space and the outside of the cell [210]. These proteins are expressed by the bacteria in response to environmental signals such as the osmolarity of the medium and the temperature. In the case of hydrophobic antibacterials, the outer membrane has been shown to be a barrier to drug diffusion. This property has been linked to the lipopolysaccharide (LPS) on the external surface of the outer membrane which may block drug access. It has been proposed that the more hydrophobic quinolones may promote their own diffusion by virtue of chelating magnesium which is essential for LPS stabilisation in the outer membrane [213].

The most frequent mutation associated with porin change is linked to OmpF. Other mutations have been described; thus *nfxB*, *norB*, *cfxB* and *marA* are also associated with decreased cell penetration by quinolones. All of these mutations yield a similar resistance pattern; i.e., a slight increase in MIC (2–32-fold), which depends on each compound's hydrophobicity. In many cases, there is cross-resistance with unrelated antibiotics, such as trimethoprim, tetracycline and chloramphenicol [214].

The process of movement of a drug from within the cell to the periplasm or cell exterior is known as efflux. It is an energy-dependent process located in the inner membrane. The increased efflux of quinolones is another mechanism associated with quinolone resistance. In such mutants, Ohshita *et al.* [215] have demonstrated that a single point mutation at Asp-27 (for Ala) led to increased efflux. Subsequently, Kaatz and Seo [216] showed that hyper-expression of *norA* may be the real cause of resistance. This mutation led to a 50% reduction in quinolone uptake [209]. Again, the overall level of resistance conferred by elevated efflux is less than that seen with gyrase changes. However, as *norA* is dominant over susceptible wild-type alleles, it may be feasible for this mutation to become plasmid mediated. In the laboratory setting, the *norA* gene of *S. aureus* has been expressed in *E. coli* [217], thereby suggesting that this transfer is possible.

It is important to realise that quinolone resistance implies cross-resistance to all quinolones, i.e., the MIC of individual quinolones will increase, but to varying degrees; the significance of this will be discussed later. In summary, gyrase changes cause greater MIC changes than other mutations.

How much impact have these resistance mechanisms had in almost 10 years of quinolone usage? It is acknowledged that the fluoroquinolones are highly active against members of the Enterobacteriaceae and respiratory gram-negative organisms such as *Haemophilus*, *Moraxella* and *Neisseria* spp. Of the quinolone class, only ciprofloxacin has good predictable activity against *P. aeruginosa*. In gram-positive terms, methicillin-susceptible *S. aureus* are fairly sensitive to quinolones, with *Str. pneumoniae* showing an intermediate level of susceptibility. Not surprisingly, with most gram-positive species and *P. aeruginosa*, the distribution of MICs tends to straddle the 'intermediate' range of values, i.e., ciprofloxacin MICs of 1.0–4.0 mg/L. Clearly, with appropriate dosing of the antibiotic for the appropriate site of infection, these infecting isolates would be eradicated. It is the use (or misuse) of low dose, infrequent or short course therapy for systemic infections that has contributed to the emergence of resistant strains. The reasons for such misuse may be false economy, ignorance of the clinical situation, drug pharmacokinetics or (increas-

ingly commonly) the use of illicit ('pirate') versions of quinolones in poorly pharmaceutically regulated areas.

It is essential that data are collected from the same centres with a similar technique over time. Table 9 shows the percentage of ciprofloxacin-susceptible strains for a range of (mainly) gram-negative pathogens isolated in three countries.

The USA is the largest consumer of antimicrobial agents, accounting for some 30% of the world's monitored consumption [198]. Virtually all of this use is through legitimate physician prescriptions; there is little or no 'black market' use of pharmaceutical agents in this country. The data shown in Table 9 [218–221] were collated from 150 hospitals and laboratories distributed throughout the contiguous states. The methods of testing used NCCLS criteria. The number of isolates examined ranged from < 10 for *Edward-siella tarda* (data not given) to > 14 000 for *E. coli*. In general terms, there have been few significant changes over the period 1992–1994. The most cause for concern occurs with *P. aeruginosa*, particularly as ciprofloxacin has provided the only oral therapeutic regimen for this problem pathogen. Conversely, of particular reassurance is the general susceptibility of the Enterobacteriaceae, while the respiratory pathogens *H. influenzae* and *M. catarrhalis* also remain highly susceptible. Interestingly, *Str. pneumoniae* has remained relatively susceptible to ciprofloxacin (1991, 92.5%; 1992, 92.6%; 1993, 92.5%; 1994, 89.9% [220, 221]), despite burgeoning penicillin resistance among pneumococci in the USA (23.6% of *Str. pneumoniae* isolates are insusceptible to penicillin [222]). Another change causing concern has been the continued emergence of quinolone-resistant, methicillin-resistant staphylococci. Generally, these organisms are isolated in outbreak situations in closed institutions such as nursing homes [223, 224]. Monotherapy with the currently available quinolones should not form the

therapeutic or prophylactic regimen for MRSA. Another gram-positive group giving cause for concern are the enterococci [225]; however, with the exception of urinary infections and a few specific systemic infections, quinolones should not be considered as first-line agents for the treatment of enterococcal infections.

Ciprofloxacin susceptibility testing has been undertaken and data collated at St Paul's Hospital, Saskatoon, Canada, since 1992 (J. Blondeau, personal communication). Additionally, susceptibilities for over 4500 isolates from 15 centres in Canada have been recorded [219]. Enterobacteriaceae appear to be fairly stable in their ciprofloxacin susceptibility; only *Ser. marcescens* seems to be diminishing in susceptibility (Table 9). The most prevalent species—*E. coli*, klebsiellae and *Morganella* spp.—are still highly susceptible, with 97% of Enterobacteraceae being susceptible. The degree of susceptibility among *P. aeruginosa* strains varied among the various centres; for four institutions, the rates ranged from 28% to 40%.

The UK data presented in Table 9 show the ciprofloxacin susceptibility of isolates tested at 12 laboratories over an 8-year period. The isolates were from both community and hospital sources and were tested by either the disk or breakpoint (abbreviated MIC) techniques. We have previously tested the validity of these data [226]. In overall terms, the strength of these data lies in the consistency of the sites involved and the methodologies employed. Table 9 shows the levels of ciprofloxacin susceptibility observed at the time of the introduction of ciprofloxacin (1987) and the most recently collated information from the participating centres. The species showing the greatest changes are *P. aeruginosa*, *S. aureus* and *Ser. marcescens*, an observation substantiated by other studies [227–229].

Over the period 1991–1994, Frost *et al.* [230] noted that resistance to ciprofloxacin among *Salmonella*

Table 9. Longitudinal comparison of ciprofloxacin susceptibility data

Species	USA*		Percentage of strains susceptible to ciprofloxacin				Europe [§]	
	1992	1994	Canada [†]	UK [‡]	1995	1987	1995	1990
<i>E. coli</i>	99.4	99.3	100	100	99	99.8	99.8	98.7
<i>Klebsiella</i> spp.	95.4	95.5	96	100	96	99.7	99.1	94.4
<i>Enterobacter</i> spp.	93.9	95.1	100	100	97	99.8	97.9	98.1
<i>Ser. marcescens</i>	83.3	87.4	100	98	92	99.0	91.8	83.1
<i>Morganella morganii</i>	97.0	93.1	100	100	97	99.7	99.2	98.0
<i>Proteus</i> spp.	97.6	95.0	—	—	98	99.7	99.5	98.6
<i>P. aeruginosa</i>	81.8	79.0	98	89	79	98.6	93.1	84.1
<i>H. influenzae</i>	99.8	99.8	100	100	99	100	99.5	—
<i>M. catarrhalis</i>	100	100	—	—	99	100	100	—
<i>S. aureus</i>	—	—	99	93	96	99.8	91.8	87.6
<i>Str. pneumoniae</i>	92.6	89.9	—	—	—	—	—	—

— No data available.

*Thornsberry [218]; B. Painter, personal communication.

[†]Blondeau *et al.* [219].

[‡]Tillotson and Herbert [220]; unpublished results.

[§]Kresken *et al.* [221].

serotypes had increased from 0.3% to 2.1%; in particular, serotype Hadar exhibited 39.6% resistance. Fortunately, this serotype represents < 1% of salmonellae isolated. The most prevalent serotype, Enteritidis (> 50% of isolates) demonstrated only 0.4% resistance to ciprofloxacin. In the commonly occurring serotypes, there was a marked variation in the relationship between ciprofloxacin resistance and foreign travel, with serotypes from India and Turkey showing the highest resistance rates.

The distribution of ciprofloxacin susceptibility in Europe mirrors earlier antibiotic susceptibility surveys; i.e., quinolone resistance was higher in Southern Europe than in Northern or Central Europe (Table 10) [221]. *P. aeruginosa* is the most striking example of this difference, with resistance levels of 43.3% in Italy and 19% in Greece, whilst in all other countries the incidence was < 10%. The authors of this multicentre survey concluded that different use of antibiotics clearly affected the degrees of resistance development. This may be associated, in part, with which quinolones were used most extensively the soonest (e.g., France with pefloxacin, while ciprofloxacin and ofloxacin were both used more frequently and earlier in Germany). This ambitious project should now be repeated as the data presented were collated in 1990.

Finally, in geographical terms, the largest population centres are in the developing nations, where there are regions in which quinolone resistance is emerging among normally very sensitive strains (e.g., *Salmonella* Typhi in India [231]). Ciprofloxacin resistance has been seen in *K. pneumoniae* isolates from several South American nations, including Chile (9–18%), Mexico (21%) and Peru (28%) [223]. However, enteric bacteria isolated in Tanzania showed no quinolone resistance [232]; similar patterns have been seen in other Central African countries (C. F. Gilks, personal communication). One can only assume that these differences are related to the relative ease with which quinolone antibiotics can be obtained. Thus in India and some Central and South American countries, there are a multiplicity of pharmaceutical groups producing a variety of quinolones at a range of prices, while in

Africa and some other Asian countries, there is not the wholesale availability of quinolones at affordable prices.

Most of the difficulties observed with quinolone resistance have been seen with gram-positive species, including staphylococci, some streptococci, enterococci and corynebacteria. As mentioned previously, MRSA has shown the greatest propensity to become quinolone-resistant. Often this is the result of infection control problems rather than widespread chromosomal mutations among individual isolates [233]. Gram-negative species that mutate more readily include *Pseudomonas* spp., *Campylobacter* spp. [234] and a few enteric species such as the salmonellae. However, for the most prevalent pathogens (i.e., *E. coli*, *H. influenzae*, etc.), quinolone susceptibility is still high.

Undoubtedly, quinolone resistance is emerging among some bacterial species, often for a variety of reasons, thereby stimulating the development of new quinolones. Such agents possess superior anti-gram-positive activity, improved (or prolonged) pharmacokinetic properties and, hopefully, a comparable safety profile. Table 11 shows the MICs of four new agents: CP 99,219 or trovafloxacin [235]; OPC 17116 or grepafloxacin [236]; Du6859a [235]; and the new class, 2-naphthypyridone, ABT-719 [237]. Only the latter shows significant, 'across-the-board' activity compared to ciprofloxacin. This improved activity against gram-positive and anaerobic species may be a result of action on topoisomerase IV as well as DNA gyrase [238]. Several of these agents are now in Phase II and III clinical development. The outcome of this research is awaited, with the hope that the toxicity seen with previous fluoroquinolones (e.g., temafloxacin and sparfloxacin) does not become an issue. Overall, the emergence of resistance has been as anticipated and most of this resistance has occurred among hospitalised patients, perhaps reflecting horizontal cross-infection of many patients by a single resistant clone. The use of appropriate dosages and routes of administration in suitable patients, in combination with surveillance programmes, should minimise these emerging problems until new, more potent quinolones, are licensed.

Table 10. Ciprofloxacin resistance in Europe by region [221]

Species	Percentage of resistant isolates		
	Northern	Central	Southern
<i>E. coli</i>	0.4	0.2	1.4
<i>Pr. mirabilis</i>	0.5	2.0	2.7
<i>Ent. cloacae</i>	2.5	0	2.3
<i>K. pneumoniae</i>	8.5	0.8	6.0
<i>P. aeruginosa</i>	6.9	6.4	30.6
<i>S. aureus</i>	5.6	6.2	23.6

Northern: Belgium, Netherlands, UK.
 Central: Austria, Germany, Switzerland.
 Southern: France, Greece, Italy.

Table 11. Comparison of antibacterial activity of ciprofloxacin with new compounds currently under investigation

Species	MIC90 values (mg/L)				
	Cip	Trova	Grepa	Du6859a	ABT-719
<i>E. coli</i>	< 0.12	0.5	0.12	0.06	0.008
<i>Klebsiella</i> spp.	0.25	0.5	0.12	0.12	0.03
<i>Enterobacter</i> spp.	0.25	0.12	0.25	0.06	0.03
<i>P. aeruginosa</i>	0.5	1.0	4.0	0.5	0.12
MSSA	1.0	0.03	0.12	0.03	0.015
MRSA	> 8.0	1.0	4.0	1.0	1.0
<i>En. faecalis</i>	2.0	0.25	4.0	0.25	0.12
<i>Str. pneumoniae</i>	2.0	0.12	0.5	0.06	0.03

Cip, ciprofloxacin; Trova, trovafloxacin; Grepa, grepafloxacin.

Who or what is the source of antibiotic resistance?

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Introduction

Development and spread of bacterial resistance is usually attributed to overuse or misuse of antibiotics. However, antibiotic resistance is not a single phenomenon, and although many resistance mechanisms have been identified and analysed for most clinical pathogens and almost all antibiotics for clinical use, the origin and sources of antibiotic resistance have remained neglected subjects of investigation. One of the factors responsible for our current limited knowledge of the origin(s) of antibiotic resistance might be the rather limited communication between microbiologists working with clinical isolates and those who deal with industrial, agricultural or environmental microbiology. In this review, identified sources of bacterial resistance and modes of spread are analysed, as well as potential future ways to overcome resistance.

Bacterial resistance in the environment

The pre-antibiotic era. Antibiotic resistance probably has different origins in nature and must be as ancient as antibiotic synthesis [239]. Resistance genes pre-existed in nature, in soil and water, and their presence was probably related to the production of antibacterial agents, synthesised naturally in the environment by saprophytic organisms such as actinomycetes. About 10^6 cfu of these organisms are found per g of dry soil and they constitute 10–50% of all soil microorganisms. Biologically active substances are synthesised by the actinomycetes, including *c.* 70% of the antibiotics in use today [240, 241]. Thus, self-protection of these organisms is essential. An example of this is the aminoglycoside-modifying enzymes present in aminoglycoside producers that are active against the auto-genous drugs (Table 12 summarises a few of the mechanisms of self-protection described in antibiotic-producing-organisms). Chromosomal resistance genes have been characterised and cloned for many of the examples shown in Table 12. Genes encoding aminoglycoside acetyltransferase enzymes of the AAC(3) type are found in actinomycetes such as the lindmycin-producer *Streptomyces lividus* [242].

Table 12. Mechanisms of self-protection in antibiotic-producing organisms

Organism	Antibiotic produced	Mechanism of resistance	Corresponding gene
Aminoglycoside-producers			
<i>Streptomyces fradiae</i>	Neomycin	APH(3')*, AAC(3)	<i>aph</i> , <i>aac</i> [†]
<i>Streptomyces rimosus</i>	Paromycin	APH(3'), AAC(3)	—
<i>Streptomyces lividus</i>	Lividomycin	APH(3'), AAC(3)	—
<i>Streptomyces kanamyceticus</i>	Kanamycin	AAC(6')	—
Macrolide–lincosamide-producers			
<i>Streptomyces erythraeus</i>	Erythromycin	MLS	<i>ermE</i> (constitutive)
		Ribosomal methylation (23 S)	
<i>Streptomyces fradiae</i>	Tylosin	MLS	<i>tlrA</i> (<i>ermSF</i>) (inducible)
<i>Streptomyces thermotolerans</i>	Carbomycin	Clone specific	<i>carB</i>
<i>Streptomyces lincolnensis</i>	Lincomycin	Ribosomal methylase activity	<i>clr</i> (constitutive)
Tetracycline producers			
<i>Streptomyces rimosus</i>	Tetracycline	Tetracycline efflux	<i>tetA</i>
<i>Streptomyces griseus</i>	Tetracycline	Tetracycline efflux	<i>tetA</i>
<i>Streptomyces aureofaciens</i>	Chlortetracycline	Tetracycline efflux	<i>tetB</i>
Novobiocin producer			
<i>Streptomyces sphaeroides</i>	Novobiocin	Altered DNA gyrase	<i>gyrB</i> (inducible)

This information is simplified from Cundliffe [240].

*Antibiotic-modifying enzymes: AAC, acetyltransferase; APH, phosphotransferase.

[†]Genes expressed best in *S. lividans*.

Plasmids in nature. Extrachromosomal resistance plasmids have been recovered from bacteria isolated and stored in the pre-antibiotic era [243] and organisms producing β -lactamases have been revived from plant specimens stored in the 17th century [244]. Plasmids are known to be the major vehicles for the natural transfer of resistance genes between bacteria [244, 245]. Plasmids are particularly frequent in actinomycetes; their sizes vary from 2 to 200 kb with variable numbers of copies (up to 100). Plasmids in *Streptomyces* spp. are often implicated in resistance to the antibiotics these organisms produce [241]. As a result, soil and the natural environment constitute a huge reservoir of antibiotic resistance genes, and various potential mechanisms of resistance existed long before clinical use of antibiotics commenced 50 years ago with the advent of penicillin.

Factors contributing to the development of resistance

The advent of antibiotics in medicine and the production and use of large quantities of antibiotics have undoubtedly contributed to the selection of bacterial clones possessing resistance genes.

'Non-clinical' use of antibiotics. This plays a significant role in the spread of resistance genes. Approximately half the antimicrobial agents produced in industrialised countries are used for agricultural purposes and food supplementation for chickens, turkeys, cattle and sheep [246, 247]. Antibiotics in animals are used: (i) to restrict the spread of *Salmonella* spp. and other agents between animals; (ii) to treat infections in animals and for prophylaxis of infections; and (iii) to improve animal growth (antibio-supplementation). The use of avoparcin (a glycopeptide) as a growth-promoter may have played a role in the emergence and spread of glycopeptide-resistance in enterococci [248]. The veterinary use of apramycin (an aminoglycoside) may have resulted in the development of acetyltransferase (AAC(3)IV) production in commensal strains [249]. Quinolone resistance in bacteria isolated from poultry is an increasing problem resulting from use of fluoroquinolones in veterinary medicine [250]. Resistance to quinolones (80%), tetracyclines (47%) and kanamycin (39%) has been observed in *Campylobacter* strains in Spain (F. Baquero, personal communication).

The normal intestinal flora in healthy animals. This constitutes a reservoir of resistance genes [251]. Micro-organisms may transfer their plasmids to pathogenic or non-pathogenic intestinal flora and then to the intestinal flora of other individuals [252]. As the risk of infection increases when large numbers of animals or poultry are concentrated together, large quantities of antibiotics are used for prevention of infection, with increased risk of resistance selection. Thus, antibiotic resistance in

animal enteric flora might be considered as a threat to human health *via* the food chain.

Contaminated food for animals. This has been implicated as a source of antibiotic-resistant bacteria. Even in the absence of selective pressure resulting from antibiotic use for growth promotion, animals carry large numbers of resistant strains coming from the environment and feed. Contaminated water (faecal contamination), irrigation of agricultural land by animal or human waste, and contaminated food for animals are unavoidable factors favouring plasmid carriage and transfer of resistance genes in the intestinal flora of animals [251].

Aquatic environments. These are contaminated directly by antibiotic-resistant bacteria excreted by animals or man, as well as *via* the practice of raw sewage discharge in waters. The assessment of water quality by identifying faecal contamination of water has identified the presence of antibiotic multiresistant *E. coli* in urban and rural drinking water sources [251, 253]. Gene transfer in the aquatic environment seems to play a significant role as a reservoir of clinical resistance genes, and it seems likely that gene transfer by transduction, transformation or conjugation may occur in water. In gram-negative bacilli such as *P. aeruginosa* and *E. coli*, conjugation is the main mechanism of transfer of R-plasmids in wastewater and sewage-contaminated samples [253].

Resistant bacteria in the human flora. The sources of resistant bacteria that can be transferred to man are multiple; transfer of antibiotic-resistant bacteria such as *E. coli*, *Campylobacter* spp. and enterococci can occur *via* the food chain or close contact with animals carrying resistant organisms [254]. There have been a few surveys in remote communities which have shown the natural presence of faecal plasmids coding for tetracycline and streptomycin resistance (e.g., in South Africa and in the Solomon Islands). Carriage of resistant enterobacteria in the gut of normal populations in the community has been observed [243, 251, 254]. In healthy individuals, carriage of resistant bacteria has no obvious effect, but may constitute a significant threat when infection occurs.

Development of resistance in man

The hospital environment. The development of resistance in the hospital environment is the result of multiple factors that can be summarised as follows:

1. Epidemic gram-negative bacilli are known to be inherently resistant to many antibiotics. They possess a tough outer membrane layer outside the cell wall that renders them impermeable to most antibiotic molecules so that they can be selected by antibiotic selective pressure and emerge as opportunist multiresistant pathogens. This is the case for

P. aeruginosa, *St. maltophilia* and *Acinetobacter* spp. [255]; because of the selective conditions in the hospital environment, spread occurs between patients, particularly in intensive care units (ICUs), and these organisms can be carried by the hands of personnel and contaminate respiratory devices.

2. Emergence of resistance in pathogens during antibiotic treatment can occur by means of chromosomal mutations occurring at variable rates (e.g., 10^{-4} /cfu in *Ent. cloacae* for derepression of cephalosporinase conferring resistance to third-generation cephalosporins [256]; 10^{-6} /cfu in *S. aureus* for fosfomycin when the drug is used in monotherapy; 10^{-7} /cfu in *P. aeruginosa* for fluoroquinolones used to treat cystic fibrosis patients). Transfer of these resistant mutants may result in epidemics of nosocomial resistant pathogens in ICUs.
3. In-vivo transfer of plasmids coding for multiple resistance may occur into species or genera previously susceptible. Thus spread of plasmid-borne ESBLs – first described in *K. pneumoniae* – to new bacterial hosts, and the subsequent epidemics of infection, have been described extensively in the medical literature [244].
4. Spread of resistant gram-positive organisms, such as MRSA and penicillin-resistant pneumococci, is an increasing problem. Such PBP-mediated resistance mechanisms can spread by dissemination of resistant organisms in the hospital environment or by means of gene transfer (horizontal spread) [245].

In the community. An increasing prevalence of resistance has been observed in *E. coli*, with ampicillin resistance reaching c. 40%, and spread of the TEM-1 β -lactamase gene to new hosts such as *H. influenzae* and *N. gonorrhoeae*. Approximately 20% of *H. influenzae* strains in the normal population are now ampicillin-resistant [254].

The relationship between resistance and antibiotic usage

Inappropriate use of antibiotics. In medical practice, drugs are often prescribed to treat either the wrong kind of infection or to treat unnecessary indications (e.g., in healthy individuals with a mild viral

respiratory infection that may respond spontaneously) [257]. Inappropriate doses of oral antibiotics may achieve serum and tissue concentrations that are lower than the MICs for the infecting pathogens. Sub-MIC concentrations exert a potent selective pressure for the emergence of resistant clones that pre-exist in the bacterial population. Together with the wrong dosage, a duration of treatment that is too short also contributes to clinical failure and to emergence of drug resistance.

Antibiotic consumption. An association between changes in prevalence of resistant organisms with time and the use of antimicrobial agents is difficult to establish. Some studies have shown that antibiotic usage has contributed directly to an increased prevalence of resistance. A 7-year survey of 7385 clinical isolates in a French hospital showed changes not only in the species found but also in their antibiotic susceptibilities as a function of increased consumption of third-generation cephalosporins from 1 kg to 14 kg/year from 1980 to 1987 [258]. A study of nosocomial outbreaks of amikacin-resistant strains of *Acinetobacter* spp. showed a clear correlation between amikacin resistance and amikacin usage [259]. In otitis media, the increase in prevalence of β -lactamase production by *M. catarrhalis* and *H. influenzae* between 1978 and 1993 has been correlated with a substantial increase in the consumption of cephalosporins in the community [260]. Paediatric bacterial isolates are more often resistant to antibiotics than those from adults, because of more frequent infections requiring antibiotic therapy in childhood; thus an urban population carried resistant enterobacteria in 42% of cases in adults compared to 64% in children [254].

Evolution of available antibacterial drugs. As seen in Table 13, the antibacterial drugs available for clinical use in France have increased from 5 to 102 molecules within 36 years. Until the mid-1970s, the small number of available antimicrobial agents limited antibiotic usage in hospitals and in the community. Since the early 1980s, the 'explosion' of newer potent β -lactam agents (ureidopenicillins, second and third generation cephalosporins, cephamycins) and the increased usage of oral aminopenicillins, cephalosporins and macrolides, have certainly exerted a potent selective pressure on the commensal flora in hospitals and the general population. During the last 10 years, antibiotic

Table 13. Evolution of available antibacterial agents in France

Date	Numbers of antibiotics available	Drugs available in medical practice
1959	Five antibiotics	Penicillin G, chloramphenicol, tetracycline, streptomycin, erythromycin (plus sulphonamides)
1970	Five new important molecules (plus a few minor ones)	Oxacillin, ampicillin, cephalothin, gentamicin, rifampicin
1995	102 molecules 182 presentations 14 antibiotic classes 41 β -lactams in 73 presentations (36 new presentations since 1987)	Amoxycillin ($\pm$ clavulanic acid), macrolides, co-trimoxazole, oral cephalosporins, only one parenteral cephalosporin (ceftriaxone), tetracyclines, quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin, ofloxacin, sparfloxacin)

prescriptions in France have increased 50–80% for otitis media and sinusitis, and 70–85% for bronchitis (P. Choutet, personal communication).

Ways to overcome resistance problems

As it seems that there is no immediate way either to control dissemination of resistance genes or to restrict medical use as well as non-clinical use of antibiotics sufficiently to diminish the spread of resistance significantly, new ways to overcome resistance problems are needed. Several solutions are proposed.

1. *Efforts to control antimicrobial resistance.* Better surveillance systems are needed in hospitals and in the community to identify risk factors for spread of resistance and hence to define preventive strategies [257].

2. *Definition of newer strategies for antibiotic therapy.* This is required to avoid sub-therapeutic doses of antibacterial agents, one of the major factors responsible for development of resistance. New strategies can be based on the use of existing antibiotics.

(i) *Development of new derivatives of existing drugs* with prolonged half-lives, resulting in once-daily dosing and, as a result, better patient compliance and a reduced risk of selecting bacteria during periods at which drug levels are sub-MIC.

(ii) *Sequential antibiotic therapy (transitional therapy)* in which use of the same antibiotic, but switching from i.v. to oral administration, results in a reduced risk of acquisition of nosocomial multiresistant pathogens. This also results in lower costs, thanks to shorter hospital stays, and a reduction in nursing time. Another way would be to switch from initial i.v. therapy with a given drug to an antibiotic of another class to limit a 'one-way selective pressure' [261, 262].

(iii) *Targeted antibiotic delivery with newer molecules* that are highly concentrated and persistent intracellularly (intracellular quinolones or macrolides) [263] or by use of liposome-encapsulated drugs or nanoparticles that allow hydrophilic drugs to cross the lipid-permeable membranes of cells [264]. This may increase antibiotic efficacy in the many intracellular infections, while diminishing the ecological impact on the commensal flora.

3. *Newer antibiotics and new perspectives in anti-infective agents.* In 1940, only three genera of actinomycetes were known: *Nocardia*, *Micromonospora* and *Streptomyces*. Today, 80 genera have been identified, all of which have the capability to synthesise various biologically active metabolites and antibiotics of therapeutic and commercial importance. Molecular genetic analysis of genes encoding the biosynthesis of antibiotics may lead to the discovery of new antibacterial agents.

Conclusion

Man has to learn to live with resistant bacteria. A 'prudent use' of antibiotics is always recommended, but is difficult to apply. Progress in antibiotic therapy may result from newer perspectives in the control of resistance problems, but for the future there is promise in the development of new biotechnologies with drugs produced by genetic engineering. Currently, 12.5% of all new drugs are for infectious diseases. The bio-synthetic attributes of bacteria, together with genetic engineering techniques and their extremely rapid growth and potent metabolic activities, permits synthesis of newer bioactive compounds. These drugs, synthesised on the basis of a better knowledge of structure–activity relationships, may provide new hope to overcome resistance problems.

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