

Review Article

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ANTIMICROBIAL-DRUG RESISTANCE

HOWARD S. GOLD, M.D.,
AND ROBERT C. MOELLERING, JR., M.D.

SINCE their discovery, antimicrobial drugs have proved remarkably effective for the control of bacterial infections. However, it was soon evident that bacterial pathogens were unlikely to surrender unconditionally, because some pathogens rapidly became resistant to many of the first effective drugs. For example, the development of resistance to penicillin in *Staphylococcus aureus* by the production of a β -lactamase quickly decreased the usefulness of penicillin for serious staphylococcal infections, especially among hospitalized patients, in whom resistant strains are frequently found before they spread to the community.¹ Initially, the problem of bacterial resistance to antimicrobial drugs was solved by the discovery of new classes of drugs, such as the aminoglycosides, macrolides, and glycopeptides, as well as by the chemical modification of previously existing drugs. Unfortunately, there is no assurance that the development of new antimicrobial drugs can keep pace with the ability of bacterial pathogens to develop resistance.

As we have learned more about the mechanisms and epidemiology of resistance to antimicrobial drugs, it has become clear that bacteria have a remarkable array of tools at their disposal to overcome antibiotics. A single genetic mutation may lead to resistance without altering the pathogenicity or viability of a bacterial strain. The development of resistance to antituberculous drugs such as streptomycin is a classic example of this type of change.² Theoretically, it should be possible to overcome mutational resistance by administering a combination of drugs in sufficient dosage and long enough to eradicate the infection, thus preventing person-to-person dissemination of resistant bacteria. The worldwide emer-

gence of multidrug-resistant *Mycobacterium tuberculosis* demonstrates that this goal may not be easy to achieve.³ Another important example of mutational resistance is the development of fluoroquinolone resistance in staphylococci, *Pseudomonas aeruginosa*, and other pathogens through alterations in DNA topoisomerase.⁴ Mutational events may also alter existing mechanisms of resistance to make them more active or give them a broader spectrum of activity. An example is changes in existing plasmid-mediated β -lactamases that result in extended-spectrum β -lactamases, which will be discussed in this article.

Of potentially greater concern is the fact that bacteria may acquire exogenous genetic material that leads to antimicrobial resistance. Species such as pneumococci and meningococci can take up foreign DNA and incorporate it into their chromosomes.⁵ Many of the genes that mediate resistance are found on transferable plasmids or on transposons that can be disseminated among various bacteria by conjugation.⁶ Transposons are mobile pieces of DNA that can insert themselves into various locations on the bacterial chromosome, as well as move into plasmids or bacteriophage DNA. Some transposons or plasmids have genetic elements termed integrons that enable them to capture exogenous genes.⁷ A number of genes may therefore be inserted into a given integron, resulting in resistance to multiple antimicrobial drugs⁸ or possibly allowing the accumulation of both regulatory and structural genes in the same transposon. A similar mechanism may have been involved in the assembly of the genetic elements that code for vancomycin resistance in enterococci.⁹

It appears that many of the genes determining resistance have been present in nature and predate the clinical use of antimicrobial drugs.¹⁰ Some of these genes are similar to those found in antibiotic-producing organisms themselves.¹¹ It is the use of antimicrobial drugs for prophylactic or therapeutic purposes in humans or for veterinary or agricultural purposes that provides the selective pressure favoring the overgrowth of resistant organisms.^{1,12} In a number of countries, many antimicrobial drugs are freely available without prescription. However, overuse and inappropriate use of these drugs are hardly unique to these countries. A recent survey by the Centers for Disease Control and Prevention documented increasing use of broader-spectrum, more expensive antimicrobial drugs by office-based physicians in the United States to treat otitis media, sinusitis, and other common infections.¹³ The widespread use of antimicrobial drugs for immunocompromised patients and in the intensive care units of modern hospitals clearly results

From the Division of Infectious Diseases (H.S.G.) and the Department of Medicine (H.S.G., R.C.M.), Deaconess Hospital and Harvard Medical School, Boston. Address reprint requests to Dr. Moellering at the Department of Medicine, Deaconess Hospital, 1 Deaconess Rd., Boston, MA 02215.

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in the selection of the multidrug-resistant organisms that cause serious nosocomial infections.

We now find ourselves following two seemingly opposite trends. The prevalence of antimicrobial-resistant human pathogens is rapidly increasing, but the discovery and development of new antimicrobial drugs that are active against multidrug-resistant organisms have slowed dramatically.¹⁴ There are many reasons for this, including the current cost of bringing a new antibiotic from discovery to the market (between \$100 million and \$350 million in the United States). Moreover, most of the obvious bacterial targets for antimicrobial agents have been discovered, so that it will take new methods and increased effort to discover novel agents.

Although the extent to which bacteria develop resistance to antimicrobial drugs and the speed with which they do so vary with different types of drugs, so far resistance has developed to all antimicrobial drugs. Moreover, there are increasingly frequent reports of clinical problems caused by bacteria resistant to multiple antimicrobial drugs.¹⁵ Tables 1 and 2 provide an overview of some of the recent problems with antibiotic resistance according to drug and organism. Here, we will highlight three mechanisms of resistance that have caused major clinical problems or have the potential to do so in the near future. These are the emergence of extended-spectrum β -lactamases in gram-negative bacilli, the worldwide dissemination of penicillin-resistant (often multidrug-resistant) pneumococci, and the development of transferable resistance to vancomycin in enterococci.

EXTENDED-SPECTRUM β -LACTAMASES AS A CAUSE OF ANTIMICROBIAL-DRUG RESISTANCE

Although there is a variety of mechanisms of bacterial resistance to β -lactam antibiotics, the most important are the β -lactamases, which are enzymes capable of hydrolyzing the β -lactam ring of penicillins, cephalosporins, and related antimicrobial drugs, rendering them inactive. There are dozens of β -lactamases, which vary in substrate specificity and host range.^{16,17} Much of the impetus to develop new β -lactam antibiotics has been the emergence of bacteria that produce β -lactamases capable of destroying existing antibiotics. The earliest cephalosporins (for example, cephalothin) are susceptible to cleavage by a variety of β -lactamases commonly found in gram-negative bacilli, including the chromosomal cephalosporinases of pseudomonas, enterobacter, and other genera, as well as the common plasmid-borne enzymes of Enterobacteriaceae. The latter enzymes also hydrolyze a variety of penicillins and, unlike the chromosomal cephalosporinases, are usually inactivated by β -lactamase inhibitors such as clavulanic acid.¹⁶

Modifications of the structure of cephalosporins produced the cephamycins, including cefotetan and

TABLE 1. SELECTED CURRENT PROBLEMS WITH ANTIMICROBIAL-DRUG RESISTANCE, ACCORDING TO DRUG CLASS.

ANTIBIOTIC CLASS	MECHANISM OF RESISTANCE
Cephalosporins	Extended-spectrum β -lactamases, chromosomal cephalosporinases
β -Lactamase inhibitors	Hyperproducers of β -lactamases, new β -lactamases resistant to inhibitors, chromosomal cephalosporinases
Carbapenems	Zinc metalloenzymes and other β -lactamases
Vancomycin, teicoplanin	Modified cell-wall precursors with decreased affinity for vancomycin
Quinolones	Alterations in DNA topoisomerase, efflux mechanisms, permeability changes
Trimethoprim-sulfamethoxazole	Resistant enzymes in folate-synthesis pathway
Erythromycin, new macrolides	Methylation of the bacterial ribosome producing resistance to macrolides, clindamycin, and streptogramin B antibiotics
Aminoglycosides	Aminoglycoside-modifying enzymes

cefexitin, which are resistant to many plasmid-mediated β -lactamases.¹⁸ Further development resulted in the extended-spectrum cephalosporins ceftazidime, cefotaxime, and ceftriaxone, as well as aztreonam (a monobactam), which have better stability against many β -lactamases. Because of their safety, efficacy, and favorable pharmacokinetics, the extended-spectrum cephalosporins have been used extensively. In the early 1980s, resistance to these drugs appeared in gram-negative bacilli with chromosomally encoded β -lactamases, most often as the result of mutations that led to the constitutive production of these normally inducible enzymes.¹⁹

Enteric gram-negative bacilli with transferable resistance to extended-spectrum cephalosporins were first detected in the mid-1980s in Western Europe.²⁰ The majority of these strains, predominantly *Klebsiella pneumoniae*, other klebsiella species, and *Escherichia coli*, were resistant to all β -lactam antibiotics except cephamycins and carbapenems.²¹ This resistance phenotype was identified first in the United States and not long afterward elsewhere.²¹ Genes encoding these extended-spectrum β -lactamases were typically carried on self-transferable plasmids that often carried other determinants of antibiotic resistance.^{22,23} Because these genes may be located on transposable elements, they may move into various plasmids, permitting the dissemination of extended-spectrum β -lactamases among gram-negative bacilli.²⁴

Studies of outbreaks of nosocomial infections with Enterobacteriaceae that produce extended-spectrum β -lactamases suggest that these strains arose in response to the selective pressure created by the use of extended-spectrum cephalosporins.^{22,25-27} Colonization and infection with these bacteria have also been associated with lengthy hospital stays, location in an intensive care or oncology unit,

TABLE 2. SELECTED CURRENT PROBLEMS WITH ANTIMICROBIAL DRUGS, ACCORDING TO ORGANISM.

ORGANISM	PROBLEM
Gram-positive cocci	Methicillin-resistant <i>Staph. aureus</i> and coagulase-negative staphylococci; penicillin-resistant pneumococci; macrolide-resistant streptococci; vancomycin-resistant enterococci
Gram-negative cocci	Penicillin-resistant meningococci; quinolone-resistant gonococci
Gram-negative bacilli	Enterobacter and other Enterobacteriaceae with chromosomal β -lactamases; multidrug-resistant <i>P. aeruginosa</i> , <i>Stenotrophomonas maltophilia</i> ; acinetobacter species with novel β -lactamases, aminoglycoside-modifying enzymes, and other resistance mechanisms; Enterobacteriaceae with extended-spectrum β -lactamases; multidrug-resistant diarrheal pathogens (shigella species, salmonella species, <i>Escherichia coli</i> , campylobacter species)
Acid-fast bacilli	Multidrug-resistant <i>Mycobacterium tuberculosis</i> ; multidrug-resistant <i>M. avium</i> complex

and catheterization of the urinary bladder.^{22,23,25-27} Strains producing extended-spectrum β -lactamases have caused hospital outbreaks involving the infection or colonization of large numbers of patients.^{23,26}

The prevalence of extended-spectrum β -lactamase production among gram-negative bacilli varies from country to country and among institutions within a country, at least in part because of patterns of antibiotic use. In a large study of clinical isolates in the United States, between 1.3 and 8.6 percent of *E. coli* and *K. pneumoniae* isolates were resistant or intermediately susceptible to ceftazidime.²⁸ A subgroup of isolates from that study was examined more closely; approximately half of these genetically diverse strains were resistant because they produced extended-spectrum β -lactamases.²⁹ Molecular epidemiologic studies have suggested that there is dissemination of strains producing extended-spectrum β -lactamases within and between hospitals, as well as transfer of plasmids encoding these enzymes among strains.^{22,25,29}

Cloning and sequencing of the genes encoding extended-spectrum β -lactamases revealed that these genes differed from those encoding common plasmid-borne enzymes with more limited activity by substitutions of only a few nucleotides.³⁰ To date, more than 20 extended-spectrum β -lactamases have been identified,¹⁶ the result of simple point mutations that alter amino acids near the active site of the enzyme, presumably facilitating the hydrolysis of extended-spectrum β -lactam antibiotics.

Clavulanic acid, sulbactam, and tazobactam are β -lactamase inhibitors that are currently available in the United States in combination with β -lactam antibiotics. In general, clavulanic acid and tazobactam have better inhibitory activity than sulbactam against extended-spectrum β -lactamases and the enzymes from which they evolved.³¹ There have been reports of Enterobacteriaceae resistant to combinations of β -lactams with β -lactamase inhibitors as a result of overproduction or mutation of β -lactamases, and

bacteria producing extended-spectrum β -lactamases could also acquire these properties.^{32,33}

Because routine susceptibility testing may miss their effects, a variety of maneuvers have been used to improve the identification of bacteria producing extended-spectrum β -lactamases, including testing with larger-than-normal inocula, lower breakpoints for resistance, and in vitro tests for synergy between β -lactams and β -lactamase inhibitors.^{34,35} The optimal therapy for infections caused by organisms producing extended-spectrum β -lactamases is currently evolving. In studies of the treatment of animals with infections caused by Enterobacteriaceae that produce extended-spectrum β -lactamases, extended-spectrum cephalosporins were usually not effective, even when they were active against the organism in vitro.³⁵⁻³⁹ Imipenem or a combination of a β -lactam with a β -lactamase inhibitor was generally more effective.

There have been no clinical trials of antimicrobial therapy against infections caused by bacteria producing extended-spectrum β -lactamases, and the body of data on the treatment of these pathogens consists only of case reports and limited retrospective information from epidemiologic studies. Treatment data from studies of nosocomial outbreaks caused by enteric gram-negative bacilli that produce these enzymes indicate that certain minor infections (for example, urinary tract infections) may resolve during therapy with extended-spectrum cephalosporins, but that more serious infections often do not.^{26,27} Despite reports that cephamycins have good inhibitory activity against these bacteria in vitro,³⁴ there are no studies addressing the efficacy of these drugs in vivo, other than a single case report of a treatment failure.⁴⁰ Treatment recommendations based on the limited data currently available are shown in Table 3, as are preventive strategies.

PENICILLIN-RESISTANT PNEUMOCOCCI

In the 1940s, all *Streptococcus pneumoniae* were exquisitely susceptible to penicillin. Concentrations

TABLE 3. SUMMARY OF THERAPEUTIC AND PREVENTIVE STRATEGIES FOR INFECTIONS CAUSED BY SELECTED ANTIMICROBIAL-DRUG-RESISTANT PATHOGENS.*

PATHOGEN	INTERVENTION	COMMENT	REFERENCE
ESBL-producing gram-negative bacilli	Treatment options		
	Carbapenems	A drug of choice	Meyer et al. ²⁶ and Rice et al. ³⁷
	β -Lactam- β -lactamase inhibitor combination	May be effective (must be given in relatively high doses)	Rice et al. ³⁷ and Mentec et al. ³⁸
	Fluoroquinolone, aminoglycoside, trimethoprim-sulfamethoxazole	May be useful if organism is susceptible; however, many ESBL-producing Enterobacteriaceae are multidrug-resistant	Rice et al. ²² and Sader et al. ²⁹
	Prevention and infection control		
	Prudent use of extended-spectrum cephalosporins	Outbreak strains may be multidrug-resistant, prompting restrictions on other antibiotics	Naumovski et al., ²⁵ Meyer et al., ²⁶ and Brun-Buisson et al. ²⁷
	Isolation of colonized or infected patients		Meyer et al. ²⁶
	Targeted surveillance of high-risk areas in the hospital		Bryce and Smith ⁴¹
	PRSP		
PRSP	Treatment options		
	Penicillin	High-dose intravenous therapy probably effective for pneumonia unless organism is very highly resistant	Pallares et al. ⁴²
	Extended-spectrum cephalosporins	Probably effective for most PRSP infections; meningitis may be an exception; also, oral β -lactams may be less effective in treatment of otitis media	Gehanno et al. ⁴³ and Paris et al. ⁴⁴
	Vancomycin	May be useful for meningitis caused by highly resistant organism (must be given in high doses [minimum of 40 mg/kg of body weight/day])	Paris et al. ⁴⁴
	Carbapenems	Very active against PRSP (imipenem not useful for meningitis)	Ward and Moellering ⁴⁵
	Rifampin	May be useful as adjunctive therapy for meningitis (based only on studies in animals)	Paris et al. ⁴⁴
	Clindamycin	May be useful for otitis media (if strain is susceptible)	
	Prevention		
	23-Valent vaccine	Wider use in adults should prevent some PRSP infections	Munford and Murphy ⁴⁶
	Possible new heptavalent vaccine	Should be administered to all persons ≥ 65 yr of age and children ≥ 2 yr of age at increased risk for serious pneumococcal infection	
	Proposed vaccine for children < 2 yr of age; must be sufficiently immunogenic and directed against the most frequently occurring serotypes of PRSP (not currently available)	Munford and Murphy ⁴⁶	
VRE	Treatment options		
	Ampicillin or penicillin with an aminoglycoside	Therapy of choice for serious enterococcal infection, but many VRE are resistant to one or both	Moellering ⁴⁷
	Teicoplanin	May be useful against VanB and VanC enterococci, particularly if used in higher-than-normal doses and combined with an aminoglycoside; currently not available in the United States; resistance may develop with therapy	Fantin et al. ⁴⁸ and Hayden et al. ⁴⁹
	Combinations of β -lactam or β -lactams and a glycopeptide	May have some efficacy, but resistance to synergy may be present or develop with therapy	Bingen et al., ⁵⁰ Fraimow and Venuti, ⁵¹ and Caron et al. ⁵²
	Chloramphenicol, fluoroquinolone, tetracycline, rifampin	May have some value for susceptible organisms, but these agents are not bactericidal	Norris et al. ⁵³ and Únal et al. ⁵⁴
	Novobiocin, bacitracin	Limited success in clearing fecal carriage of VRE	O'Donovan et al. ⁵⁵ and Montecalvo et al. ⁵⁶
	Quinupristin-dalfopristin	An investigational streptogramin antibiotic having some efficacy against vancomycin-resistant <i>Enterococcus faecium</i> (not active against <i>Ent. faecalis</i>)	
	Nitrofurantoin	Useful for urinary tract infection due to susceptible organism	
	Prevention and infection control		
	Prudent use of vancomycin	Includes parenteral and oral use	HICPAC ⁵⁷
Susceptibility testing with approved methods	Particularly important in detecting moderate and low levels of glycopeptide resistance	HICPAC ⁵⁷ and Tenover et al. ⁵⁸	
Isolation of colonized or infected patients	Critical to note that long-term fecal carriage is the norm	HICPAC ⁵⁷	
Surveillance of antibiotic susceptibility of nosocomial enterococcal isolates	Particularly in hospitals with little or no vancomycin resistance among enterococci	HICPAC ⁵⁷	

*ESBL denotes extended-spectrum β -lactamase, PRSP penicillin-resistant *Streptococcus pneumoniae*, VRE vancomycin-resistant enterococci, and HICPAC Hospital Infections Control Practices Advisory Committee. VanB and VanC are phenotypes of glycopeptide-resistant enterococci.

of penicillin of less than 0.1 μg per milliliter not only inhibited the growth of these organisms but also killed them by rapid lysis. It was not until the 1960s that reports of strains of pneumococci with intermediate levels of penicillin resistance (minimal inhibitory concentrations of 0.1 to 0.6 μg per milliliter) began to appear.⁵⁹ More highly resistant pneumococci (requiring minimal inhibitory concentrations of penicillin of up to 4 to 8 μg per milliliter and often resistant to other antimicrobial drugs) were described in South Africa in the mid-1970s.⁶⁰ Subsequently, penicillin-resistant *S. pneumoniae* have been found virtually worldwide.

In a study of more than 1500 isolates of *S. pneumoniae* collected from outpatients at medical centers in the United States between 1994 and 1995, 23.6 percent of isolates were not susceptible to penicillin: 14.1 percent had intermediate resistance (minimal inhibitory concentration of penicillin, 0.1 to 1.0 μg per milliliter), and 9.5 percent were highly resistant (minimal inhibitory concentration of penicillin, ≥ 2.0 μg per milliliter).⁶¹ There was marked geographic variation in the rates of penicillin resistance among pneumococci, ranging from 2.1 to 53 percent. Unfortunately, it appears that the overall prevalence of resistant strains in the United States is rising steadily, with the most dramatic increases among highly resistant pneumococci.^{61,62} To complicate matters, many of these penicillin-resistant strains are resistant to other antimicrobial drugs, including erythromycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole.^{59,61}

The mechanism of pneumococcal resistance to penicillin and other β -lactam antibiotics involves alterations in one or more of the penicillin-binding proteins that are important in the synthesis of the bacterial cell wall and that bind β -lactam antibiotics. These alterations cause decreased affinity for penicillin and related drugs. The genes that code for the altered penicillin-binding proteins are termed "mosaics" because they consist of segments of native pneumococcal DNA mixed with segments of foreign DNA, presumably from more penicillin-resistant organisms, such as viridans streptococci, that have been taken up by the pneumococcus and incorporated into the chromosome.⁶³ There is also evidence that these hybrid genes may have been transferred to other pneumococci and even to other gram-positive organisms, such as *S. oralis*.⁶⁴ Although these alterations in pneumococcal penicillin-binding proteins lead to decreased affinity for all β -lactam antimicrobial drugs, the extended-spectrum cephalosporins and carbapenems have greater activity than penicillin G against the resistant strains.⁴⁵ Pneumococci more resistant to the extended-spectrum cephalosporins than to penicillin G have been described; this pattern of resistance appears to be due to unique alterations in penicillin-binding proteins.⁶⁵ Determi-

nation of penicillin susceptibility alone may fail to identify these strains, although they are not currently prevalent.

Penicillin resistance occurs in a number of different pneumococcal serotypes but appears to be much more prevalent among those serotypes that most frequently cause disease in children.⁴⁶ This is consistent with the hypothesis that many of these organisms originate in children and then spread to adults,⁶⁶ a concept supported by the occurrence of outbreaks of resistant pneumococci in child-care facilities. These facilities provide conditions thought to favor the emergence and dissemination of resistant pneumococci: large numbers of children with frequent, close contact who often receive antimicrobial drugs.^{66,67} There is substantial evidence of global spread of clones of resistant pneumococci.⁶⁸

Pneumococci are easily spread from person to person by respiratory droplets or through direct inoculation of secretions. The organism may spread from patients to hospital staff,⁴⁶ and the carriage rates among nurses caring for patients with pneumococcal pneumonia can be high.⁶⁹ This raises the very real specter of nosocomial dissemination of resistant pneumococci, especially if infection with a resistant strain is not detected when the patient is admitted.

The acquisition of penicillin resistance by pneumococci is not accompanied by any decline in virulence.⁷⁰ Thus, preventing infection by immunization,⁴⁶ as well as therapy with appropriate antimicrobial drugs, assumes great importance (Table 3).

Penicillin resistance has its most dramatic effects in patients with pneumococcal meningitis. The poor penetration of penicillin into the cerebrospinal fluid makes it difficult to achieve predictably effective drug concentrations in the cerebrospinal fluid (8 to 10 times the minimal bactericidal concentration for the infecting organism) against relatively penicillin-resistant strains, and especially against highly penicillin-resistant strains. This difficulty, combined with the intrinsic virulence of pneumococci, undoubtedly accounts for the reported failures of ampicillin and penicillin in such patients, and most experts believe that even high doses of these drugs should not be used to treat meningitis caused by penicillin-resistant pneumococci. At the present time, cefotaxime and ceftriaxone appear to be the drugs of choice for the initial treatment of these infections. However, because there have been treatment failures with these drugs, many authorities suggest the routine addition of vancomycin in areas where there is a high prevalence of strains with minimal inhibitory concentrations of penicillin greater than 1.0 μg per milliliter.⁴⁴

The value of corticosteroid therapy for pneumococcal meningitis in adults remains controversial, but in a recent study of children with pneumococcal meningitis, it decreased the frequency of adverse sequelae of the infection.⁷¹ In animals, corticosteroid

therapy markedly decreased the penetration of vancomycin into the cerebrospinal fluid, caused a lesser reduction in ceftriaxone penetration, and had no effect on rifampin penetration.⁷² Some of these effects of corticosteroids may be offset by the fact that combinations of ceftriaxone and vancomycin exhibit synergistic killing of penicillin-resistant pneumococci in vitro and in an animal model of meningitis.⁷³

In addition to being tested for susceptibility to penicillin, cerebrospinal fluid isolates of pneumococci should be tested for susceptibility to cefotaxime or ceftriaxone by a quantitative agar-diffusion method, such as the E test, or by the broth-dilution method.^{74,75} Strains with minimal inhibitory concentrations of cefotaxime or ceftriaxone of 0.5 μg per milliliter or less are likely to be eradicated, but strains with higher minimal inhibitory concentrations should be considered resistant.⁷⁵ More detailed recommendations for the treatment of meningitis caused by penicillin-resistant pneumococci may be found in the recent review by Paris et al.⁴⁴

Although new drugs with high levels of activity against penicillin-resistant pneumococci may solve this problem, antimicrobial drugs alone will probably not provide the final answer. Because of the worldwide increase in multidrug-resistant pneumococci, there is growing interest in immunization to prevent infections with these organisms (Table 3).⁴⁶

VANCOMYCIN RESISTANCE IN ENTEROCOCCI

Enterococci are currently ascendant nosocomial pathogens,⁷⁶ having become the second most common organisms recovered from nosocomial urinary tract and wound infections and the third most common cause of nosocomial bacteremia in the United States.⁷⁷ One of the major reasons these organisms have thrived in the hospital environment is their intrinsic resistance to several commonly used antibiotics and, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (Table 4).^{78,79} Because most enterococci are tolerant to the bactericidal activity of β -lactam and glycopeptide antibiotics,⁸⁰ bactericidal synergy between one of these antibiotics and an aminoglycoside is needed to treat the most serious enterococcal infections, such as endocarditis and meningitis.⁴⁷ This effect is lost if there is high-level resistance to either class of drug. Resistance to high concentrations of aminoglycoside antibiotics, usually due to aminoglycoside-modifying enzymes, is widespread among enterococci (more than 50 percent of isolates in some centers).⁸¹ Also, many isolates of *Enterococcus faecium* are highly resistant to penicillins by virtue of their low-affinity penicillin-binding proteins.⁸² Until recently, vancomycin was virtually the

TABLE 4. INTRINSIC AND ACQUIRED
ANTIMICROBIAL-DRUG RESISTANCE
IN ENTEROCOCCI.*

Intrinsic resistance
β -Lactams (particularly cephalosporins and penicillinase-resistant penicillins)
Low concentrations of aminoglycosides
Clindamycin
Fluoroquinolones
Trimethoprim-sulfamethoxazole
Acquired resistance
High concentrations of β -lactams, through penicillin-binding proteins or β -lactamase
High concentrations of aminoglycosides
Glycopeptides (vancomycin and teicoplanin)
Tetracycline
Erythromycin
Fluoroquinolones
Rifampin
Chloramphenicol
Fusidic acid
Nitrofurantoin

*Data were adapted from Moellering and Krogstad.⁷⁸

only drug that could be consistently relied on for the treatment of infections caused by multidrug-resistant enterococci.

Vancomycin had been in clinical use for more than 30 years without the emergence of marked resistance.⁸³ Teicoplanin, the other glycopeptide antibiotic in clinical use, is not available in the United States, but it has been used in Europe. Because of their activity against methicillin-resistant staphylococci and other gram-positive bacteria, these drugs have been widely used for therapy and prophylaxis against infections due to these organisms. Also, oral vancomycin, which is poorly absorbed, has been used extensively for the treatment of *Clostridium difficile* enterocolitis. The use of glycopeptides is escalating; at one teaching hospital, vancomycin use increased 20-fold from 1981 to 1991.⁸⁴

It was against this backdrop that reports of acquired vancomycin resistance in enterococci began to appear in the mid-1980s.⁸⁵ Initially reported in Europe, resistant organisms have become an important problem in an ever-growing number of centers in the United States. Data reported to the National Nosocomial Infection Survey of the Centers for Disease Control and Prevention revealed that vancomycin resistance had increased more than 20-fold among nosocomial isolates of enterococci, from less than 0.5 percent in 1989 to more than 10 percent in 1995.⁸⁶

These results are alarming, because vancomycin-resistant organisms, initially concentrated in intensive care units, have spread throughout hospitals. Among patients with bacteremia due to vancomycin-resistant enterococci, many of whom have serious underlying disease, the mortality rate attributable to the bacteremia may approach 50 percent.⁸⁷ The acquisition of vancomycin-resistant enterococci by hospitalized pa-

tients has been associated with a number of factors, including the length of hospital stay, underlying disease, intensity of antibiotic exposure, and exposure to particular antibiotics, including broad-spectrum drugs and parenteral and oral vancomycin.⁸⁸⁻⁹⁰

Outbreaks of vancomycin-resistant enterococci may be monoclonal^{90,91} or due to multiple strains.⁸⁹ In the United States, isolates of vancomycin-resistant enterococci have been genetically diverse, but dissemination of single strains between hospitals has been reported.⁹² The original source of vancomycin-resistant enterococci is unknown, but these bacteria have been isolated from farm animals, poultry for human consumption, and sewage.^{93,94} The use of glycopeptide antibiotics in animal husbandry may be linked to the recovery of vancomycin-resistant enterococci from environmental sites.⁹⁴

The treatment of multidrug-resistant enterococcal infections poses a challenge for clinicians, and the organism's potential to serve as a reservoir for resistance genes is of great concern. In the laboratory, resistance to glycopeptide antibiotics has been transferred between enterococcal species and from enterococci to other gram-positive organisms, including streptococci, *Listeria monocytogenes*, and, most ominously, *Staph. aureus*.^{95,96}

Glycopeptide-resistant enterococci are divided into phenotypes primarily on the basis of their patterns of resistance to specific drugs (Table 5).⁹⁷ Acquired glycopeptide resistance in enterococci is mediated by complex operons encoding an alternative biosynthetic pathway for the production of a modified cell-wall component (a peptidoglycan precursor) that binds vancomycin with a very small fraction of the avidity of the normal precursor; however, polymerization of the cell-wall peptidoglycan proceeds unimpeded.⁹⁸ The mechanism of resistance is similar in gram-positive bacteria that are intrinsically resistant to these drugs, such as leuconostoc, pediococcus, and glycopeptide-resistant lactobacilli,⁹⁹ but these bacteria do not appear to be the source of the genes encoding acquired resistance in enterococci.^{100,101}

Laboratory detection of glycopeptide resistance in enterococci has improved as a result of revised breakpoints for reading disk-diffusion susceptibility tests¹⁰² and updated software for some automated testing systems,¹⁰³ although neither technique may be optimal for the detection of moderate and low-level vancomycin resistance.⁵⁸ A number of broth-dilution and agar-dilution tests, as well as E-test strips, may be used to test for vancomycin susceptibility in enterococci without the occurrence of major errors.⁵⁸ The addition of a standardized screening method, with 6 μg of vancomycin per milliliter in brain-heart infusion agar plates, provides a useful supplement to the other techniques.¹⁰⁴

Despite the variety of regimens used to treat infections with glycopeptide-resistant enterococci, there

TABLE 5. CHARACTERISTICS OF PHENOTYPES OF GLYCOPEPTIDE-RESISTANT ENTEROCOCCI.*

CHARACTERISTIC	PHENOTYPE		
	VAN A	VAN B	VAN C
Vancomycin MIC ($\mu\text{g}/\text{ml}$)	64 to >1000	4 to 1024	2 to 32
Teicoplanin MIC ($\mu\text{g}/\text{ml}$)	16 to 512	≤ 0.5	≤ 0.5
Most frequent enterococcal species	<i>Ent. faecium</i> <i>Ent. faecalis</i>	<i>Ent. faecalis</i> <i>Ent. faecium</i>	<i>Ent. gallinarum</i> <i>Ent. casseliflavus</i>
Genetic determinant	Acquired	Acquired	Intrinsic
Transferable	Yes	Yes	No

*Data are for the majority of reported isolates. MIC denotes minimal inhibitory concentration.

are few conclusive in vitro or in vivo studies and no published clinical trials. Although current therapy for these infections has been based largely on susceptibility data and empiricism, a few recommendations based on in vitro and animal studies and limited clinical data are shown in Table 3. Infection-control measures are crucial.⁵⁷ It is disconcerting that, through mutation and the acquisition of foreign genetic material, enterococci seem to have become resistant to all currently available antibiotics.

CONCLUSIONS

The therapeutic problems caused by gram-negative bacilli that produce extended-spectrum β -lactamases, by penicillin-resistant pneumococci, and by vancomycin-resistant enterococci are but three of the many problems being caused by drug-resistant bacteria. The development of some resistance is almost certainly an inevitable consequence of the clinical use of antimicrobial drugs. The variety of mechanisms by which bacteria acquire resistance to antimicrobial drugs is astonishing. More research is urgently needed to define mechanisms of resistance, to look for new targets for antimicrobial drugs, to discover more effective ways of using our existing drugs, to minimize the development of resistance, to ascertain the most useful therapy for infections due to multidrug-resistant organisms, and to learn how to prevent these infections. These are important challenges, and if they are not met we are in danger of entering the 21st century with a less effective armamentarium against bacterial pathogens than we have at present.

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