improved diagnostics, $0.18 million.
91. The NIAID increase for TB research proposed in the 1993 federal budget is $200,000 (Hearing on Tuberculosis, Government Operations Subcommittee, House of Representatives, 2 April 1992).
92. Fiscal year 1993, Justification of Appropriation Estimates for the Committee on Appropriations.
93. N. Machiavelli, *The Prince*, L. Ricci, Ed. (English edition, Oxford Univ. Press, Oxford, 1933), chap. 3, p. 1513 [transl. in G. B. Webb (*12*), p. 178].
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The Crisis in Antibiotic Resistance

Harold C. Neu

The synthesis of large numbers of antibiotics over the past three decades has caused complacency about the threat of bacterial resistance. Bacteria have become resistant to antimicrobial agents as a result of chromosomal changes or the exchange of genetic material via plasmids and transposons. *Streptococcus pneumoniae, Streptococcus pyogenes*, and staphylococci, organisms that cause respiratory and cutaneous infections, and members of the *Enterobacteriaceae* and *Pseudomonas* families, organisms that cause diarrhea, urinary infection, and sepsis, are now resistant to virtually all of the older antibiotics. The extensive use of antibiotics in the community and hospitals has fueled this crisis. Mechanisms such as antibiotic control programs, better hygiene, and synthesis of agents with improved antimicrobial activity need to be adopted in order to limit bacterial resistance.

The stunning success of the pharmaceutical industry in the United States, Japan, the United Kingdom, France, and Germany in creating new antibiotics over the past three decades has caused society and the scientific community to become complacent about the potential of bacterial resistance. There are countless antibiotics: more than 50 penicillins, 70 cephalosporins, 12 tetracyclines, 8 aminoglycosides, 1 monobactam, 3 carbapenems, 9 macrolides, 2 new streptogramins, and 3 dihydrofolate reductase inhibitors (*1*). Despite all these antibiotics, a person could die in a hospital in New York, San Francisco, Paris, Barcelona, Tokyo, or Singapore as a result of a resistant bacterial infection.

Antibiotics are available that effectively inhibit bacterial cell wall synthesis, protein synthesis, and DNA replication (Fig. 1 and Table 1). Bacteria can resist antibiotics as a result of chromosomal mutation or inductive expression of a latent chromosomal gene or by exchange of genetic material through transformation (the exchange of DNA), transduction (bacteriophage), or conjugation by plasmids (extrachromosomal DNA) (*2*). Conjugation with plasmid transfer of DNA is particularly common among the *Enterobacteriaceae*, *Pseudomonas*, and anaerobic species (*2, 3*). In addition to conjugative plasmids, bacteria may possess transposons, the so-called jumping genes, that have the ability to enter transmissible plasmids or chromosomes (*4*). Resistance can be transferred horizontally by plasmids or by chromosomally located conjugative transposons that spread the resistance to other species. It has been postulated that *Escherichia coli* transferred the ability to produce β-lactamase enzymes that destroy compounds with a β-lactam nucleus (Fig. 2) into *Haemophilus influenzae* by initially infecting *Haemophilus parainfluenzae* (*5*). Intergenus spread of resistance can occur between Gram-positive species such as staphylococci and enterococci and between *Enterobacteriaceae* and *Pseudomonas* or anaerobes such as *Bacteroides* (*6*). Gram-positive species can transfer resistance to Gram-negative species, but the reverse is uncommon.

Antimicrobial agents are rendered inactive by three major mechanisms: (i) inactivation of the antibiotic by destruction or modification, (ii) prevention of access to the target, and (iii) alteration of the antibiotic target site (*3*). Some examples of inactivation are β-lactamase and aminoglycoside-inactivating enzymes (*7, 8*) (Table 2). The alteration of permeability or efflux of an agent occurs for β-lactams, aminoglycosides, and tetracyclines (*9*), and a single amino acid change in an enzyme alters the sensitivity of the targets for β-lactams, macrolides, and folate synthesis antagonists (*3, 10, 11*).

Staphylococci

In 1941, virtually all strains of *Staphylococcus aureus* worldwide were susceptible to penicillin G, but by 1944 *S. aureus* was

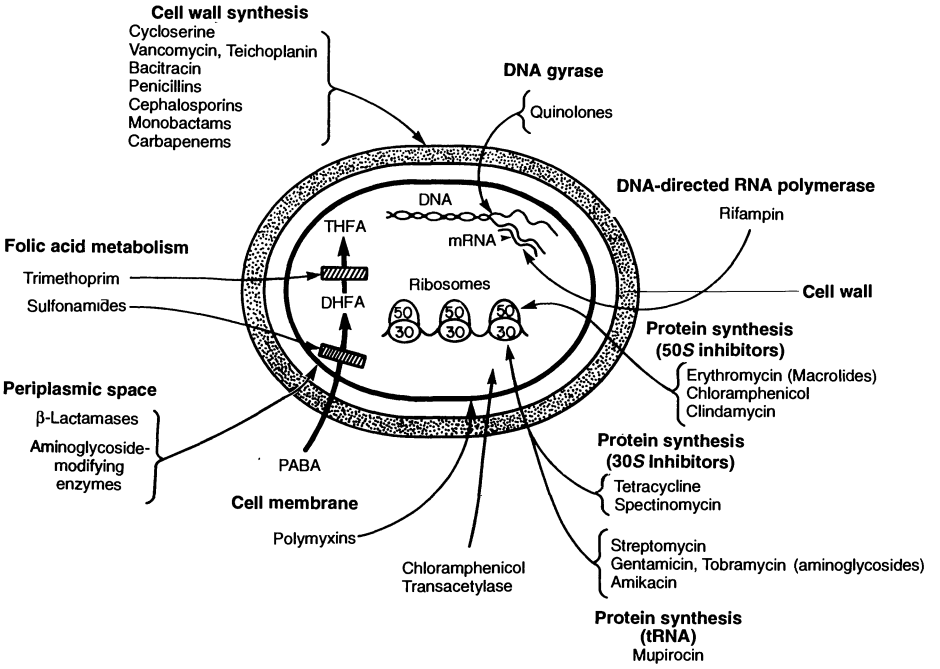

Fig. 1. Sites of action of various antimicrobial agents; mRNA, messenger RNA; tRNA, transfer RNA; PABA, *p*-aminobenzoic acid; DHFA, dihydrofolic acid; THFA, tetrahydrofolic acid.

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capable of destroying penicillin by means of penicillinase, which today is called a β-lactamase (Fig. 3). Today, in excess of 95% of *S. aureus* worldwide is resistant to penicillin, ampicillin, and the antipseudomonas penicillins (*12*).

The pharmaceutical industry responded to this challenge with the synthesis of methicillin, a semisynthetic penicillin. Resistance to methicillin was noted in hospitals in the United Kingdom, Poland, and Denmark, but such resistance was rare in the United States until the 1980s when methicillin-resistant *S. aureus* (MRSA) became a problem, particularly in long-term care facilities and among narcotics abusers (*12, 13*).

MRSA is resistant to all β-lactams, penicillins, cephalosporins, carbapenems, and penems (Fig. 2) because of a *mec*A gene that produces a new penicillin binding protein (PBP) 2a that has a low affinity for β-lactam antibiotics (*12, 14*). PBPs are transpeptidases that cross-link the cell wall of bacteria. They are called PBPs because radiolabeled penicillin binds to these proteins and is used as a marker to which the β-lactam compounds bind, thus inhibiting cell wall synthesis.

In burn centers, there is a ninefold increase in the chance of acquiring or developing a methicillin-resistant strain if the patient is treated with a β-lactam agent, and 14% of burn patients who become colonized with MRSA will develop bacteremia (*15*). In long-term care facilities, patients and staff carry these organisms in their nares or on their hands, and approximately 15% of patients will become infected per 100 days once they are colonized with MRSA (*13*). Approximately 25% of patients who carry MRSA will have an episode of infection, compared to 4% of those who are colonized with susceptible *S. aureus* (*16*). As a result of transposition and site-specific integration in many MRSA, the chromosome mediates resistance not only to β-lactam antibiotics but to other antibiotics such as erythromycin, fusidic acid, tetracycline, minocycline, streptomycin, spectinomycin, and sulfonamides and to disinfectants and toxic metals such as cadmium and mercury (*12, 17*). Resistance to these compounds is a result of different mechanisms. Tetracyclines undergo efflux that is a result of synthesis of a protein that removes the antibiotic. Aminoglycosides are modified through acetylation, adenylation, or phosphorylation and do not bind to ribosomes, and toxic metals are converted to other forms and excluded from the interior of the bacteria. The emergence of MRSA as a major problem worldwide has resulted in an increased use of vancomycin, the only agent that effectively treats these bacteria, but this increased use of vancomycin has created vancomycin resistance in other species such as enterococci.

MRSA is a classic example of the downfall of a new antimicrobial agent with respect to one species. In the mid-1980s, the new fluoroquinolone antimicrobial agents, such as ciprofloxacin, inhibited MRSA at <2 μg/ml (*18*). Fluoroquinolones eradicated the nasal carrier state of MRSA and cured serious infection that was a result of MRSA (*18*). It was assumed that the problem of MRSA was rapidly drawing to a conclusion. This was far from the case. Today at the Columbia-Presbyterian Medical Center in New York City, less than 20% of MRSA is inhibited by any of the commercially available fluoroquinolone antibiotics. A study by the Centers for Disease Control showed that ciprofloxacin resistance of MRSA went from less than 5% to greater than 80% within 1 year, and in the Veterans Administration Hospital in Portland, Oregon, the MRSA that colonized in elderly men hospitalized there were all resistant to ciprofloxacin (*19*). Fluoroquinolone-resistant MRSA is present in the United Kingdom, Europe, Japan, and other parts of the world. Fluoroquinolone resistance of MRSA is a result of an altered codon 84 or codon 85 in DNA gyrase and hence an alteration of the target (*20*).

Mupirocin, a pseudomonic acid derivative that interferes with protein biosynthesis, is highly effective against MRSA (*1*). Application of mupirocin to the nares results in eradication of the nasal carriage of *S. aureus*. This is extremely important because the nares are the site where MRSA and susceptible staphylococci persist. Hands are colonized with staphylococci, and unfortunately because most individuals touch their noses many times each day, the organisms are spread from hospital workers to patients and from patient to patient. Mupirocin has been available as a topical agent for only a short time in the United States. However, in the United Kingdom where the agent has been available for a number of years, MRSA with plasmid resistance to mupirocin has been found (*21*). These aspects about staphylococcal resistance illustrate the rapid ability of bacteria to become resistant to virtually all antibacterial agents, whether of natural origin, such as penicillin G or aminoglycosides, partially synthetic (cephalosporins), or totally synthetic, such as fluoroquinolones.

Staphylococci have acquired resistance to aminoglycosides because of aminoglycoside-modifying enzymes (*12*). Aminoglycosides can be phosphorylated, adenylated, or acetylated (Fig. 4). This changes the three-dimensional configuration of the compounds so that they do not bind adequately to the receptor proteins on the ribosomes and hence prevents normal protein biosynthesis. Aminoglycosides are not routinely used to treat staphylococcal infections but have been used for synergism which is provided by the combination of aminoglycosides and antistaphylococcal penicillins. Synergy means that a concentration fourfold less than the minimal inhibitory concentration (MIC) of each agent inhibits the bacteria. MRSA in which the entire population of bacteria is resistant to β-lactam antibiotics are usually resistant to aminoglycosides, and the combination of an aminoglycoside with vancomycin, another cell wall–inhibiting antibiotic, does not show synergy against these MRSA.

Table 1. Classification of antimicrobial agents by mechanism of action.

Mechanism of action	Agent
Inhibition of synthesis or damage to cell wall	Penicillins Cephalosporins Monobactams Carbapenems Bacitracin Vancomycin Cycloserine Fosfomycin
Inhibition of synthesis or damage to cytoplasmic membrane	Polymyxins Polyene antifungals
Inhibition of synthesis or metabolism of nucleic acids	Quinolones Rifampin Nitrofurantoins Nitroimidazoles
Protein biosynthesis	Aminoglycosides Tetracyclines Chloramphenicol Erythromycin Clindamycin Spectinomycin Mupirocin Fusidic acid
Modification of energy metabolism	Sulfonamides Trimethoprim Dapsone Isoniazid

Coagulase-negative staphylococci such as *Staphylococcus epidermidis, S. hemolyticus*, and *S. hominis* emerged as significant pathogens at the end of the 1970s (*22, 23*). They produce β-lactamase, and many of them that have low affinity for β-lactams possess PBP2a (*24*). Advances in medical technology have made *S. epidermidis* infection of prosthetic orthopedic devices and heart valves, peritoneal dialysis catheters, central nervous system shunts, and intravenous lines a major problem both in the hospital and in the community as therapy is administered at home for patients with malignancy or infection.

Staphylococcus hemolyticus has been a particular problem because it can be resistant to vancomycin (*25*). Vancomycin and teicoplanin are glycopeptides that inhibit late steps in bacterial cell wall peptidoglycan

synthesis (*1*). Most *S. epidermidis* that cause prosthetic valve endocarditis are resistant to methicillin because of altered PBP2a, resistant to aminoglycosides because of phosphorylating and adenylating enzymes, and resistant to fluoroquinolones because of an altered DNA gyrase A protein (*22, 23*). Although rifampin, which inhibits DNA-directed RNA polymerase activity, inhibits many *S. epidermidis*, resistance routinely develops when rifampin is used singly because most populations of staphylococci contain bacteria that have one of the four subunits of the enzyme with low affinity for rifampin, and even when rifampin is combined with vancomycin to achieve synergy, resistance to rifampin develops. *S. saprophyticus*, a cause of 10% of uncomplicated urinary tract infections in young women, is resistant to amoxicillin because of β-lactamases and is resistant to erythromycin because of the presence of an enzyme that methylates an adenine in the 16S component of the 23S ribosomal RNA (rRNA).

Coagulase-negative staphylococci are felt to be a reservoir of resistance genes amplified through antibiotic selection that occurs when antibiotics administered to patients achieve low concentrations in skin. The resistance genes can be transferred to *S. aureus*, making it resistant to multiple agents.

Streptococcus pneumoniae

In 1941, 10,000 units of penicillin administered four times a day for 4 days cured patients of pneumococcal pneumonia. Today, a patient could receive 24 million units of penicillin a day and die of pneumococcal meningitis. Pneumococci remain the most important cause of community-acquired pneumonia and a major cause of otitis media, sinusitis, and meningitis both in children and adults.

The resistance of *S. pneumoniae* was first noted in 1977 in South Africa but has recently become a worldwide problem. In Spain and other mediterranean countries, the majority of *S. pneumoniae* are resistant or relatively resistant to penicillin (*26–28*). Recently, highly resistant pneumococcal strains causing meningitis, bacteremia, empyema, and septic arthritis have been sent to the Centers for Disease Control (*29*). These strains are also resistant to cephalosporin antibiotics such as cefotaxime or ceftriaxone, which have been used to treat infection of the nervous system when the infection is caused by relatively resistant strains.

The mechanism of penicillin resistance of *S. pneumoniae* involves the development of altered forms of PBPs that have decreased affinity for β-lactam antibiotics (*30*). Strains with the highest level of resistance show reduction in the affinity of the five high molecular weight PBPs, 1a, 1b, 2a, 2x, and 2b. The PBP 2b and 2x genes of penicillin-resistant pneumococci differ extensively from the genes of susceptible pneumococci. It appears that the altered PBP genes arose by interspecies recombinational events in which segments of the PBP structural genes had been replaced by re-

Table 2. Resistance mechanisms. MRSE, methicillin-resistant *S. epidermidis*.

Antibiotic(s)	Mechanisms	Genetic basis	Present in pathogens	Crisis now	Future crisis
β-lactams Penicillins Cephalosporins Monobactams Carbapenems	Altered penicillin-binding proteins	Chromosomal	*S. aureus* *S. epidermidis* *S. pneumoniae* *S. sanguis* *H. influenzae* *N. gonorrhoeae* *N. meningitidis* *E. coli* *P. aeruginosa*	*S. pneumoniae* *S. epidermidis*	*N. meningitidis*
	Reduced permeability	Chromosomal	*P. aeruginosa* *E. cloacae* *S. marcescens* *K. pneumoniae* *K. oxytoca*	*P. aeruginosa* *E. cloacae*	*S. marcescens* *K. pneumoniae*
	β-lactamase	Plasmid and chromosomal	*S. aureus* *S. epidermidis* *Enterococci* *P. aeruginosa* *Enterobacteriaceae* *N. gonorrhoeae* *N. meningitidis* *Moraxella* *Bacteroides* *Acinetobacter*	*Xanthomonas* *Acinetobacter*	*Bacteroides* *N. meningitidis* *Enterobacteriaceae* *Salmonella* *Shigella* *Haemophilus* *Enterococci*
Fluoroquinolones Norfloxacin Ofloxacin Ciprofloxacin	Altered DNA gyrase	Chromosomal	*S. aureus* *S. epidermidis* *Enterobacteriaceae* *Pseudomonads*	MRSA	*Pseudomonads* *Enterobacteriaceae* *Haemophilus* *N. gonorrhoeae*
Lomefloxacin	Reduced permeability	Chromosomal	*Enterobacteriaceae* *P. aeruginosa*	*Serratia* *P. aeruginosa*	*Enterobacteriaceae*
Aminoglycosides Gentamicin Tobramycin Amikacin	Decreased ribosomal binding	Chromosomal	*Streptococci*	*Streptococci*	
	Reduced uptake	Chromosomal	*Bacteroides* *Pseudomonas* *Enterobacteriaceae*	*Pseudomonas*	*Enterobacteriaceae*
	Modifying enzymes	Plasmid	*Staphylococci* *Enterococci* *Streptococci* *Enterobacteriaceae* *Pseudomonads*	*Enterococci* *Pseudomonas* *Enterobacteriaceae*	*Streptococci*

gions derived from PBP genes of oral streptococcal species that are resistant to penicillins (30). A 23F serotype *S. pneumoniae* resistant to penicillin and present in children attending a day care center in Cleveland was identical to isolates from Spain in a comparison by electrophoretic analysis of the PBPs and by DNA restriction endonuclease cleavage of the PBP genes. Thus, by traveling, such organisms can be spread throughout the world (31).

High-level resistance of pneumococci has been encountered in Hungary, where in the period of 1988 to 1989, 58% of *S. pneumoniae* were resistant to penicillin, and 70% of children who were colonized with *S. pneumoniae* carried resistant strains that were also resistant to tetracycline, erythromycin, trimethoprim/sulfamethoxazole (TMP/SMX), and 30% resistant to chloramphenicol (32). In addition, resistant pneumococcal strains have emerged independently in many parts of the world.

The resistance of pneumococci to macrolides such as erythromycin averages 20 to 25% in France, 18 to 20% in Japan, and less than 10% in Spain (33). This resistance, referred to as macrolide-lincinoid-streptogramin (MLS) resistance, is a result of a plasmid-mediated production of an enzyme that methylates a crucial adenine residue in 23S rRNA. This results in the failure of binding of the erythromycin and related macrolides. The resistance can be induced by erythromycin, or bacteria can produce the methylase continuously, which is constitutive resistance. Macrolide-resistant *S. pneumoniae* are found in France, Belgium, and the United Kingdom, but are uncommon so far in the United States (33). The extensive use of macrolides in France compared to a lower use in Spain is thought to explain the difference in resistance between the two countries. It is possible that increased use of the new macrolides azithromycin, clarithromycin, and roxithromycin will cause an increase in macrolide-resistant *S. pneumoniae* in the United States. Erythromycin has been less frequently administered to adults in the United States because it causes gastrointestinal upset, but the new agents are better tolerated, and interest in using these agents to treat respiratory infections will increase resistance.

Streptococcus pyogenes

Streptococcus pyogenes, the group A streptococcus, has remained susceptible to penicillin, although the concentrations required to kill this organism have been increasing over the past two decades. MLS resistance was a problem in Japan in the early 1960s and has been a problem for years in France where there has been extensive use of erythromycin, josamycin, another oral macrolide, and pristinamycin, a closely related compound (33, 34). Outbreaks of erythromycin-resistant group A streptococci have occurred in day-care centers in Sweden (35). In western Australia, only 1% of *S. pyogenes* were resistant in 1985, but 17.6% were resistant in 1987 (36).

The use of oral erythromycin in Finland tripled from 1979 to 1989, and the frequen-

Table 2 (continued)

Antibiotic(s)	Mechanisms	Genetic basis	Present in pathogens	Crisis now	Future crisis
Macrolides-lincosamides	Methylating enzymes	Plasmid and chromosomal	*Streptococci*	*Enterococci*	*S. pneumoniae*
Erythromycin			*S. pneumoniae*		*S. pyogenes*
Clindamycin			*Enterococci*		*S. agalactiae*
			Staphylococci		*M. tuberculosis*
Chloramphenicol	Acetyltransferase	Plasmid and chromosomal	*Staphylococci*		*N. meningitidis*
			Streptococci		*S. pneumoniae*
			S. pneumoniae		
			Enterobacteriaceae		
			Neisseria		
Tetracyclines	Efflux	Plasmid	*Staphylococci*	*Enterobacteriaceae*	
Tetracycline			*Streptococci*	*Cholera*	
Minocycline			*Enterococci*		
Doxycycline			*Enterobacteriaceae*		
			Bacteroides		
	Ribosomal protein altered	Plasmid	*N. gonorrhoeae*		Mycoplasma
			Mycoplasma		
			Ureaplasma		
Rifampin	Reduced DNA polymerase binding	Chromosomal	*Staphylococci*	MRSA	*S. aureus*
			Enterococci		*M. tuberculosis*
			Streptococci		
			Enterobacteriaceae		
			Pseudomonads		
Folate-inhibitors	Altered targets	Plasmid and chromosomal	*Staphylococci*	*Enterobacteriaceae*	*H. influenzae*
TMP/SMX			*Streptococci*	*Shigella*	*P. cepacia*
			S. pneumoniae	*Salmonella*	MRSA
			Enterobacteriaceae	*Neisseria*	*S. pneumoniae*
			Neisseria	*Haemophilus*	
	Reduced permeability	Chromosomal	*Pseudomonads*		
			Campylobacter		
Glycopeptides	Altered target	Plasmid and chromosomal	*Enterococci*	*E. faecium*	MRSA
Vancomycin			*Leuconostac*		MRSE
Teicoplanin			*Lactococcus*		*Streptococci*
			Pediococcus		
			Lactobacillus		
			S. hemolyticus		
Mupirocin	Altered target	Plasmid			*Staphylococci*
Fusidic acid	Altered target	Chromosomal			*Staphylococci*
Fosfomycin	Altered transport	Chromosomal	*S. aureus*		
		Plasmid	*Serratia*		

cy of erythromycin resistance of group A streptococci from throat swabs increased from 7 to 20% and in cutaneous lesions increased from 11 to 31% in 1990 (*37*). Resistance was of multiclonal origin, which indicates that it appeared simultaneously in many areas and was not a result of one strain spreading across Finland. This resistance was associated with the failure of therapy for pharyngitis and skin infection, which indicates that the resistance was of clinical significance and not just a laboratory phenomenon.

Macrolide resistance of group A streptococci may become a serious problem. Sepsis and shock now occur as a result of infection with a particularly virulent new group A streptococci (*38*), and the increased use of new macrolide antibiotics clarithromycin, azithromycin, and roxithromycin will increase the likelihood that resistance will increase (*39*).

Enterococci

There currently are 12 enterococcal species, but *Enterococcus faecalis* accounts for 90% and *E. faecium* for 5% of cases around the world of endocarditis and urinary tract, wound, intra-abdominal, and pelvic infections. Enterococci have become the third most commonly encountered, hospital-acquired organism in the United States (*23, 40*).

Since 1983, penicillinase-producing *E. faecalis* have been found in many parts of the United States and Europe, and localized outbreaks have occurred (*23, 40, 41*). Its β-lactamase is similar to the β-lactamase of staphylococci. Aminoglycosides are used with penicillin, ampicillin, or vancomycin to treat serious enterococcal infections to achieve synergistic bactericidal activity; penicillin, ampicillin, and vancomycin by themselves are not bactericidal for enterococci. In Wisconsin in 1987, 35% of enterococci showed high-level gentamicin resistance (MIC >2000 μg/ml). Nationwide, 25% of enterococci possess high-level aminoglycoside-inactivating enzymes that cause resistance, and in other countries the percent of resistant isolates is higher (*23, 40–42*).

Glycopeptides such as vancomycin and teicoplanin inhibit late stages in bacterial cell wall peptidoglycan synthesis (*1*). Vancomycin- and teicoplanin-resistant enterococci are found in Europe and the United States (*43, 44*). There are three major phenotypes of vancomycin resistance (*44, 45*) (Table 3). High-level resistance to glycopeptides is mediated by a 34-kb plasmid and is transferable from *E. faecium* by conjugation to different Gram-positive bacteria such as oral streptococci, group A streptococci, and *Listeria monocytogenes* (*44, 45*).

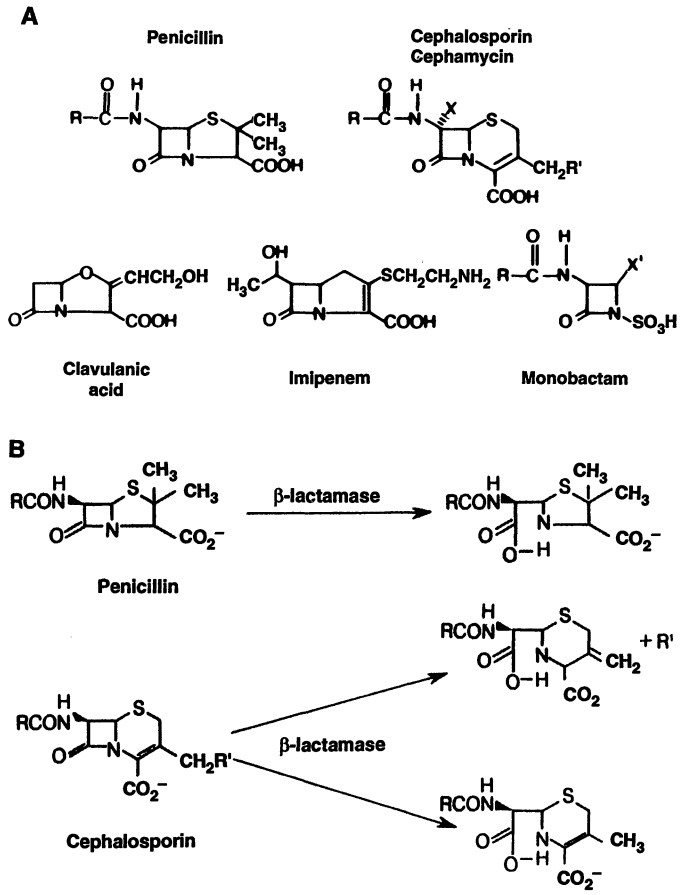

Fig. 2. (A) Basic structures of currently used β-lactam antibiotics. Modification of the penicillins, cephalosporins, and monobactams at R by addition of different moieties alters the antibacterial activity and β-lactamase stability of the compounds. Addition of different moieties to the R′ position of the cephalosporins alters both pharmacological and, to a lesser degree, antimicrobial activity. Placement of an O–CH_3, group at X in cephalosporins and –CH_3, at X′ in the monobactam provides β-lactamase stability. **(B)** Effect of β-lactamase attack on penicillins and cephalosporins. In the case of penicillins, a stable penicilloic acid is formed. The structures shown for the cephalosporins are postulated because the molecules undergo rapid decomposition to small fragments (*64*). R = β-acyl side chain.

A new protein of approximately 39 kD encoded by the gene *van*A is functionally related to the D-Ala-D-alanine ligases involved in cell wall synthesis in *E. coli*. It preferentially condenses D-alanine with D-phenylalanine or D-methionine, producing a modified cell wall in the Gram-positive bacteria (*11, 46*). Another gene, *van*H, encodes a D-keto acid reductase related to the D-lactate dehydrogenases of *Lactobacillus* and *Leuconostoc*, which are Gram-positive genera of bacteria intrinsically resistant to glycopeptides. The protein encoded by *van*A catalyses ester bond formation between the 2-hydroxy acid products of the protein encoded by the gene *van*H. These peptidoglycan precursors bind glycopeptides with a reduced affinity compared to the D-Ala-D-alanine ligases of susceptible enterococci. Vancomycin resistance has not spread so far to staphylococci, group A and B streptococci, or *S. pneumoniae*, but it may in the future.

Haemophilus influenzae

Ampicillin had been a major antibiotic used to treat *Haemophilus influenzae* meningitis from 1960 to the 1970s. In 1974, a plasmid-mediated β-lactamase was first noted in *H. influenzae*. Since then, resistance to ampicillin has continued to increase (*28, 47*). The β-lactamase is called TEM after a Greek girl from whom an *E. coli* strain was isolated that produced a plasmid-mediated β-lactamase (Fig. 3). A few *Haemophilus* strains contain a plasmid that mediates synthesis of another β-lactamase, Rob 1 (*28, 47*). β-lactamase production by *Haemophilus* ranges from 5 to 55%, and worldwide resistance of type b *Haemophilus* is approximately 20% (*28*). In Barcelona, Spain, 50% of *H. influenzae* b are resistant to five or more antibiotics, including chloramphenicol and TMP/SMX (*44*). The resistance of *Haemophilus* to chloramphenicol is a result of a plasmid-mediated enzyme, chloramphenicol transacetylase, that acetylates the two hydroxyl groups on the compound so that it will not bind to its ribosomal receptor protein. Resistance to TMP/SMX is a result of plasmid production of new pteridine synthetase and dihydrofolate reductase enzymes with a low affinity for these two antibacterial agents.

In the United States, most *H. influenzae* are susceptible to cephalosporins that possess an iminomethoxy or propyl carboxy moiety on the β-acyl side chain and to TMP/SMX (*28*). *Haemophilus* resistance to rifampin has developed in patients receiving it as chemoprophylaxis to prevent meningococcal or *Haemophilus* meningitis.

Neisseria and *Moraxella*

Most strains of *Neisseria meningitidis*, an important cause of meningitis, worldwide are susceptible to penicillin, but in Spain the concentration of penicillin needed to kill meningococci has increased by tenfold. *Neisseria* have acquired new PBPs from commensal organisms by gene transfer (*48*), and plasmids that mediate β-lactamase production have been found in *N. meningitidis* in Europe and North America.

Moraxella (Branhamella) *catarrhalis* causes otitis media and bacterial bronchitis in elderly individuals with chronic lung disease. In the 1970s, virtually 100% of isolates were susceptible to ampicillin, but now in excess of 75% produce β-lactamases that inactivate ampicillin, amoxicillin, and cefaclor, oral antibiotics widely used to treat ear and sinus infections (*49*).

Penicillin was the drug of choice to treat gonorrhea, but over the years the concentration of penicillin needed to achieve cure rose as a result of a chromosomal mutation that reduced the affinity of PBPs for penicillin. In 1976, the plasmid-mediated TEM β-lactamase of *E. coli* was found in *N. gonorrhoeae* in Africa and Asia. Today in the Philippines and Thailand, in excess of 90% of *N. gonorrhoeae* produce β-lactamase (*50*). In Washington Heights in New York City, we have found 42% of *N. gonorrhoeae* to be resistant to penicillin. In 1986, a plasmid that contains the gene *tet*M and mediates tetracycline resistance in enterococci, *Mycoplasma hominis*, *Ureaplasma urealytica*, and *Gardnerella vaginalis*, appeared in gonococci. This plasmid produces a protein that causes efflux of tetracyclines so that the drug does not bind to its 30S ribosome receptor site. The concentrations of β-lactamase–stable cephalosporins such as ceftriaxone and cefixime and of fluoroquinolone agents required to kill *N. gonorrhoeae* isolates from Asia have been increasing yearly.

Enteric Pathogens

That plasmids encode for resistance was first recognized in Japan in 1959. *Shigella*, a major cause of dysentery particularly in Asia and Central and South America, now possess plasmids that mediate production of resistance to ampicillin, chloramphenicol, tetracycline, aminoglycosides, and TMP/SMX. *Shigella dysenteriae* type 1, for which it has not been possible to make a vaccine, in the 1970s killed more individuals in Central America and Mexico than were killed in the various conflicts in Central America in the 1980s (*51*).

Nontyphoidal *Salmonella*, which have caused outbreaks of diarrhea infecting as many as 100,000 people in the United States, are resistant to multiple antibiotics (*51*). In the Far East *Salmonella typhi*, the cause of typhoid, is resistant to chloramphenicol, ampicillin, and TMP/SMX, the antibiotics that had been used to treat this serious infection. *Salmonella* have recently been found in Europe and Asia that produce modified β-lactamases (Table 2). These enzymes are related to the TEM enzyme. A change of one, two, or three amino acids in the enzyme changes the affinity of the β-lactamase for the cephalosporins that contain either an iminomethoxy or propylcarboxy β-acyl side chain. Currently, only fluoroquinolones can be used to treat some of these *Salmonella* infections.

Antibiotics decrease the length of illness and decrease intestinal shedding of *Vibrio cholera*, helping to reduce the spread of cholera. Cholera is currently a major problem in South and Central America, where isolates are resistant to tetracycline, sulfonamides, chloramphenicol, kanamycin, and TMP/SMX. This means that inexpensive antibiotics that would decrease the spread of cholera by decreasing the organism burden are no longer effective.

Campylobacter jejuni is an important cause of diarrhea in both industrialized and developing countries. It is intrinsically resistant to TMP/SMX because the dihydrofolate reductase is not competitively inhibited by trimethoprim. Recently, *Campylobacter* has become tetracycline-resistant as a result of acquiring the *tet*M gene from en-

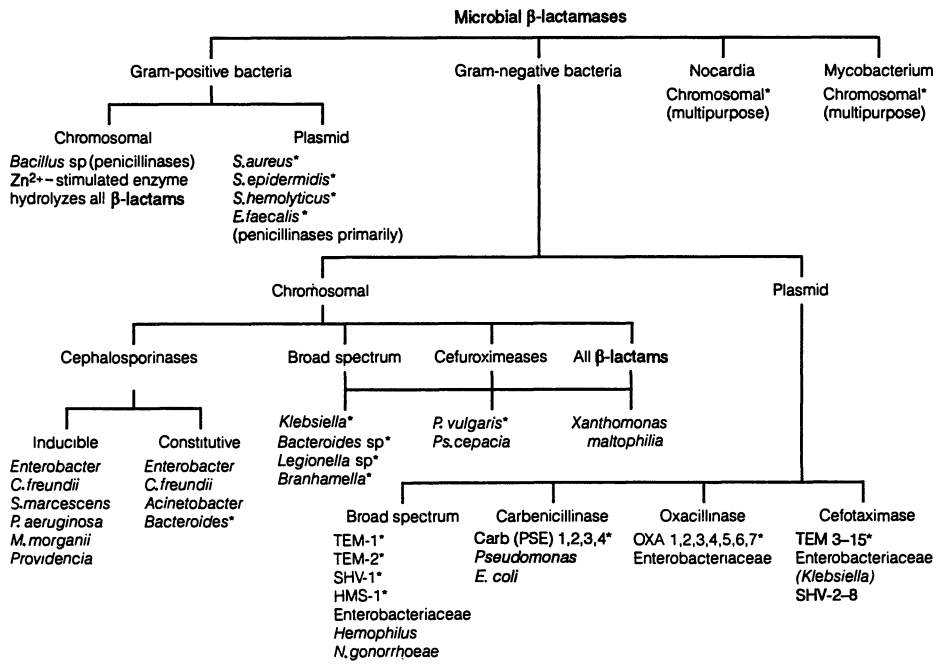

Fig. 3. β-lactamases and their distribution in nature. Asterisks indicate that these bacteria are inhibited by clavulanate, sulbactam, and tazobactam.

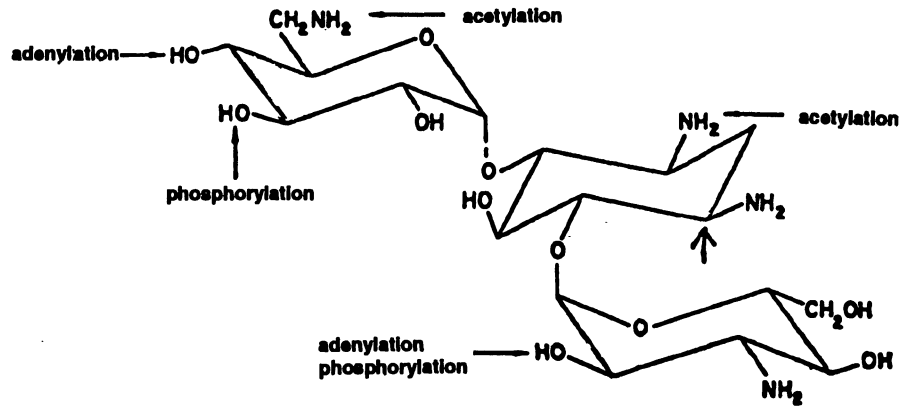

Fig. 4. A prototype aminoglycoside. The activity of the compounds depends on the position of –OH and $-NH_2$ groups as well as substitutions at positions indicated by the arrow. Bacterial resistance to aminoglycoids is the result of acetylation, adenylation, or phosphorylation by enzymes produced by plasmids present in bacteria.

terococci. In addition, resistance to ciprofloxacin, a fluoroquinolone, is the result of an altered DNA gyrase A subunit (*18*).

Hospital Bacteria

Escherichia coli is an important cause of community- and hospital-acquired infections such as uncomplicated urinary tract infections, pyelonephritis, and hospital bacteremia. *Klebsiella, Serratia, Proteus, Enterobacter*, and other aerobic Gram-negative bacteria have become increasingly important causes of nosocomial infections and have unfortunately acquired multiple resistance to antibiotics (*51*). As many as 30% of community-isolated *E. coli* and 40 to 50% of hospital-acquired *E. coli* are resistant to amoxicillin, one of the most widely used oral semisynthetic penicillins. *E. coli* with uropathogenic abilities—that is, the ability to attach to epithelial cells of the bladder or kidney—are resistant to multiple antibiotics. TMP/SMX resistance of *E. coli* from the urine of patients in geriatric units in the United States averages 40%. In the United States, *E. coli* isolated from the feces of children in day-care centers show high resistance to amoxicillin. Seventy-five percent of community-isolated *E. coli* in India are resistant to ampicillin and TMP/SMX. Of greatest concern is the fact that some of these *E. coli* are resistant to ampicillin/clavulanate and ampicillin/sulbactam. Clavulanate and sulbactam are β-lactam compounds that inhibit β-lactamases. Inexpensive drugs administered orally or parenterally are no longer useful in the treatment of urinary tract infections or other infections in which *E. coli* is a likely pathogen. This is particularly a problem in developing countries.

In the mid-1980s in France and Germany, the failure of therapy for *Klebsiella* infections suddenly occurred because *Klebsiella pneumoniae* were resistant to cefotaxime, ceftriaxone, or ceftazidime, agents considered totally β-lactamase–stable. Resistance was a result of new β-lactamases initially referred to by the compound that they preferentially destroyed, such as CTX-1 for cefotaxime or CAZ-1 for ceftazidime (*7, 8*) (Fig. 3). As already noted, these β-lactamases differ from the original TEM-1 enzyme by one, two, or three amino acids, and have been designated as TEM-3, -4, and -5, up to TEM-15. There are also amino acid variants of another β-lactamase, SHV-1 (sulfohydrovariable inhibited). There are now eight SHV enzymes (*7, 8*).

β-lactamases of these types have been found in *E. coli, Klebsiella, Proteus, Serratia, Salmonella*, and *Enterobacter* species (*8, 52*). In vivo transfer of resistance from *K. pneumoniae* to other organisms can occur, and a patient may have different organisms that contain the same or different β-lactamases as a result of gene transfer. In vivo dissemination has been demonstrated that involves transposable elements that translocate multiresistance genes, including these β-lactamases that destroy cephalosporins and the monobactam aztreonam. β-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam inhibit these new β-lactamases, but the antibiotics with which β-lactamase inhibitors are combined often are not effective in eradicating *Klebsiella* species that contain the new TEM and SHV β-lactamases (*53*).

Klebsiella pneumoniae that contain β-lactamases that destroy new cephalosporins and also possess aminoglycoside-modifying enzymes have caused hospital outbreaks of wound infection and septicemia. The gastrointestinal tract of hospitalized patients may contain *Klebsiella* that carry a plasmid encoding both adhesion to intestinal cells and β-lactamase production (*54*). This allows the organisms to colonize the intestine of both patients and hospital personnel. In this, *Klebsiella* are similar to the *Shigella dysenteriae* responsible for outbreaks of diarrhea in Central America in the 1970s, in which invasiveness and resistance was on the same plasmid.

Undoubtedly, widespread use of extended spectrum cephalosporins, albeit appropriate, has resulted in the selection of mutants of the original TEM-1 β-lactamase. With increased use in the coming years of oral cephalosporins, which are only partially absorbed in the gastrointestinal tract and which have chemical moieties that have been associated with the selection of bacteria that possess the new β-lactamases, there will be marked selective pressure in the gastrointestinal tract. We probably will see more bacteria that contain β-lactamases that readily destroy all of the currently available cephalosporins and monobactams.

Another resistance crisis is the appearance of *Enterobacter* species as a significant cause of nosocomial infections. They have replaced *K. pneumoniae* as the third leading cause of Gram-negative nosocomial infections in the United States behind *E. coli* and *Pseudomonas aeruginosa* (*55*). Why did this occur? *Enterobacter* species were not even considered important organisms until the end of the 1960s and throughout the 1970s were thought to be of minor significance.

Enterobacter and several other members of the *Enterobacteriaceae* such as *Citrobacter freundii, Serratia marcescens, Morganella morganii*, and *Proteus vulgaris* are not truly susceptible to the new cephalosporin antibiotics that were not destroyed by the plasmid-mediated TEM-1 β-lactamases or the chromosomally mediated β-lactamase of *Klebsiella*. These organisms, particularly *E. cloacae*, possess a cephalosporinase referred to as a type 1 enzyme (*7, 8, 56*). The β-lactamases are of chromosomal origin and not normally expressed, but expression of high concentrations of the enzyme is triggered by exposure to certain cephalosporins (*56*). Most resistance of *Enterobacter* to

Table 3. Resistance of Gram-positive bacteria to vancomycin and teicoplanin. ND, not determined; R, resistant; S, susceptible.

Relevant characteristics	Acquired resistance		Intrinsic resistance		
	High level	Low level	Low level		High level
MIC (μg/ml)					
Vancomycin	≥64 (R)	16 to 32 (R)	8 to 16 (R)	2 to 32 (R)	≥1000 (R)
Teicoplanin	≥16 (R)	0.5 (S)	0.5 (S)	0.5 to 1 (S)	≥ 250 (R)
Transferable by plasmid	Yes	No	No	No	No
Inducibility by:					
Vancomycin	Yes	Yes	No	ND	No
Teicoplanin	some strains	No	No	ND	No
Molecular size of resistance protein (kD)	39 to 40	39.5	ND	ND	ND
Microorganisms	*E. faecium* *E. faecalis*	*E. faecium* *E. faecalis* *S. epidermidis* *S. hemolyticus*	*E. gallinarum*	*E. casseliflavus*	*Leuconostoc* spp. *Pediococcus* spp. *Lactobacillus* spp. *E. rhusopathiae* *Actinomycetes*

β-lactams is a result of selection of preexisting mutant organisms that produce large amounts of β-lactamase constitutively. Some of these organisms also have decreased expression of the outer membrane protein porin F, the channel through which cephalosporins cross the outer cell wall of a Gram-negative bacterium, coupled with increased expression of porin C, a channel through which the cephalosporins do not readily pass. This results in inhibition of the entry of the cephalosporins or of penicillins. High affinity of the β-lactamase for the cephalosporins with a decreased amount of drug coming into the periplasmic space where the β-lactamase resides results in destruction of the cephalosporin (Fig. 5).

The β-lactamase inhibitors clavulanate, sulbactam, and tazobactam do not inhibit the type 1 β-lactamases found in *Enterobacter* (*7*, *8*). Extensive use of β-lactamase–stable cephalosporins, particularly in intensive care units in hospitals, acts as a selection pressure to cause the appearance of *Enterobacter*, and numerous serious infections have occurred, including lethal bacteremias (*55*).

The carbapenem imipenem inhibits *Enterobacter* species because it enters the periplasmic space and reaches the PBPs via a different porin channel than that used by cephalosporins, and it is very β-lactamase–stable. Imipenem is a very effective inducer of the production of type 1 β-lactamase. Clinical isolates recently have been found that are resistant to imipenem. They lack the major outer membrane porins and have increased β-lactamase production in the periplasmic space with destruction of imipenem (*57*). I would anticipate that by the end of the decade, we will see increasing numbers of imipenem-resistant *Enterobacter*.

Cephalosporin resistance can also occur with *Proteus* and *Serratia* species, albeit so far a less frequent problem. *Serratia marcescens* have become increasingly common as a cause of nosocomial wound, urinary tract, pulmonary, and even bacteremic infections. In general, *S. marcescens* in the United States are susceptible to fluoroquinolones (MICs <1 μg/ml). Extensive resistance of *Serratia* to the fluoroquinolones has been found in Japan (*18*), and we have similar organisms in our hospital. The mechanism of the resistance is a mutation that results in an amino acid change at a critical turn in the A subunit of the DNA gyrase.

Outbreaks of infection that are a result of aminoglycoside-resistant *S. marcescens* have occurred. These isolates possess enzymes that adenylate, phosphorylate, or acetylate the aminoglycosides (Fig. 4). This causes the aminoglycoside to bind less well to its receptor protein and also results in the failure to produce a protein that causes rapid uptake of aminoglycosides, as occurs

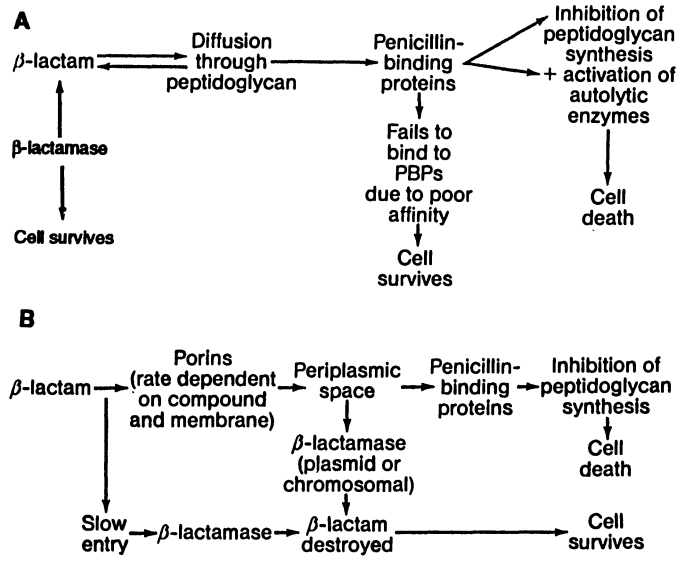

Fig. 5. The interaction of β-lactam antibiotics with Gram-positive and Gram-negative bacteria. (**A**) Gram-positive bacteria. (**B**) Gram-negative bacteria. In resistant Gram-positive bacteria such as *Staphylococci*, β-lactamases are excreted as exoenzymes and destroy the β-lactams, or the bacteria contain transpeptidases, which fail to bind the β-lactams. In Gram-negative bacteria, β-lactam antibiotics enter the periplasmic space of bacteria via outer membrane proteins, porins. In the space between the outer wall and the cytoplasmic membrane, β-lactamases can destroy the β-lactams before they reach and bind to PBPs.

in susceptible bacteria. The aminoglycoside-modifying enzymes exist in the periplasmic space of Gram-negative bacteria and are highly efficient at producing resistance. *Serratia* resistant to β-lactams, aminoglycosides, fluoroquinolones, and even TMP/SMX have been found in hospitals in the United States, Europe, and Japan.

Pseudomonas aeruginosa is a major cause of serious infection in many patients, particularly those who lack white cells as a result of hematologic malignancy or chemotherapy (*58*). Resistance usually is a combination of poor entry of antibiotics and the presence of enzymes that inactivate or modify the antibiotics (*7–9*). All *P. aeruginosa* contain an inducible, chromosomally mediated cephalosporinase (*7*, *8*). Some otherwise β-lactamase–stable cephalosporins induce the production of large amounts of the β-lactamase, which results in their own destruction. The resistance of *Pseudomonas* to antipseudomonas penicillins is usually a result of the presence of the plasmid-mediated TEM-1 β-lactamase. Other plasmid-mediated β-lactamases will hydrolyze antipseudomonas penicillins and cefoperazone and cefsulodin, two antipseudomonas cephalosporins; these β-lactamases are abbreviated PSE for *Pseudomonas* and are numbered 1 to 4. Ceftazidime and aztreonam, a monobactam, are not hydrolyzed by these β-lactamases, but ceftazidime can cause selection of hyperproducers of β-lactamase that render the organism resistant to it and to aztreonam (*59*). Aminoglycoside-inactivating enzymes are common in *Pseudomonas*, and a 6′-*N*-acyl-transferase enzyme occurs on a transposon in *Pseudomonas* and mediates resistance to the most stable aminoglycoside, amikacin (*2*). Clinical isolates of *P. aeruginosa* that are resistant to all aminoglycosides because of altered cell wall lipopolysaccharide and porins cause the lack of accumulation of drugs and are an extremely common cause of respiratory infection in patients with cystic fibrosis or in burn patients.

The resistance of *P. aeruginosa* to the carbapenem, imipenem, has increased (*59*). A recent study from the Centers for Disease Control revealed that resistance to imipenem isolated from the respiratory tract of patients in intensive care units and in teaching hospitals had increased by 25% in the period from 1986 through 1990 (*59*). Similar resistance has been noted in Japan. The resistant *P. aeruginosa* lack a D2 outer membrane protein. Two new carbapenems, meropenem and a drug under early clinical investigation, LJC 10,627, are less effective inducers of the β-lactamase. They inhibit some imipenem-resistant *P. aeruginosa*, but *P. aeruginosa* that produce large amounts of β-lactamase and that have lost the D2 outer membrane protein are highly resistant to all the carbapenems because inadequate numbers of molecules reach the PBPs.

In 1984, virtually 100% of *P. aeruginosa* in the United States, Europe, and Japan were inhibited by <1 μg/ml of ciprofloxacin (*18*). Today in some institutions, 25% of *P. aeruginosa* are resistant to all the fluoroquinolones (*18*). This has been particularly true in isolates from individuals with respiratory infection with cystic fibrosis or severe wound infections. Resistance in these strains has been shown to be a result of a combination of alteration of the A subunit of the DNA gyrase and changes in outer membrane proteins as well as changes in cell wall lipopolysaccharide (*58*, *60*).

Pseudomonas cepacia, an important nosocomial pathogen and a cause of lung abscesses in cystic fibrosis patients, is resistant to β-lactams, fluoroquinolones, and aminoglycosides (*58*). *P. pseudomallei*, a major pathogen in the Far East, is resistant to

many aminoglycosides and many other agents and has developed new β-lactamases, which make it resistant to the broad-spectrum cephalosporins and even to β-lactamase inhibitors.

Xanthomonas maltophilia, formerly called *Pseudomonas maltophilia*, produces two inducible β-lactamases, one of which is a zinc metallo enzyme that destroys all β-lactam compounds (Fig. 3). *Xanthomonas* is resistant to virtually all antibiotics, including the new fluoroquinolones and aminoglycosides (*61*). *Xanthomonas* has caused outbreaks in intensive care units in which there has been extensive use of imipenem (*61*).

Acinetobacter calcoaceticus anitratus has become an important cause of nosocomial infections in the hospital (*58*). It is resistant to β-lactams, aminoglycosides, tetracyclines, and aminoglycosides. Most *Acinetobacter* have been susceptible to carbapenems such as imipenem, but *Acinetobacter* probably will become resistant to these agents as have many other organisms.

Anaerobic Bacteria

Twenty years ago, all anaerobic bacteria in the mouth that were capable of causing aspiration pneumonia were susceptible to penicillin G. This is no longer the case. *Bacteroides melaninogenicus* and other oral *Bacteroides* make β-lactamases that destroy penicillins (*62*) (Fig. 3). Resistance of *B. fragilis*, the major anaerobic organism in the large bowel, to β-lactams ranges from 3 to 30%, depending upon whether it is in the United States, Canada, or Europe. Some *B. thetaiotaomicron* hydrolyze highly β-lactamase–stable compounds such as the cephalosporin cefoxitin and even imipenem (*7, 8, 62*). Because genetic material can be exchanged between *Bacteroides* and *E. coli* and vice versa, it is conceivable that within this decade we will see *Enterobacteriaceae* resistant to carbapenems such as imipenem and meropenem.

Before 1970, less than 30% of *Bacteroides* that caused intra-abdominal or gynecological infection were resistant to tetracycline. Now, it is rare to find a susceptible strain because most organisms possess a plasmid-mediated protein that causes efflux of tetracyclines. Resistance to metronidazole, a nitroimidazole, has been exceedingly rare, but plasmid resistance to metronidazole in *Clostridium* species has been noted.

Summary

Antibiotic use has contributed significantly to the increased resistance found in many *Enterobacteriaceae*, particularly the new β-lactamases found in *Klebsiella* species, and to the appearance of *Enterobacter* and *Pseudomonas* that produce large amounts of β-lactamase (*63*). It is probable that the extensive use of vancomycin to treat MRSA, and as therapy of *Clostridium difficile* diarrhea, has contributed to the development of plasmid resistance in enterococci. The extensive use of antibiotics in animal feeds may explain the high antibiotic resistance of *Salmonella* species.

I believe that, for the remainder of the 1990s, we will see increased numbers of penicillin-resistant *S. pneumoniae*. Increased use of macrolides will result in increased resistance in *S. pneumoniae* and *S. pyogenes*. Continued use of cephalosporins, even that which is necessary, will cause an increase of new β-lactamases and of infections that are a result of *Enterobacter*, *Citrobacter*, and *Serratia* that carry type 1 β-lactamases. Resistance due to these organisms will undoubtedly result in increased use of carbapenems, such as imipenem and meropenem, with increased resistance of *P. aeruginosa* to carbapenems and the appearance of *Xanthomonas* resistant to carbapenems in intensive care units.

Mechanisms to overcome bacterial resistance range from obvious hygienic practices to stop the spread of bacteria in a hospital to the synthesis of agents with improved antimicrobial activity (*58*). Antibiotic control programs have proved to be an effective way to reduce inappropriate use of antibiotics in hospitals. Such programs are sorely needed in the community, particularly in residence facilities for the elderly.

The need for new antibiotics will continue because bacteria have a remarkable ability to overcome each new agent synthesized. Appropriate use of antibiotics will delay and in many cases prevent the emergence of the resistance discussed herein. The responsibility of reducing resistance lies with the physician who uses antimicrobial agents and with patients who demand antibiotics when the illness is viral and when antibiotics are not indicated. It also is critical for the pharmaceutical industry not to promote inappropriate use of antibiotics for humans or for animals because this selective pressure has been what has brought us to this crisis.

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The Origin of Plagues: Old and New

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Viruses and bacteria emerge in new and old forms to cause disease epidemics. Some microorganisms recur when changing life-styles (including increased international travel) offer new opportunities; others arise from new genetic variations. These various epidemics connect the future with the past, offering lessons for guarding the health of generations to come—lessons learned from diseases such as tuberculosis, toxic shock syndrome, Lyme disease, streptococcal infection, influenza, and acquired immunodeficiency syndrome (AIDS). The public must be vigilant to the possibility of new epidemics, learn more about the biology and epidemiology of microbes, and strengthen systems of surveillance and detection.

Acquired immunodeficiency syndrome is the most contemporary example of human vulnerability to the microbial world, and there is genuine concern that another "new" microbe, or a genetic variation of an old one, can and will "go global" as AIDS has done. A new epidemic may be incubating even now in the crowded, unsanitary mega-cities of the developing world, or in remote jungles in Africa, South America, or Asia—once sparsely inhabited regions that have recently been altered by modern civilization (*1–4*).

AIDS is not the only case of a microbial threat to human beings in the late 20th century. Since the 1970s, a series of unanticipated outbreaks of microbial diseases startled inhabitants of the United States. Legionnaires' disease, toxic shock syndrome, and Lyme disease all happened before the recognition of AIDS in 1981.

In addition to these threats within the United States, strange epidemics have been occurring elsewhere in the world. In Africa, outbreaks of the deadly Ebola virus took the lives of 50% of the people who became infected. A majority of the doctors and nurses who treated these patients also died of the disease. After fatal but localized outbreaks of Ebola fever, the disease failed to become a worldwide epidemic. At the same time, however, the AIDS virus, human immunodeficiency virus (HIV), was spreading from the rural communities of Africa to the towns and cities, infecting thousands of people in Central Africa alone. After a few years, HIV, which has a long latency period, manifested itself when those who were infected developed the medical symptoms of full-blown disease. By that time, AIDS had spread from Africa to the United States, Europe, Asia, and elsewhere.

Many factors interact and contribute to the reemergence of old infectious diseases or the emergence of new ones. More often than not, epidemics occur because of changes in the patterns of human behavior, social organization, urbanization, and agriculture. However, the most important factor is the spread of microbial organisms from points of origin as a result of the migration and travel of their human hosts (*5*).

In ancient times, infectious diseases spread slowly but steadily along caravan routes throughout the Roman and Asian world (*6*, *7*). From A.D. 165 to 180, measles was spread along the caravan routes, and from A.D. 251 to 266 smallpox was carried. One-third of the population died. Such a catastrophe did not recur until the bubonic plague spread from Asia to Europe in the 13th and 14th centuries. This occurred when the horsemen of the Mongol armies raced across the steppes of Asia, transmitting the disease from the point of origin in northern Burma. They carried fleas infected with plague bacillus. From there the plague moved farther eastward via the caravan routes to Europe and elsewhere. After 1492, the oceans became highways that further extended the dispersal of disease agents. It became possible for plagues such as smallpox and measles to circle the globe within a year. The oceans remained the predominant route of transmission until the present era of mass air travel. Today, airborne travelers incubating infections can reach any point on the globe within 24 hours. As a consequence, worldwide exposure to a highly infectious virus, such as influenza, occurs in a matter of weeks.

However, microbes are not idle bystanders, waiting for new opportunities offered by human mobility, ignorance, or neglect. Microbes possess remarkable genetic versatility that enables them to develop new pathogenic vigor, to escape population immunity by acquiring new antigens, and to develop antibiotic resistance.

For these reasons, it is necessary to be prepared for new epidemics caused by old

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