Bacterial Resistance: Origins, Epidemiology, and Impact

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The basic mechanisms of antibacterial resistance are well known, but critical new aspects continue to be discovered. Recently discovered factors with major implications for the emergence, dissemination, and maintenance of resistance include multidrug efflux, hypermutability, integrons, and plasmid addiction. Some resistances are widespread and others local, with prevalence rates often worst in newly prosperous countries and in those specialist units where antibacterial use is heaviest. Multidrug-resistant epidemic strains are critical to the total accumulation of resistance (e.g., among Streptococcus pneumoniae, methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae), but it remains unclear why some bacterial lineages achieve epidemic spread whereas others that are equally resistant do not. The correlation between in vitro resistance and treatment failure is imperfect, but resistance undoubtedly increases mortality, morbidity, and costs in many settings. Recent concern has led to a plethora of governmental and agency reports advocating less antibacterial use, better antibacterial use, better infection control, and the development of new antibacterials. The evidence that better prescribing can reduce resistance rates is mixed, and although changes to hospital regimens may reduce one resistance problem, other opportunistic bacteria may fill the vacant niche. Overall, the best that can reasonably be anticipated is an improved balance between the accumulation of resistance and new antibacterial development.

Most bacteria have multiple routes to resistance to any drug and, once resistant, can rapidly give rise to vast numbers of resistant progeny. Natural selection favors mechanisms that confer resistance with the least fitness cost and those strains that are least burdened by their resistance. Selection may also favor determinants that prevent their own counterselection and resistant strains with enhanced survival ability or virulence. To this genetic and biochemical potential must be added the wide variety of bacteria that cause opportunistic infections in vulnerable human patients and the fact that the numbers of vulnerable patients grow steadily with advances in other fields of medicine. In short, the emergence of resistance is profoundly unsurprising; what is remarkable is how long it has taken for the problem to become a source of public, as well as scientific, concern.

Resistance can result from modification of an antibacterial’s target or from functional bypassing of that target, or it can be contingent on impermeability, efflux, or enzymatic inactivation. All members of a species may be resistant. Alternatively, resistance may arise in hitherto susceptible organisms via mutation or DNA transfer. The aim of this article is not to catalog individual mechanisms or their prevalence—that has been done elsewhere—but to emphasize the continuing dynamism of resistance, its impact on therapy, and the difficulty—but also the potential—for combating the problem.

SELECTION OF SPECIES WITH INHERENT RESISTANCE

Antibacterial use disrupts the microbial ecology of the patient, unit, or population. Entire species may be selected. The increasing role of enterococci as opportunistic pathogens in the past 20 years partly reflects increasing
use of fluoroquinolones and cephalosporins, to which these organisms are inherently resistant [1, 2]. The increasing role of coagulase-negative staphylococci (especially) and α-hemolytic streptococci in hematology patients also may reflect antimicrobial use: α-hemolytic streptococci are resistant to fluoroquinolones, which are often used as prophylaxis in these patients, and coagulase-negative staphylococci frequently have acquired multidrug resistance [3]. A greater factor behind the rise of coagulase-negative staphylococci is, however, the increased use of indwelling lines, which provide entry portals for the skin microflora. Among gram-negative pathogens, Acinetobacter baumannii and Stenotrophomonas maltophilia are increasingly prevalent in many intensive care units (ICUs), with A. baumannii notoriously associated with ventilator-associated pneumonias [4]. A. baumannii commonly has acquired resistance to all antibacterials except carbapenems, minocycline, sulbactam, and colistin, whereas S. maltophilia is often resistant to all antibacterials except co-trimoxazole (and perhaps ticarcillin-clavulanate). Some publications associate an increasing incidence of S. maltophilia infections with carbapenem use [5], but others find a less specific relationship to the use of multiple antibacterials, including those (e.g., erythromycin) with predominantly anti-gram-positive spectra [6, 7].

**RESISTANCE VIA MUTATION**

As DNA is replicated, uncorrected base substitutions occur randomly, at a frequency of \(10^{-6}\) to \(10^{-9}\) per gene [8]. In addition, copying errors may lead to the partial or complete deletion of individual genes [8]. As a result, the targets of antibacterials may be altered, drug-inactivation or efflux systems may be up- or downregulated, and uptake pathways (porins and active transporters) may be lost or activated. Resistance genes or their repressors also can be activated or inactivated by the migration of insertion sequences. Approximately 3% of *Bacteroides fragilis* isolates have the carbapenemase gene *ccrA* (*cfiA*), but its enzyme product is expressed only if an insertion sequence has migrated upstream of this structural gene [9].

Classical experiments showed that antibacterials cause the selection of preexisting variants, not the emergence of new mutants. This observation entirely agrees with the precepts of Darwinian evolution, but a twist is given by the observations that bacteria can become hypermutable through inactivation of the proofreading and DNA mismatch–repair systems that normally correct DNA copying errors [8]. Hypermutators have up to 200-fold higher mutation rates than normal cells and so are more likely to become resistant to a first antibacterial by mutation. Once selected by this first drug, they are then “primed” to develop resistance to further agents. To this extent, antibacterials may cause the emergence of variants with an increased propensity to develop further resistance. Hypermutability also may arise transiently through induction of the SOS system, a stress response that involves the expression and function of alternative DNA polymerases with reduced copying fidelity [8, 10]. The SOS system is induced, inter alia, by starvation, and it is also notable that the spectrum of mutations seen in non- or slow-growing cells differs from those among logarithmic-phase organisms. Also relevant is the fact that DNA-damaging forms of reactive oxygen accumulate in non-growing cells [8, 11], perhaps acting as mutagens.

The selection or transient induction of hypermutability may explain why variants with multiple mutations have emerged more rapidly than was predicted from laboratory studies. Nonclonal strains of fluoroquinolone-resistant *Escherichia coli* have emerged worldwide and have become highly prevalent, for example, in India [12], Spain [13], and China [14]. This is despite the fact that substantive fluoroquinolone resistance in Enterobacteriaceae requires mutations to the genes for subunits of topoisomerases II and IV (gyrA and parC, respectively), together with further mutations that upregulate efflux and reduce permeability, or both [14–17]. Perhaps the emergence of these multiple mutants is favored by the quinolone-mediated induction of the SOS response, with its contingent hypermutability [18]. This is speculation, but there is hard evidence of the role of hypermutators in the cystic fibrosis lung, where Oliver et al. [19] found that 36% of 30 chronically colonized patients carried hypermutable *Pseudomonas aeruginosa*. Hypermutators were not found in 75 control (noncystic) patients with acute *P. aeruginosa* infections.

Most mutations affect only a single antibacterial class, but those affecting impermeability or efflux may have a pleiotropic effect. The potential for porin loss to affect multiple drug classes by restricting nonspecific permeability is self-evident, but the role of broad-spectrum efflux has come as a surprise. Its function is best understood in *P. aeruginosa*, where mutation at *mexR* upregulates the *mexA-mexB-oPrM* operon and raises the MICs of β-lactams (except imipenem), fluoroquinolones, tetracyclines, chloramphenicol, macrolides, various disinfectants, detergents, and organic solvents [20]. MexAB-OprM also plays a role—perhaps coincidentally—in excretion of the quorum-sensing mediator, homoserine lactone. Even in typical *P. aeruginosa* isolates, MexAB-OprM is expressed to some degree and accounts for much of the resistance previously ascribed to impermeability [21]. Isolates with further upregulation have additional resistance to substrate drugs (table 1). *P. aeruginosa* strains have 3 further well-characterized efflux systems, 2 of which (MexCD-Opr J and MexEF-Opr N) are normally repressed but may be activated by mutation (table 1). The genomic sequence for *P. aeruginosa* suggests the presence of at least 5 further efflux systems, which await even preliminary characterization [20]. Multidrug efflux systems are also being reported in many other gram-negative bacteria, including En-
Table 1. Drug efflux systems of Pseudomonas aeruginosa.

<table>
<thead>
<tr>
<th>System</th>
<th>Regulator gene</th>
<th>Mutation causing upregulation</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MexAB-OprM</td>
<td>mexR</td>
<td>nalB (affects mexR) and nalC</td>
<td>β-Lac (not imipenem), Cm, Em, Fq, Nv, Tm, Su, Tc, ethidium bromide, acriflavine, SDS, aromatic hydrocarbons, irgasan, triclosan, homoserine lactone</td>
</tr>
<tr>
<td>MexCD-OprJ</td>
<td>nfxB</td>
<td>nfxB</td>
<td>β-Lac (not imipenem), Cm, Em, Fq, Nv, Tm, Tc, ethidium bromide, acriflavine, SDS, aromatic hydrocarbons, triclosan</td>
</tr>
<tr>
<td>MexEF-OprN</td>
<td>mexT</td>
<td>nfxC</td>
<td>Cm, Fq, Tm, aromatic hydrocarbons, triclosan</td>
</tr>
<tr>
<td>MexXY-OprM</td>
<td>mexZ</td>
<td>Agl, β-lac (not carbenicillin, ceftazidime or imipenem), Cm, Em, Fq, Nv, Tc</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Agl, aminoglycosides; β-lac, β-lactam; Cm, chloramphenicol; Em, erythromycin; Fq, fluoroquinolones; Nv, novobiocin; SDS, sodium dodecyl sulfate; Su, sulfonamides; Tc, tetracycline; Tm, trimethoprim. Data from [20, 21].

terobacteriaceae as well as other nonfermenters [20, 22]. At a functional level, the role of broad-spectrum efflux pumps may be to remove amphipathic substances (i.e., those with hydrophilic and hydrophobic parts) from membranes, preventing disorganization. Such a “cleaning” role would account for their broad activity and wide distribution of these pumps, but their substrate recognition sites await elucidation.

Efflux systems can be coregulated with porin expression. In P. aeruginosa, the nfxC mutation at mexT upregulates MexEF-OprN–mediated efflux (table 1), and also reduces expression of OprD (“D2 porin” in older literature), thereby reducing OprN–mediated efflux (table 1), and also reduces expression of the OprD porin, which provides carbapenem-specific pores through the outer membrane [29, 30]. Most recently, there has been concern about linezolid resistance emerging in enterococci or, more rarely, in Staphylococcus aureus via modification of the domain V of 23S rRNA, which contains the drug’s binding site. Mutational resistance to linezolid is difficult to obtain in vitro, apparently because the bacteria have multiple copies of the encoding gene, with modification of more than one copy required for resistance. Nevertheless, linezolid resistance can be selected in vivo, particularly in difficult-to-reach infections, those requiring prolonged therapy and if the drug is underdosed [31, 32]. It is not clear whether DNA recombination events follow the mutation of one gene copy or whether multiple gene copies undergo sequential mutations, perhaps by induction of a transient hypermutability (above).

**ACQUISITION OF RESISTANCE BY DNA TRANSFER**

DNA transfer among bacteria is critical to the dissemination of resistance and has recently been reviewed [33]. Transfer of DNA is most often via plasmids. These existed long before humans used antibacterials but did not then carry resistance determinants, or rarely did so [34]. Since the advent of the antibacterial era, plasmids have, however, proved to be the ideal vehicles for recruitment and dissemination of resistance genes. Within plasmids, resistance genes are often carried by transposons, which can shuttle determinants between more and less promiscuous plasmids, or into and out of the chromosome. Some transposons are directly transmissible between bacteria, particularly (but not exclusively) among gram-positive species. Resistance genes also may be transferred by lysogenic bacteriophage. This latter mechanism seems likely with the mecA determinant staphylococci, which has never been located on mobile DNA but which has spread among a few S. aureus lineages, and among different coagulase-negative species [35].

Integrons are natural recombination systems that facilitate the acquisition and expression of resistance determinants behind a single promoter. They are widely distributed among gram-negative bacteria, often occurring within plasmids and
transposons, and are particularly important in the dissemination of resistance genes to sulfonamides (sul1), and streptomycin (aadA3) [36]. Other genes often found in integrons include those for various OXA, PSE, VIM, and IMP β-lactamas and for many aminoglycoside-modifying enzymes [36, 37]. In principle, integrons have a fearsome capacity for the recruitment, spread, and expression of resistance genes, and surveys show that they are widespread among gram-negative bacteria in countries as far apart as The Netherlands and Taiwan [38, 39]. Nevertheless, it is striking that the most successful β-lactamase genes (i.e., blαTEM derivatives) are carried directly by transposons, not within integrons, whereas the integron-associated OXA and PSE β-lactamases are considerably rarer [27]. Similarly with sulfonamide resistance: sul2, which is not integron-associated, is increasing in prevalence among E. coli in the UK, whereas sul1, which is integron-determined, is stable in prevalence, or declining [40]. It is notable also that the composition of integrons is more stable over time than might be expected [38]. In short, integrons are important, but their importance should not be overplayed relative to that of other vehicles of resistance.

The dissemination of plasmids, transposons, and integrons among bacteria and species give rise to so-called gene epidemics. Plasmids encoding the TEM plasmid-mediated β-lactamases were first recognized in 1965 but have since spread—varying with the country, unit, and species—to 30%–50% of clinical Enterobacteriaceae, to a few P. aeruginosa, and to anywhere between 1%–50% of Haemophilus influenzae and Neisseria gonorrhoeae isolates [27]. Other determinants that have spread extremely widely include erm, sul1, sul2 strA, strB, aadA3, tetA, and tetM. tetM has spread in both gram-positive and gram-negative organisms, but more generally, the genes of gram-positive and gram-negative species are distinct [41]. The factors that determine whether a mobile gene will spread widely are poorly understood. TEM-2 β-lactamase differs from TEM-1 by a single amino acid substitution, confers similar resistance, is coded by similarly promiscuous plasmids and transposons, and has been known for almost as long. Nevertheless, for no obvious reason, it is 10-fold less prevalent that TEM-1 β-lactamase in every country and species surveyed [27].

Many of the resistance determinants now found on plasmids are believed to have originated in the chromosomes of other species, although only a few of their source organisms have been identified definitively. The plasmid-mediated SHV β-lactamases are derived from the chromosomal β-lactamases of Klebsiella pneumoniae, the plasmid-borne AmpC enzymes emerging in Klebsiella spp. and E. coli are chromosomal escapes from Citrobacter freundii, Hafnia alvei, Morganella morganii, and Enterobacter cloacae [42, 43], and several CTX-M cephalosporinases are chromosomal escapes from Kluvyera spp. [44] (table 2). Some resistance determinants to non–β-lactam drugs seemingly originated in antibiotic-producing streptomycetes, which must protect themselves against their own products. Examples include several aminoglycoside-modifying enzymes and the erm determinants, which have products that methylate 23S rRNA so as to block binding of macrolides, lincosamides, and group B streptogramins [45]. Also, the genes that encode the d-ala-d-ala ligases are critical to glycopeptide resistance in enterococci (and now also S. aureus [46, 47]). Many plasmids and transposons carry multiple resistance genes conferring resistance to different antibacterials, and selection for any one determinant may conserve the entire plasmid and its resistances. The frequent consequence is multidrug resistance, as illustrated in table 3, which shows that klebsiellae with extended-spectrum β-lactamases (ESBL) were more often resistant to aminoglycosides and fluoroquinolones than those without ESBLs [48, 49]. ESBLs and aminoglycoside-modifying enzymes may be encoded by single plasmids, but the fluoroquinolone resistance, also seen in an excess of the ESBIL producers, is chromosomal and independent.

The problems of multiresistance are increasing and becoming more complex. When plasmid electrophoresis was developed in the 1970s, it was uncommon to see isolates with >2–3 plasmids, and plasmid profiles were used to define strain epide-

### Table 2. Sources of β-lactamase resistance genes now found on transferable DNA.

<table>
<thead>
<tr>
<th>Genes or product(s)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHV β-lactamases</td>
<td>Klebsiella pneumoniae chromosome</td>
</tr>
<tr>
<td>CTX-M2,4,5,6,7 and Toho-1 β-lactamases</td>
<td>Klyvera spp. chromosome</td>
</tr>
<tr>
<td>CMY2, 3, 4, 5, 6, 7, LAT-1, -2, -3, -4, BIL-1 AmpC β-lactamases</td>
<td>Citrobacter freundii chromosome</td>
</tr>
<tr>
<td>ACC-1 AmpC β-lactamases</td>
<td>Hafnia alvei chromosome</td>
</tr>
<tr>
<td>DHA-1 and -2 AmpC β-lactamases</td>
<td>Morganella morganii chromosome</td>
</tr>
<tr>
<td>TEM, OXA, PSE, staphylococcal penicillinase</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

* Although source organisms for plasmid-mediated OXA enzymes have not been identified, some Aeromonas spp. have chromosomal enzymes belonging to this family.
miology. Nowadays, it is common to encounter Enterobacteriaceae with 5–6 plasmids; moreover, plasmid profiles often vary greatly within the "strains" defined by PFGE [50]. These observations (which complicate epidemiological analysis) reflect the gain and loss of plasmids by different representatives of the "same" strain and the gain and loss of genes among different representation of the "same" plasmid. Even when genes are not gained or lost, multiple copies of the "same" plasmid may carry different variants of a β-lactamase gene, reflecting mutation. Thus, for example, some members of a K. pneumoniae strain from Turkey had SHV-3 β-lactamase, whereas others had SHV-5 (table 4) [50]. More strikingly, 84 blaTEM and blaSHV copies were found among just 25 K. pneumoniae isolates collected at one hospital in Durban, South Africa [51].

A few species—Neisseria spp., Haemophilus spp., and α-hemolytic streptococci—can absorb and incorporate DNA released by dead cells of related organisms, allowing the generation of "mosaic" genes [52]. This mechanism is absent from most other species, which restrict incoming DNA. Mosaic gene formation, acting in combination with mutation, is the basis of emerging penicillin resistance in pneumococci and of β-lactamase–independent ampicillin or penicillin resistance in H. influenzae and Neisseria spp.; it is also implicated in sulfonamide resistance in H. influenzae [53].

**EPIDEMIOLOGY OF RESISTANCE: LOCAL, NATIONAL, AND INTERNATIONAL**

At one level, the epidemiology of resistance is extremely local. Most outbreaks and clusters involve a few patients in a unit, and the prevalence of resistance is often highest in those units where the most vulnerable patients are congregated and where antibacterial use consequently is heaviest. Archibald et al. [54] found 2-fold higher rates of methicillin resistance among staphylococci, ceftazidime resistance among E. cloacae and P. aeruginosa, imipenem resistance among P. aeruginosa, and vancomycin resistance among enterococci in patients in ICUs than in patients in general wards or outpatients at the same hospitals. In virtually all European countries, the prevalence of methicillin-resistant S. aureus is higher in ICUs than in general wards [55].

At another level, the epidemiology of resistance is national. In Europe, the common pattern is for resistance to increase in prevalence as one moves southward: it is lowest in Scandinavia and highest in the Mediterranean countries. In North America, resistance rates are mostly higher in the United States than in Canada. Some of the worst resistance rates are in the newly prosperous countries of East Asia and South America. A few examples: methicillin-resistant strains comprise 30%–45% of all S. aureus from bacteremias in Spain, Portugal, Italy, France, and the United Kingdom and 10%–15% in Germany and Austria, but <1% in the Netherlands and Scandinavia (see, e.g., European Animicrobial Resistance Surveillance System, http://www.ears.s.rivm.nl). In Korea, Japan, Taiwan, and Vietnam, 70%–80% of S. pneumoniae are resistant or intermediately resistant to penicillin, compared with 30%–40% in France and Spain, 5%–10% in the United Kingdom, and 1%–2% in Scandinavia [56–58]. As a final example, gentamicin resistance in E. coli remains considerably more frequent in most Southern European countries and the United States than in the United Kingdom, where it occurs in <3% of isolates [57].

Finally, the epidemiology of resistance is partly international, with some transferable determinants prevalent worldwide. The epidemiology is also international to the extent that some resistant strains spread between countries and continents. Multi-drug-resistant pneumococci of serotype 6B were imported from Spain into Iceland, apparently by nasopharyngeal carriage in the children of returning holidaymakers [59]. These pneumococci then became established in child care centers in Iceland, causing an increase in the penicillin resistance rate from 1% in 1988 to 17% in 1993. Other penicillin-resistant pneumococci of serotype 23F have spread from Spain to the Far East, the Americas, and South Africa [60]. On a smaller scale, many of the few E. coli and Klebsiella spp. with plasmid-mediated AmpC β-lactamases in the United Kingdom are epig—

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**Table 3. Multidrug resistance among Klebsiella spp. collected in 2 European surveys of isolates from intensive care units.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL positive (n = 220)</td>
<td>ESBL negative (n = 748)</td>
</tr>
<tr>
<td>Klebsiellae with the indicated ESBL phenotype</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>Gentamicin resistance</td>
<td>76</td>
<td>8</td>
</tr>
<tr>
<td>Amikacin resistance</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>Ciprofloxacin resistant</td>
<td>31a</td>
<td>2</td>
</tr>
</tbody>
</table>

**NOTE.** Data are percentage of isolates. ESBL, extended-spectrum β-lactamase. Data from [42, 43].

a Declined to 10% if members of a single widespread clone were discounted.
demographically linked to the Indian subcontinent, where there is evidence of local frequency in Punjab [61, 62]. Last, PER-1 ESBL was first recorded from a *P. aeruginosa* isolate collected in France [63] and shortly afterward was found in numerous *P. aeruginosa*, *Salmonella*, and *Acinetobacter* spp. isolates from several cities in Turkey [64]. An inquiry revealed that the original source patient in France was a Turk, visiting for treatment [65].

### EPIDEMIC RESISTANT STRAINS

Successful epidemic strains are critical to the accumulation of many resistances. Common vectors in hospitals are contact with staff members, contact with nonsterile devices, or procedures. Spread in the community is favored by those factors that have aided epidemics throughout history: crowding and travel. Many strains, resistant or otherwise, spread locally, but a few achieve a much wider distribution. The international spread of penicillin-resistant pneumococcal lineages was mentioned above, but further examples abound. In England and Wales, the proportion of *S. aureus* bactereamias caused by methicillin-resistant *S. aureus* (MRSA) was steady at 1%–3% from 1989 to 1993 but increased rapidly afterward, reaching 42% by 2000 [57].

The beginning of this increase coincided with the emergence of 2 new epidemic (E) strains, EMRSA 15 and 16, and recent analysis shows that these now account for >93% of all *S. aureus* bactereamias in England and Wales [66]. In France, a serotype K25 *K. pneumoniae* strain with SHV-4 β-lactamase and cross-resistance to amikacin and ciprofloxacin has disseminated widely, having been reported repeatedly since 1988, first around Paris and subsequently in hospitals from the Atlantic coast to the Mediterranean, with reports also from Ghent in Belgium [50, 67, 68]. A survey of ESBLs among 966 klebsiellae in 1994 included 35 centers, only 5 of them in France, yet this single strain accounted for 52 of 220 ESBL producers collected [50]. Also in France and Belgium, an *Enterobacter aerogenes* strain with TEM-24 β-lactamase and multiresistance to aminoglycosides, quinolones, and, occasionally (via porin loss), carbapenems has become widely established [69]. Other examples where resistance has a major clonal element include *Burkholderia cepacia* from cystic fibrosis patients [70] and *Salmonella typhi-murium*, where major recent problems (now perhaps declining) have been associated with the intercontinental spread of multidrug-resistant lineages of definitive type DT104 [71, 72].

In the case of vancomycin-resistant *Enterococcus faecium*, recent studies by amplified DNA restriction fragment-length polymorphism suggest that epidemic strains from hospitals in Europe, the United States, and Australia, although differing from place to place, are more closely related to each other than to sporadic and agricultural isolates [73].

Other strains with similar resistances to these epidemic organisms are recorded, often in the same hospitals or patient groups, but these fail to spread extensively or fail to spread at all. The reason for epidemic success remain obscure, but potential factors—not mutually exclusive—including the following: (1) increased adherence to host cells or prosthetic materials, (2) greater tolerance of desiccation, (3) elevated resistance to disinfectants, (4) faster growth rates, and (5) better adaptation to the fitness cost of resistance. Hard evidence for the role of any of these factors is scanty for many particular strains, and studies are confounded by the fact that researchers, having found one fact that may contribute to epidemic success, then concentrate on that factor in isolation. In the case of the successful serotype K25 *K. pneumoniae* strain from France and Belgium, one report suggests a plasmid-mediated fimbrial antigen that aids adherence to the gut mucosa [74].

Local variation, national variation, and role of epidemic strains should be borne in mind when undertaking and assessing prevalence surveys. Grandiloquent statements about resistance rates “in Europe” or the “Western Pacific” abound in the literature, but often are based on surveys with a very few centers per country, and with the extent of resistance differing greatly among the countries included within a region. The variation in resistance prevalence between Stockholm and Madrid, it should be noted, is vastly greater than between Boston and San Francisco. Inferences on the general distribution of resistance likewise should not be made on the basis of data for

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**Table 4. Diverse β-lactamases and resistances of multiple representatives of a serotype K62 *Klebsiella pneumoniae* strain collected at an intensive care unit in Istanbul, Turkey.**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Gentamicin</th>
<th>Aztreonam</th>
<th>Ceftazidime</th>
<th>Ceftriaxone</th>
<th>Piperacillin-tazobactam</th>
<th>β-Lactamase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>8</td>
<td>512</td>
<td>1024</td>
<td>256</td>
<td>4</td>
<td>SHV-5</td>
</tr>
<tr>
<td>1953</td>
<td>16</td>
<td>512</td>
<td>1024</td>
<td>256</td>
<td>4</td>
<td>SHV-3 + TEM</td>
</tr>
<tr>
<td>1954</td>
<td>8</td>
<td>8</td>
<td>32</td>
<td>0.5</td>
<td>1024</td>
<td>SHV-3 + TEM</td>
</tr>
<tr>
<td>1959</td>
<td>16</td>
<td>512</td>
<td>512</td>
<td>512</td>
<td>1024</td>
<td>SHV-5 + TEM</td>
</tr>
<tr>
<td>1964</td>
<td>16</td>
<td>512</td>
<td>512</td>
<td>256</td>
<td>512</td>
<td>SHV-3 + TEM</td>
</tr>
</tbody>
</table>

**NOTE.** Data from [50]. All 5 representatives had the same DNA profile as investigated by PFGE.
isolates from ICUs, where resistance rates are commonly higher than among isolates overall [54, 55]. A final—and bigger—source of bias, particularly for surveys of community isolates, is that microbiological investigations are more likely to be performed for recalcitrant infections, which may be recalcitrant because they are caused by resistant bacteria. Thus, MacGowan et al. [75] found that only 3% of patients presenting with respiratory symptoms to general practitioners in Bristol received microbiological investigation and that the apparent prevalence of ampicillin resistance among *H. influenzae* isolates decreased from 22% to 11% when all the presenting patients had sputum culture performed.

**IMPACT OF RESISTANCE**

The consequences of resistance are harder to define than microbiologists, health care managers, and politicians might wish. Some patients recover despite inadequate treatment, exactly as some recovered before antibacterials were available. The infection of others failed to respond despite appropriate therapy. In compromised patients, it often remains debatable whether infection or an underlying disease was ultimately fatal. Perhaps the clearest link between in vitro resistance and in vivo responses for penicillin and gonorrhea. Classical strains with MIC ≤ 0.06 mg/L respond to penicillin at low doses; those with chromosomally mediated resistance (MIC 0.25–2 mg/L) respond to high-dose penicillin; and those with β-lactamase cannot be cured by any achievable level of penicillin. However, gonorrhea is an uncomplicated infection mostly caught by those in otherwise good health! Elsewhere, relationships are less clear and pseudomonal infection in cystic fibrosis presents the opposite extreme. Drugs that are active in vitro consistently fail to eliminate infection, whereas some of those that entirely lack in vitro antipseudomonal activity (e.g., erythromycin) often ameliorate the symptoms of infection [76].

Most correlations fall between these extremes. Analysis by Mosdell et al. [77] showed that the incidence of complications, including reoperation, abscess formation, and wound infection, increased ~2-fold if empirical therapy for intra-abdominal sepsis failed to cover all the pathogens subsequently isolated and that the length of hospital stay was likewise extended (figure 1). The incidence of complications rose further if inadequate empirical regimen was not modified when resistant pathogens were isolated. In recent analyses, Kollef and Ward [78] and Ibrahim et al. [79] found 2-fold higher mortality among ICU patients and those with ventilator-associated pneumonia when the pathogens proved resistant to the antibacterials used empirically (figure 2). Many of the failures in these series reflected infection by *P. aeruginosa* or MRSA, which were not well covered by the empirical regimens routinely used. Risk factors for isolation of these pathogens and so for poor outcomes included previous use of antibacterials and previous hospitalization. Such factors, as well as the likely pathogens and their likely local resistance patterns, should always be taken into account when designing empirical regimens for hospital units. It should be added that the science of pharmacodynamics is allowing more precise modeling of the probability of cure of a given pathogen in a given infection, underscoring the fact that for many infection types, there is a strong relationship between in vitro susceptibility and outcome. These aspects are addressed elsewhere in this supplement by Drusano [80].

Increased morbidity and mortality are the most dramatic...
Figure 2. Mortality among critically ill patients in intensive care (n = 655) in relation to whether empirical therapy was appropriate (gray) or inappropriate (black) in relation to the resistances of the bacteria subsequently isolated. Reproduced with permission from Ibrahim et al. [79].

consequence of resistance. Other effects are more insidious. Physicians and surgeons are forced to use previously reserved agents as first-line therapy. These may be inherently less potent or more toxic that classical regimens: vancomycin is increasingly used as a first-line antistaphylococcal (and for prophylaxis) but is less convenient to administer safely and less bactericidal than the semisynthetic antistaphylococcal penicillins, which themselves are 100-fold less active than benzylpenicillin against fully susceptible staphylococci. Previously reserved agents—now used earlier—may be undermined by resistance. The accumulation of cephapirin-resistant bacteria is driving the earlier clinical use of carbapenems and is a reasonable justification for the development of oral and long half-life carbapenems. Nevertheless, the selection pressure that mass use of these will cause is disturbing, coming precisely when the number of reported carbapenemases is growing sharply [81].

Finally, resistance adds cost: treatment failures extend the length of hospital stay or demand repeated physician visits; hospital beds are blocked to new patients, and productive time is lost. If new or hitherto reserved antibacterials are needed as therapy, these are usually more expensive than previous regimens. These costs seem unlikely to decline in the future, especially with the growing demand of regulators and the new costs of genomics-based drug discovery. A new antibacterial already costs ~$0.5 billion to develop; this sum and the financing costs (for the ~$0.5 billion is spent before income is generated) must be recouped in the 10–12 years of patent life remaining after the compound is launched.

RESPONSES TO RESISTANCE

Concern about resistance increased in the late 1990s. Since then, many governmental and agency reports have been published, adding to those of professional societies [82–84]. These reports vary in emphasis, especially as regards the agricultural use of antibacterials, but all advise (1) less use of antibacterials, (2) more appropriate choices of antibacterials and regimens, (3) prevention of cross-infection, and (4) development of new antibacterials. Measuring the effect of these charges demands better surveillance of resistance prevalence and of prescribing. Optimists hold that it may be possible to reverse resistance trends, and pessimists hold that it may only be possible to slow the accumulation of resistance sufficiently to keep one step ahead of bacterial evolution.

EFFECTS OF REDUCED PRESCRIBING

In a few cases, reductions in prescribing at a national level have been followed by a reduced prevalence of resistance. In one example of success, the prevalence of penicillin-resistant pneumococci in Iceland was reduced from 19.3% (1993) to 14% (1998–2000) after a 12.9% reduction in drug use [85]. In Finland, a national advisory to reduce prescribing of macrolides was followed by a decline in use from 3 doses/1000 population per month in 1988 to 1.1 doses/1000 population per month in 1994 and by a decrease in the prevalence of erythromycin-resistant Streptococcus pyogenes from 19% in 1993 to 8.5% in 1996 [86]. By 1998, however, macrolide use had increased to 2.1 doses/1000 population per month, and the resistance rate among S. pyogenes was back to 18% (Bacterial Resistance to Antimicrobial Agents in Finland FINRES 1999; http://www.mmm.fi/el/julk/finres99en.htm). In both these cases, the resistances displaced were associated with clonal strains—two widely disseminated S. pyogenes lineages in Finland [87]—and, in Iceland, the serotype 6B S. pneumoniae strain mentioned earlier. Displacement seems more difficult when resistances are multiple and linked and when they have disseminated among different strains. Resistance to streptomycin and chloramphenicol remains frequent in gram-negative bacteria, although these drugs have fallen into virtual disuse in humans, and are compromised by mechanisms that do not directly affect any antibacterial that remains in extensive human use [88].
example of the difficulty of displacing resistance, sulfonamide
use in the United Kingdom declined by ∼97% during
1991–1999 after national advice to prescribe trimethoprim in
stead of co-trimoxazole, but the prevalence of sulfonamide re-
sistance among *E. coli* in London remained virtually constant,
at 39%–45% [40].

The continued prevalence of resistance in disused antibac-
terials begs the question, Why? Potential contributory factors
include continued agricultural use and—as was shown with the
sulfonamide resistance in *E. coli* from London [78]— linkage
of resistance determinants within large multiresistance plas-
mids. More generally, evolution acts to favor those determi-
nants that exert the least fitness cost on their host bacteria and
those strain variants in which this cost is minimized. Thus,
once antibacterials have been used heavily for long periods,
evolution seems likely to have honed the resistant strains. Lab-

eratory experiments illustrate how this process may arise. An
*E. coli* strain with a tetracycline resistance plasmid initially grew
more slowly than its plasmid-free parent, but after 500 gen-
erations, it grew 6% more rapidly [89]. Similarly, the growth
rate of a streptomycin-resistant *E. coli* mutant was initially sup-
pressed by 12%–14%, but after repeated subculture, it grew
only 6% more slowly than the parent strain [90]. Such well-
adapted strains are unlikely to be swiftly displaced. Plasmids
themselves should perhaps be seen as selfish genomes, seeking
their own perpetuation, and not as mere “possessions” of their
host bacteria. Some plasmids have specific binding sites to the
chromosome, favoring segregation with each daughter cell. Others
encode “plasmid addition” systems determining both a
long half-life toxin and a homologous but less stable antitoxin.
So long as the plasmid is present, antitoxin is manufactured
and the cell survives, but residual toxin kills any daughter cell
that fails to inherit a plasmid copy [91]. Such factors maintain
plasmid carriage within populations, increasing the pressure to
minimize the fitness cost. It remains plausible (but unproven)
that a *generalized* reduction in prescribing may lead to a re-
duction in resistance prevalence. An answer may be forthcom-
ing. Since an annual peak in 1996–1997 (the British financial
year runs from April to March), antibacterial prescriptions in
the community have decreased by 23.4% and veterinary use by
25%.

**FORMULARY CHANGES AND RESISTANCE PATTERNS**

Although there are few examples of reducing resistance on any
large scale, there are many examples of altered (rather than
reduced) prescribing being followed by changes in the local
epidemiology of resistance. A formulary switch to amikacin
was followed, over several years, by declining gentamicin re-
sistance among opportunistic gram-negative bacteria [92, 93].
More recently, Bradley et al. [94] found that a formulary switch
from empirical ceftazidime to piperacillin-tazobactam in neu-
tropic fevers, together with reinforced infection control, was
associated with reduced colonization and infection by vanco-
mycin-resistant enterococci (table 5). A return to empirical
ceftazidime was followed by a reemergence of vancomycin-
resistant enterococci, despite continued emphasis on infection
control. Other examples of apparent success include reductions
in the prevalence of AmpC-derepressed *Enterobacter* spp. after
a formulary switch from ceftazidime to cefepime [95] and of
cephalosporin-resistant *Enterobacteriaceae* after switches to
piperacillin-tazobactam or carbapenems [96–98].

These examples highlight positive consequence, but caveats
should be noted. First, changes that achieve positive conse-
quences are perhaps more likely to be reported than those that
fail to do so. Second, others who followed these strategies were
not always so successful. Formulary switches from gentamicin
to amikacin have been associated with increasing amikacin re-
sistance [99]. Third, authors often concentrate on one pathogen
and demote other effects; thus, deaths owing to multidrug-
resistant gram-negative bacteria occurred in both the ceftazi-
dime and piperacillin-tazobactam arms of the study outlined
in table 5 [94], and a formulary switch from cephalosporins

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**Table 5. Incidence of colonization and infection with vancomycin-resistant enterococci in a London hematology unit in relation to preferred empirical therapy**

<table>
<thead>
<tr>
<th>Phase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Empirical therapy for fever</th>
<th>Colonized with vancomycin-resistant enterococci, %</th>
<th>No. of infections with vancomycin-resistant enterococci/total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ceftazidime</td>
<td>57</td>
<td>5/75</td>
</tr>
<tr>
<td>2a</td>
<td>Pip/tazo + infection control reinforced</td>
<td>29</td>
<td>0/70</td>
</tr>
<tr>
<td>2b</td>
<td>Pip/tazo + infection control reinforced</td>
<td>8</td>
<td>0/59</td>
</tr>
<tr>
<td>3</td>
<td>Ceftazidime + infection control reinforced</td>
<td>36</td>
<td>3/58</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each phase was defined as a 4-month period; identical regimens were used in phases 2a and 2b.
to imipenem was followed by the emergence of imipenem-resistant *Acinetobacter* and *P. aeruginosa* as well as by a decline in cephalosporin-resistant *klebsiellae* [96, 100]. Among opportunistic infections in the seriously ill, the cynic can argue that resistance is like a balloon: squeeze it on one side, and it bulges on the other.

It is salutary to emphasize how much remains unknown. Does drug cycling have positive effects, or does it lead to the accumulation of multidrug-resistant strains [101]? At what prevalence of resistance should empirical therapy be changed in different types of infection? To what extent does combination therapy mitigate against resistance (except in the case of tuberculosis, where its value is beyond dispute)? What are the relative selectivities of different antibacterials, allowing that a recent Finnish study found a correlation between macrolide use and resistance in *S. pneumoniae* but not between penicillin use and resistance [102]? Is it in any way desirable to encourage all community physicians to use the same therapies in the same indications, or is it wiser to make the selection pressure more diffuse [103]? What is the ideal duration of therapy, allowing that underdosing may fail to eliminate the least susceptible members of the original population and that excessive duration may exacerbate disruption of the normal flora? Are drugs with unlinked resistances (e.g., fosfomycin, nitrofurantoin, rifampin, and fucidin) to be favored as unlikely to select multiresistance plasmids or avoided because they may select hypermutable strains “primed” to develop further resistances? The new respiratory quinolones exemplify a further conundrum. As a result of greater antipneumococcal activity, they are less likely to than ciprofloxacin to select first-step quinolone-resistant mutants of *S. pneumoniae*; however, they are less active than ciprofloxacin against Enterobacteriaceae and may be selective for resistance in the gut microflora [104], which is gratuitously exposed.

**SOCIAL CONTEXT**

The scientific answers to these questions on antibacterial use are uncertain, and to complicate matters, the whole problem of resistance is intertwined with moral, social, political, and commercial issues. Concern about resistance is used as ammunition for other agendas, most obviously including marketing by the pharmaceutical industry and cost containment within managed or socialized health care. To some extent, the individual patient gains when powerful antibacterials are used early, but the resistance risks for society are raised. In reality, matters are complex. Failed treatments with “old and simple” drugs may lead to more severe disease or to the spread of infection, resulting in a demand for further therapy, along with its contingent selection pressure. Hungary, before 1989, had a restricted list of antibacterials for community prescription, yet achieved one of the world’s highest prevalence rates for penicillin-resistant pneumococci [105]. Moreover, the argument assumes a vacuum in which no new drugs are developed. This assumption, made in many reports on resistance, is already untrue for gram-positive pathogens [106].

Concerns about resistance have led to the banning of most agricultural growth promoters in Europe. Such concerns are also used to support wider objections to intensive farming and to the genetic modification of crop plants (where unexpressed resistance genes remain within the cloning vectors). Less is said on the other aspects of modern life that potentially exacerbate resistance: large hospitals; the concentration of the very young and very old in socialized care; and increasing travel. Action on these would be socially and politically impossible, even if they are more pertinent to the sum total of resistance than the use (recently banned in the European Union) of zinc bacitracin as a agricultural growth promoter! Perhaps the contradictions are best exemplified by the World Health Organization, which argues on the one hand for effective, affordable antibacterials for all the world’s population and on the other hand expresses concern about the worldwide accumulation of resistance (World Health Organization Report on Infectious Diseases 2000, http://www.who.int/infectious-disease-report/2000/index.html). Both concerns are ethical, humane, and honorable—but counterpoised.

**CONCLUSIONS**

Antibacterial resistance is complex and dynamic. Although the major genetic and biochemical mechanisms have long been recognized, new factors continue to be discovered, including integrons, multidrug efflux, hypermutability, and plasmid addiction. Within many individual isolates, the complexity of resistance is increasing, with multiple determinants carried, and with genes being gained, amplified, and lost. Many international resistance problems reflect the spread of a few multidrug-resistant strains, but the reasons underlying the success of particular lineages remain almost universally obscure. Resistance is a significant cause of excess morbidity, mortality, and cost. Numerous reports have emphasized the need for less and better use of antibacterials, improved infection control, and the development of new agents. However, reductions in antibacterial use do not always lead to reduced resistance, perhaps because bacteria are now well adapted to the carriage of resistance. It is probably naive to anticipate reaching a grand “control” over resistance, and attempts should center on management rather than elimination, with the objective of slowing the development of new resistance while continuing to develop new agents at a sufficient rate to keep ahead of the bacteria.
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