Pathogens Resistant to Antibacterial Agents

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- Vancomycin-resistant *Enterococcus*
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- Penicillin-resistant *Streptococcus pneumoniae*
- *Klebsiella pneumoniae* carbapenemase
- *Acinetobacter baumanii*

Multidrug-resistant pathogens historically were limited to the hospital setting. In the 1990s, multidrug-resistant pathogens were described to be affecting outpatients in health care–associated settings (nursing homes, dialysis centers, infusion centers, among patients recently hospitalized). More recently, multidrug-resistant pathogens have become major issues in the community, affecting persons with limited or in many cases no contact with health care. This article reviews the molecular mechanisms by which resistance traits are conferred and disseminated and the epidemiology of such bacterial resistance.

MECHANISMS OF RESISTANCE

It is important to distinguish the many ways by which an organism may demonstrate resistance. Intrinsic resistance to an antimicrobial agent characterizes resistance that is an inherent attribute of a particular species; all organisms of the species may lack the appropriate drug-susceptible target or possess natural barriers that prevent an antimicrobial agent from reaching its target. Some examples are the natural resistance of gram-negative bacteria to vancomycin because the drug cannot penetrate the

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gram-negative outer membrane, or the intrinsic resistance of the penicillin-binding proteins (PBPs) of enterococci to the effects of the cephalosporins.

Acquired resistance, the primary focus of this article, reflects a change in the genetic composition of a bacterium so that a drug that once was effective is no longer active, resulting in clinical resistance. Sometimes genetic change results in diminished antimicrobial activity, but not complete loss of drug effectiveness.

The major strategies used by bacteria to avoid the actions of antimicrobial agents are outlined in **Table 1**. These include limiting the intracellular concentration of an antimicrobial agent by decreased influx or increased efflux, neutralization of the antimicrobial agent by enzymes, alteration of the target so that the agent no longer interferes with it, and elimination of the target altogether by the creation of new metabolic pathways.[1] Bacteria may use one or multiple mechanisms against a single agent or class of agents or a single change may result in resistance to several different agents or even multiple unrelated drug classes.

Gram-positive and gram-negative bacteria possess different structural characteristics and these differences determine the mechanisms for primary resistance. The targets of most antimicrobial agents are located either in the cell wall, cytoplasmic membrane, or within the cytoplasm. In gram-negative bacteria, the outer membrane may provide an additional intrinsic barrier that prevents drugs from reaching these targets. Additionally, modifications in outer membrane permeability by both alterations in porin channels and by upregulation of multidrug efflux pumps may contribute to resistance in many gram-negative organisms. Moreover, inactivating enzymes released across the cytoplasmic membrane can function more efficiently within the confines of the periplasmic space.

The mechanisms by which intracellular concentrations of drugs are limited include decreased outer membrane permeability, decreased uptake through the cytoplasmic

Table 1
General mechanisms of resistance to antimicrobial agents

Resistance Mechanism	Specific Examples	References
Diminished intracellular drug concentration		
Decreased outer membrane permeability	β-Lactams (eg, OmpF, OprD)	2
Decreased cytoplasmic membrane transport	Quinolones (eg, OmpF)	1
	Aminoglycosides (decreased energy)	3
Increased efflux	Tetracyclines (eg, tetA)	195
	Quinolones (eg, norA)	196
	Macrolides (eg, mefA)	196
	Multiple drugs (eg, mexAB-OprF)	2
Drug inactivation (reversible or irreversible)	β-Lactams (β-lactamases)	95
	Carbapenemases (carbapenems)	105,96
	Aminoglycosides (modifying enzymes)	3
	Chloramphenicol (inactivating enzymes)	1
Target modification	Quinolones (gyrase modifications)	6
	Rifampin (DNA polymerase binding)	6
	β-Lactams (PBP changes)	6
	Macrolides (rRNA methylation)	5
	Linezolid (23srRNA modifications)	58
Target bypass	Glycopeptides (vanA, vanB)	7
	Trimethoprim (thymidine-deficient strains)	—

membrane, and active efflux out of both the cytoplasmic membrane and the outer membrane. Acquired outer membrane permeability changes in gram-negative organisms previously attributed solely to alterations in outer membrane porin proteins are now also understood to be related to the upregulation of complex multidrug efflux pumps whose expression is linked to that of outer membrane proteins, such as the *MexAB-OprM* system of *Pseudomonas aeruginosa*.[2] These efflux systems are widely distributed among gram-negative pathogens, such as *P aeruginosa* and Enterobacteriaceae, and may be an important component of resistance to β-lactams, but usually result in high-level resistance only when associated with β-lactamase production.[2] Imipenem resistance in *P aeruginosa* can be mediated by alteration of a specific porin *OprD* that is used preferentially by this agent.[2] Decreased outer membrane permeability through porin changes and efflux may also play a role in resistance to fluoroquinolones and aminoglycosides. Resistance mediated by decreased uptake across the metabolically active cytoplasmic membrane is best demonstrated by small-colony aminoglycoside-resistant mutants of staphylococci, but this mechanism is less important than other mechanisms of aminoglycoside resistance.[3] Active antimicrobial efflux systems play a role in resistance to many different agents, including macrolides, tetracyclines, quinolones, chloramphenicol, and β-lactams.

Inactivating enzymes remain the predominant mechanism of resistance to several major classes of antimicrobial agents. Resistance to β-lactams is mediated by a wide variety of β-lactamases that hydrolytically inactivate these drugs. β-lactamases can be either plasmid or chromosomally mediated, and their expression can be constitutive or induced. Unlike those of gram-positive organisms, β-lactamases of gram-negative organisms are confined to the periplasmic space, which may explain some of the differences in their phenotypic expression and ease of laboratory detection. Of particular importance in the hospital setting are the class I chromosomal β-lactamases in organisms, such as *Enterobacter cloacae*, which are produced in high levels after exposure to an inducing β-lactam agent (particularly to third-generation cephalosporins), and the extended-spectrum β-lactamases (ESBLs), mediating resistance to third-generation cephalosporins and aztreonam.[4] Carbapenemases are an emerging, and important class of inactivating enzymes that mediate resistance to carbapenem antibiotics in gram-negative organisms. Another major class of inactivating enzymes is the family of aminoglycoside-modifying enzymes. These enzymes are widely distributed in gram-positive and gram-negative bacteria, and usually are plasmid-mediated.[3] Resistance to chloramphenicol and macrolides also can be mediated by modifying or inactivating enzymes.[5]

Target modifications are widely used by bacteria to mediate resistance to a wide variety of antimicrobial agents.[6] Some of these alterations may require as little as a single mutational event at a critical gene sequence in the primary target to create a new, functional target with reduced affinity for the antimicrobial agent. Such changes account for the relative ease of selection of rifampin-resistant mutants of staphylococci and streptococci by changes in DNA polymerase or the selection of high-level streptomycin-resistant mutants with altered ribosomes. Although some resistance secondary to target modifications can be directly selected, others, such as development of resistance to semisynthetic penicillins in staphylococci, require acquisition of novel exogenous DNA. Modification of PBPs is the primary mode of penicillin resistance in *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Enterococcus faecium*. Modification of genes encoding DNA gyrases and topoisomerases is the main mechanism of resistance to quinolones, while target modification is important in resistance to macrolides, tetracyclines, rifampin, and mupirocin.[6]

Some bacteria have gone beyond simple target modification and have acquired novel systems in which the antimicrobial target is no longer necessary for survival of the organism. This is achieved by creation of new metabolic pathways to bypass the primary target. Perhaps the most elaborate examples of this are the *vanA* and *vanB* clusters that mediate resistance to glycopeptides in enterococci.[7] Target bypass also is the major mechanism of acquired resistance to folate antagonists.

MECHANISMS OF DISSEMINATION OF RESISTANCE GENES

In addition to the complex strategies used to express resistance to antimicrobial agents, bacteria also can avail themselves of a variety of efficient mechanisms for the transfer of resistance genes to other organisms and to other species.[8] The bacterial genome consists of chromosomal DNA, and encodes the following cellular characteristics: metabolic and repair pathways, smaller circular DNA elements or plasmids that encode for supplemental bacterial activities, such as virulence factors and resistance genes, and genes essential to the independent mobilization and transmission of the plasmid elements. Most resistance genes are plasmid-mediated, but plasmid-mediated traits can interchange with chromosomal elements. Transfer of genetic material from a plasmid to the chromosome can occur by simple recombination events, but the process is greatly facilitated by transposons. Transposons are small, mobile DNA elements capable of mediating transfer of DNA by removing and inserting themselves into host chromosomal and plasmid DNA. Many resistance genes, such as plasmid-mediated β-lactamases, tetracycline-resistance genes, and aminoglycoside-modifying enzymes, are organized on transposons, which may have a broader host range than their parent plasmids. Multiple resistance transposons can then be clustered together on even larger composite transposable elements capable of simultaneously transferring multiple unrelated resistance genes.[8]

Resistance determinants carried on the chromosome are vertically transmitted by clonal dissemination. Resistance determinants on plasmids can also be vertically transferred, although plasmids may be lost from the bacterial population if they no longer confer a particular selective advantage. Plasmids also are capable of horizontal transfer by conjugation, although the efficiency of plasmid transfer both within and between species can vary tremendously. Plasmid transfer between gram-positive and gram-negative bacteria, once thought to be an unlikely event, can occur both in the laboratory and in the gastrointestinal tract of gnotobiotic mice, suggesting that such transfer events between even remotely related organisms may be important in nature.[9]

Conjugative transposons of gram-positive bacteria are capable of directly mediating gene transfer without plasmids. Transformation, direct incorporation of free DNA by bacterial cells, may also be important for the evolution of resistance in *Neisseria* and streptococcal species.[6,8]

Resistant Gram-Positive Cocci

Multidrug-resistant enterococci

Epidemiology and characteristics of resistance Enterococci are naturally tolerant of penicillins, and are resistant to cephalosporins, clindamycin, and achievable serum levels of aminoglycosides. Cephalosporin resistance is caused by poor affinity of cephalosporins for enterococcal PBPs. Natural low-level aminoglycoside resistance is attributed to the inability of aminoglycosides to penetrate the enterococcal cell wall, but activity is enhanced in the presence of cell wall–active drugs, such as ampicillin or vancomycin.[10] Enterococci are usually intrinsically tolerant, or lysis resistant, to the

effects of penicillins and glycopeptides alone. Until the emergence of drug-resistant isolates, bactericidal therapy was reliably achieved with synergistic combinations of cell wall–active drugs plus aminoglycosides.

Enterococci demonstrate several types of penicillin resistance Penicillin resistance in *E faecalis* is mediated by β-lactamase production and has been reported from a few nosocomial outbreaks both within and outside of the United States,[11] but infections with such strains are still very uncommon. Chromosomal high-level penicillin resistance is a species-specific characteristic of *E faecium*, but is occasionally found in other species.[12] *E faecium* with low-level penicillin-resistance are found in normal fecal flora, but high-level resistant strains are likely to be nosocomially acquired.[13] Ampicillin resistance to an intermediate level (minimum inhibitory concentrations [MICs] of 16–64 μg/mL) is attributable to alterations in PBPs and in most cases, is associated with the overexpression of PBP5, a PBP with low affinity for penicillins.[14] High-level penicillin-resistant *E faecium* are also resistant to imipenem and β-lactam–β-lactamase inhibitors and are often also glycopeptide-resistant.[15]

High-level gentamicin resistance, mediated by a bifunctional inactivating enzyme, first appeared in 1978 and rapidly spread worldwide. As many as 60% of enterococci from some hospitals are high-level gentamicin resistant, but resistance remains strongly associated with nosocomial acquisition.[16] High-level gentamicin resistant enterococci are highly resistant to all other aminoglycosides in clinical use in the United States, with the possible exception of streptomycin. Importantly, highly resistant strains do not demonstrate synergistic killing of enterococci when aminoglycosides are combined with penicillin or vancomycin.[17] Most high-level gentamicin resistance is carried on transposons and is plasmid mediated.[17] Detection of high-level gentamicin resistance requires either special susceptibility wells or screening plates with high concentrations of gentamicin or streptomycin (eg, $\geq$500 μg/mL for gentamicin and $\geq$1,000 μg/mL for streptomycin) (see the article on antibacterial susceptibility testing in the clinical laboratory elsewhere in this issue).

Vancomycin-resistant enterococci (VRE) were first isolated in Europe in 1988 and in the United States in 1989. Since then, VRE have spread rapidly throughout the United States and the world and have become a significant infection control problem for many hospitals.[18] VRE are established throughout the United States and Europe, but are less frequently isolated in Asia and Latin America.[19] The prevalence of VRE remains low in true community-acquired isolates in the United States.

The increase in glycopeptide resistance in the United States followed the marked increase in vancomycin usage in many hospitals, just as methicillin-resistant *Staphylococcus aureus* (MRSA) strains became established in the 1980s. Most VRE seem to have been acquired nosocomially or institutionally, and spread of epidemic strains both within and between institutions is well documented.[20] From 1989 to 2002, the proportion of enterococcal isolates from ICUs that were resistant to vancomycin increased from 0.3% in 1989 to 23.9% in 1998, and further increased to 33.3% in 2008.[18] As of October 2008, the National Nosocomial Infections Surveillance system reported that more than one third of healthcare–associated enterococcal infections were associated with organisms resistant to vancomycin.[18] Some risk factors for VRE colonization or infection include the exposure to antibiotics, such as broad-spectrum cephalosporins, fluoroquinolones, vancomycin, and antianaerobic drugs, prolonged hospital and ICU stays, intrahospital transfer between patient floors, use of enteral tube feedings, or sucralfate and liver transplant requiring surgical re-exploration.[21]

Glycopeptides are large, complex molecules that do not enter the bacterial cell. They interfere with cell wall synthesis by tightly binding to the D-alanine-D-alanine terminal dipeptide on the peptidoglycan precursor, sterically blocking the subsequent transglycosylation and transpeptidation reactions. The vancomycin-resistance mechanism involves a complex series of reactions that ultimately result in the building of the cell wall by bypassing the D-alanine-D-alanine–containing pentapeptide intermediate structure, thereby eliminating the glycopeptide target.[22]

VRE initially were characterized phenotypically as *vanA*, *vanB*, and *vanC* strains based on levels of resistance to vancomycin, cross-resistance to teicoplanin, and the inducible or constitutive nature of resistance.[23] The genotype and molecular basis for each resistance type have now been characterized (**Table 2**). The *vanA* cluster has been identified predominantly in *E faecium* and *E faecalis* but has also been found in other enterococci, streptococci, *Oerskovia*, and *Bacillus*, and most recently has been found in *S aureus*.[24–28] Furthermore, there is evidence for in vivo transfer of *vanA* resistance on plasmids.[29]

vanB is found almost exclusively in *E faecium* and *E faecalis*. *vanC1*, *C2*, *C3*, *D*, *E*, and *G* are rarely found in enterococci causing human infections.[30–32]

Resistance to linezolid in enterococci is mediated by the G2576U mutation or similar mutations of the 23S ribosome.[33] Recent data document high rates of linezolid resistance among enterococcal isolates. In recent studies, 11% to 20% of VRE colonizing or infecting isolates were resistant to linezolid.[34,35] Risk factors found to be specifically associated with isolation of linezolid-resistant VRE were receipt of a solid organ transplant; receipt of parenteral nutrition; peripheral vascular disease; and prior receipt of linezolid, piperacillin-tazobactam, or cefepime.[35]

Enterococcal resistance to daptomycin rarely occurs and is associated with previous treatment with daptomycin. The mechanism of resistance is not well understood.[36]

Multidrug-Resistant Staphylococcus Aureus

The first strain of penicillinase-producing *S aureus* was reported in 1941[37] and 90% of *S aureus* isolates in the world are now penicillin-resistant as a result of β-lactamase production. Over the ensuing decades, *S aureus* continued to predominate as a major

Table 2
Glycopeptide-resistant enterococci

Genotype	Vancomycin MIC (μg/mL)	Teicoplanin MIC (μg/mL)	Expression	Typical Location
vanA	64–1024	≥16	Inducible	Plasmid[a]
vanB	4–1024	≤1[b]	Inducible[c]	Chromosome[d]
vanC[f]	2–32	≤1	Constitutive and inducible	Chromosome
vanD	64–256	4–32	Constitutive and inducible[e]	Chromosome
vanE	16	0.5	Inducible	Chromosome
vanG	16	0.5	Inducible	Chromosome

[a] Strains with *vanA* on the chromosome have been described.
[b] Teicoplanin-resistant strains have emerged with MIC ≥16.
[c] Constitutively expressing strains have been described.
[d] Plasmids containing *vanB* have been described.
[e] Both have been described.
[f] Species-specific variants *vanC-1*, *vanC-2*, and *vanC-3* have been described.

human pathogen and it became increasingly resistant to drugs in the face of antimicrobial selection pressure. For example, since 1996, there have been several reports of infections caused by MRSA with intermediate susceptibility to vancomycin (vancomycin-intermediate *S aureus* [VISA]; MIC 4–8 μg/mL).[38] Furthermore, there are now several strains of *S aureus* in the United States that are fully resistant to vancomycin (vancomycin-resistant *S aureus* [VRSA]; MIC ≥16 μg/mL)[39] and to other recently approved antibiotics specifically developed to treat MRSA, such as linezolid[40] and daptomycin.[41]

Mechanism of resistance in MRSA

Resistance to methicillin and other β-lactam antibiotics is mediated by the *mecA* gene, which encodes for an additional PBP (PBP2a), which has low affinity for β-lactams. Strains with *mecA*-mediated methicillin resistance are classically referred to as MRSA. These *mecA* genes are situated on a mobile genetic element, known as the Staphylococcal Cassette Chromosome mec (SCCmec). To date, eight types of SCCmec (I–VIII) have been reported, and these are widely distributed in both coagulase-positive and coagulase-negative staphylococci.[42–44] The SCCmec element also includes two regulatory loci, the repressor MecI and the trans-membrane β-lactam–sensing signal-transducer MecRI.[45] Some SCCmec components may contain additional genes encoding for resistance against non–β-lactam antibiotics.

The expression of *mecA* can be either constitutive or inducible. Additionally, expression of the resistance phenotype also depends, in part, on other chromosomal genes, which are part of cellular peptidoglycan metabolism and can regulate the degree of resistance without altering levels of PBP2a.

Community-acquired MRSA Community-acquired MRSA is both phenotypically and genotypically distinct from healthcare–associated MRSA. Frequently, community-acquired MRSA isolates produce toxins including Panton-Valentine leukocidin, which is an exotoxin encoded by two cotranscribed genes: lukF-PV and lukS-PV. Although the actual virulence of the Panton-Valentine leukocidin gene has not been determined, presence of the Panton-Valentine leukocidin protein has been associated with skin and soft tissue infections and severe necrotizing pneumonia.[46] Furthermore, community-acquired MRSA strains are susceptible to non–β-lactam antibiotics[47] and this is explained by the fact that resistance genes to non–β-lactam antibiotics are not usually included in the smaller type IV SCCmec elements that are common in community-acquired MRSA isolates. In contrast to nosocomial MRSA isolates, most community-acquired MRSA strains are susceptible to multiple classes of antibiotics other than β-lactams, including trimethoprim-sulfamethoxazole (TMP-SMX), clindamycin, aminoglycosides, tetracyclines, and fluoroquinolones.

Reduced susceptibility to vancomycin Vancomycin is the mainstay of treatment of MRSA infections. Failure of vancomycin therapy, however, is not uncommon and is increasing. Reduced susceptibility of MRSA to vancomycin, which occurs predominantly among strains of healthcare–associated MRSA, is being studied in the following three contexts: (1) VRSA; (2) VISA; and (3) the trend of MRSA isolates with increased vancomycin MICs (MIC creep).

VRSA, fortunately, continues to be rare. There have only been seven reports of clinical isolates of VRSA in the United States as of this writing; five of the seven occurred within the greater Michigan area.[39] MICs of VRSA isolates to vancomycin are generally greater than 128 μg/mL. In these isolates, vancomycin resistance has been conferred by the *vanA* resistance cluster, which also mediates glycopeptide resistance in some enterococcal species. In these patients from whom VRSA was isolated, prior dual

infection with MRSA and VRE was documented in five patients, suggesting that the *vanA* gene was transferred from VRE to MRSA isolate. The transfer of the *vanA* gene has been demonstrated in vitro from enterococcus to *S aureus*.[48] Vancomycin acts by binding the D-Ala-D-Ala terminus of peptidoglycan precursors and the resistance to vancomycin in VRSA is mediated by the presence of enzymes that produce low-affinity precursors, such as D-Ala-D-lactate or D-Ala-D-Ser, or the presence of enzymes that eliminate the high-affinity peptidoglycan precursors that are normally produced in susceptible *S aureus*.[49]

Among VISA isolates, the MIC to vancomycin is 4 to 8 μg/mL. The mechanism conferring glycopeptide resistance in VISA is not well understood at the genetic level but seems to involve cell wall thickening with reduced levels of peptidoglycan cross-linking and does not seem to require the acquisition of new DNA. It is postulated that reduced levels of peptidoglycan cross-linking leads to more D-alanyl-D-alanine side chains. These side chains can bind vancomycin outside the cell membrane and prevent vancomycin from reaching its cell membrane targets.

Independent risk factors for infections caused by VISA include prior infection caused by MRSA and antecedent vancomycin use within 3 months before VISA infection. Most patients in the United States with VISA infections received repeated, prolonged exposures to vancomycin and received dialysis at the time of infection.

Several investigators have observed increased rates of vancomycin treatment failure in patients with MRSA infections where the isolates have increased vancomycin MICs but are still classified as susceptible by Clinical and Laboratory Standards Institute (CLSI) definitions.[50] One study showed less than 10% treatment success when vancomycin was used to treat bacteremia caused by MRSA strains with vancomycin MICs of 1 to 2 μg/mL, compared with 56% success when the vancomycin MIC was less than or equal to 0.5 μg/mL.[51] Partly in response to this study and others, in 2007 the susceptibility breakpoints for *S aureus* to vancomycin were lowered from 8 to 16 μg/mL to 4 to 8 μg/mL for intermediate and from less than or equal to 4 μg/mL to less than or equal to 2 μg/mL for susceptible.

Several investigators from different geographic locales have reported that the MICs of *S aureus* isolates to vancomycin have increased over time. This phenomenon has been described as ''MIC creep.''[52] Debate is ongoing with respect to the significance of the MIC creep and the degree to which it is actually occurring.[53] For example, a study of more than 35,000 *S aureus* isolates from the SENTRY database collected from sites around the world between 1997 and 2003 showed no evidence of increasing vancomycin MICs over time.[54] Another study of more than 6000 *S aureus* isolates collected over 5 years in southern California found a clear and statistically significant drift of vancomycin MICs toward reduced susceptibility, with an increase in the proportion of isolates with a MIC equal to 1 μg/mL from 19.9% in 2000 to 70.4% in 2004.[55] Despite these conflicting results, reports of vancomycin treatment failures for susceptible MRSA strains and reports of MIC creep from some geographic locales raise concerns about the future of vancomycin as an effective agent for treatment of invasive MRSA infections. Recently, guidelines were published regarding optimal dosing of vancomycin for the treatment of infections caused by *S aureus*.[56]

Resistance to other alternative agents for treatment of MRSA

Linezolid is bacteriostatic against *S aureus* and binds to the 50S subunit of the bacterial ribosome, resulting in the inhibition of bacterial protein synthesis.[57] In vitro resistance to linezolid have been reported in *S aureus*.[58] Fortunately, such resistant isolates remain a rare phenomenon. Linezolid may fail to bind its bacterial target

site in the presence of single point mutations in the bacterial 23S rRNA of the 50S subunit.[59] Notably, 23S rRNA mutations that confer resistance to linezolid can produce cross-resistance to chloramphenicol and quinupristin-dalfopristin, which also bind to the same 23S rRNA domain.[59] Prior therapy with linezolid seems to be an important risk factor for subsequent isolation of linezolid-resistant *S aureus*.

Daptomycin is rapidly bactericidal against *S aureus* and its primary mechanism of action is a calcium-dependent depolarization of bacterial cell wall.[60] Daptomycin is inactivated by alveolar surfactants, and should not be used for pulmonary infections.[61] Clinical failures caused by emergence of resistance have been reported during daptomycin for chronic infections.[62] The exact mechanisms of resistance to daptomycin have not been completely elucidated. Studies have shown that daptomycin resistance gradually emerges by a multistep process[63] and generally results in a heteroresistant bacterial subpopulation with higher MICs to daptomycin. Current evidence suggests that resistance is caused by impairment of daptomycin binding by a change in cell membrane potential leading to (1) reduced daptomycin binding to the cell membrane, (2) changes in cell membrane surface charge, and (3) reduced susceptibility to daptomycin-induced depolarization.[64] Furthermore, there is a correlation between daptomycin-resistance and vancomycin nonsusceptibility in isolates of VISA.[65] The cross-resistance between vancomycin and daptomycin in VISA is thought to be caused by the thickened cell wall, which acts as a common obstacle to daptomycin and vancomycin cellular penetration.

Quinupristin-dalfopristin is a concentration-dependent antibiotic that is bactericidal against staphylococci. Staphylococcal isolates constitutively expressing macrolide-lincosamide-streptogramin B resistance (eg, erm A or erm C) may be resistant to the quinupristin component of quinupristin-dalfopristin. In such cases, the activity of quinupristin-dalfopristin may be significantly altered.[66]

Clinical failures associated with resistance to quinupristin-dalfopristin have already been described in some isolates of MRSA even in the relatively short timeframe that the drug has been available.[67] Isolates of staphylococci that are resistant to quinupristin-dalfopristin are more likely to have resistances to the dalfopristin component, which is caused by genes encoding acetyltransferases (*vatA*, *vatB*, and *vatC*) or efflux pumps (*vgaA* and *vgaB*).[68] Resistance to quinupristin-dalfopristin can also be mediated by resistance to the quinupristin component, caused by production of ribosomal methylases encoded by *erm* genes; and the macrolide-lincosamide-streptogramin B phenotype, which also confers cross-resistance to macrolides and lincosamides.

Resistance of MRSA to other classes of antibiotics

MRSA isolates containing *mecA* are resistant to all β-lactam antibiotics. Most nosocomial and health care–associated isolates frequently carry multiple other resistance determinants. Many of these resistance determinants can be found on transferable genetic elements or plasmids, whereas others are chromosomal in origin. The plasmid-mediated determinants include aminoglycoside-modifying enzymes, tetracycline efflux genes, and macrolide-methylating enzymes, and resistance determinants for TMP-SMX. Quinolone resistance is mediated primarily by alterations in DNA topoisomerases, but also by the staphylococcal *norA* gene by active efflux. Resistance to macrolide, lincosamide, and streptogramin antibiotics may be conferred by modifications of target sites, active efflux, and by inactivating enzymes. Gentamicin resistance also can occur by selection of small-colony, membrane energy-deficient mutants.

Penicillin and Multidrug-Resistant Pneumococci

Mechanisms of resistance

Penicillin-resistance in *S pneumoniae* is caused by reduced affinity between the PBPs and β-lactam antibiotics. Such resistance is mediated through changes in genes that encode one of the six high-molecular-weight PBPs of the organism.[69] There are two notable features of PBP-mediated resistance in pneumococci. First, highly resistant strains generally show more PBP alterations than do those of low-level resistant strains.[70] Second, pneumococcal resistance can occur by homologous recombination of PBP genes of different strains, in addition to direct clonal dissemination of resistant strains.[71] Such events can occur both between different pneumococcal species and between pneumococci and the closely related viridans streptococcal species. Oral streptococci have been postulated to be the major reservoir for the novel DNA required to create the genetic sequences demonstrated by some of the altered pneumococcal PBP genes.[6]

Tolerance to penicillins in pneumococci can also be mediated by altered peptidoglycan structures. Additionally, penicillin tolerance has been found in strains with reduced penicillin susceptibility that fails to lyse at penicillin concentrations far above the MIC; however, the clinical significance of this observation remains unclear.

Changes in PBP of penicillin-resistant strains also result in diminished susceptibility to other β-lactam agents.[72] The levels of resistance to different agents vary greatly. Penicillin-resistant strains are uniformly resistant to penicillin derivatives, such as ampicillin and the ureidopenicillins, and generally are resistant to first- and second-generation cephalosporins. Certain third-generation agents, particularly cefotaxime and ceftriaxone, are often effective, in part because of their high level of activity and in part because the tissue levels attained by these agents are high. Many strains of pneumococci also harbor high-level resistance to cefotaxime and ceftriaxone (MIC $\geq$2 μg/mL) (3.3% of all strains reported in a recent United States study were ceftriaxone-resistant and 5% of all isolates in a recent Taiwanese study were ceftriaxone-resistant).[73,74] Interestingly, several studies also show that isolates of penicillin-resistant pneumococci may be disproportionately more susceptible to ceftriaxone than to cefotaxime.[75] Regardless of the pattern of β-lactam nonsusceptibility, debate is still ongoing as to infections caused by these resistant strains are associated with poorer outcomes than infections caused by susceptible strains.[76]

Resistance of pneumococci to non–β-lactam antimicrobial agents

Penicillin-resistant strains are frequently resistant to non–β-lactam antimicrobial agents and are often multidrug resistant. Resistance to erythromycin, tetracycline, TMP-SMX, and chloramphenicol are the most common.

A recent surveillance study in the United States shows that prevalence of macrolide resistance in *S pneumoniae* is approximately 26.2%.[77] The most common mechanism of macrolide resistance in *S pneumoniae* is caused by target-site modification encoded by erythromycin ribosome methylation (erm) genes that provide inducible cross-resistance to all macrolides, lincosamides, and streptogramin B. Two other mechanisms of resistance to macrolides include active efflux pump encoded by macrolide efflux genes (*mefA*, *mefE*) that result in resistance to macrolides alone (M phenotype); and ribosomal mutations in the 23S rRNA gene for ribosomal protein L4 or L22.

Although vancomycin-tolerant pneumococcal isolates have recently been isolated, no strains fully resistant to vancomycin have been reported.[78]

Fluoroquinolone resistance has developed during therapy, especially in patients with prior fluoroquinolone exposures, leading to clinical failure.[79] The older

fluoroquinolones (eg, ciprofloxacin) lack reliable activity against pneumococci (18.6% of pneumococcal isolates in a 2008 study were nonsusceptible to ciprofloxacin).[77] Newer fluoroquinolones, such as levofloxacin, moxifloxacin, and gemifloxacin, inhibit most strains at achievable levels (only 1.3% of the isolates were nonsusceptible to levofloxacin in a study by Sahm and colleagues[77]). The mechanism of decreased susceptibility to the newer fluoroquinolones is primarily caused by mutations in the *parC* gene of topoisomerase IV and the *gyrA* gene of DNA gyrase.

RESISTANT GRAM-NEGATIVE MICROORGANISMS

Escherichia Coli and Klebsiella spp Resistant to Broad-Spectrum Cephalosporins

Resistance of *Escherichia coli* and *Klebsiella* spp to broad-spectrum cephalosporins is largely mediated by ESBLs, designated as Bush-Jacoby-Medeiros Group 2be. These enzymes confer resistance to oxyimino-β-lactam antibiotics. Most ESBLs are derivatives of TEM and SHV, which have undergone amino acid substitutions at the active site of the enzyme. Such ESBL enzymes are often plasmid-borne. Depending on the location of the substitution, the resultant β-lactamase can cause variably diminished susceptibility to cefotaxime, ceftazidime, and aztreonam. ESBLs are most commonly expressed in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *E coli*, although they have been detected in other organisms including *Salmonella* spp, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and other Enterobacteriaceae. Plasmids that encode ESBLs often carry resistance to other antibiotics. The effective control and treatment of ESBLs are a growing challenge because of the role of ESBLs in outbreaks in hospitals and nursing homes, and because of the ability of ESBLs to be transferred to other bacterial species by plasmids.

Epidemiology and mechanisms of resistance

ESBLs were first discovered in Europe in 1983 and soon after in the United States. Since then their prevalence has increased. By 1990, 14% of all *K pneumoniae* isolated from French hospitals were ESBL producers, with rates approaching 50% in some hospitals.[80] In some United States hospitals, the prevalence of ESBLs in *K pneumoniae* has been reported to be as high as 40%.[81] The Centers for Disease Control and Prevention reported, in 2006 to 2007, the rate of ceftazidime-resistant *K pneumoniae* strains associated with device-related infection exceeded 20%, and the rate of ceftazidime-resistant *E coli* ranged between 5% and 11%.[82] Teaching hospitals have a higher prevalence of ESBL resistance.[83]

ESBL-producing organisms are generally found in hospitals, although nursing home outbreaks have been reported.[84] Outbreaks can occur through either clonal spread of a specific plasmid-carrying strain or through transfer of a particular plasmid to other bacterial strains, or even to different bacterial genera.[84] Resistant organisms can pass from patient to patient on the hands of healthcare providers.[85] Risk factors for acquisition of ESBLs are similar to those reported for other hospital-acquired organisms and include emergency abdominal surgery, mechanical ventilation, presence of percutaneous devices, tracheostomy, prolonged hospital stay, and increased patient morbidity.[83,84,86] Risk factors associated with antibiotics are of increasing interest to researchers. Exposure to antibiotics, particularly to ceftazidime and aztreonam, has been associated with an increased prevalence of ESBL-producing organisms.[87,88] Other studies, however, have found no association between use of third-generation cephalosporins and emergence of ESBL resistance.[84,89] Exposure to TMP-SMX has also been associated with acquisition of ESBLs.[84] Restriction of cephalosporin use has been associated with control of hospital outbreaks.[90,91]

Recently, a study showed use of piperacillin-tazobactam and vancomycin was an independent risk factor for colonization with ESBL organisms.[92]

Many different types of ESBLS have been described, including greater than 160 in the TEM class, over 100 in the SHV family, over 80 in the CTX-M class, and more than 140 in the OXA family. A Web site maintains an up-to-date, complete listing of all identified ESBLs: http://www.lahey.org/studies/.[93]

Each ESBL has unique amino acid substitutions at active sites of the enzyme, affecting its isoelectric point and affecting the enzyme's affinity for and hydrolytic activity of oxyimino-β-lactams.[83] Most ESBLs have higher hydrolytic activity against ceftazidime and aztreonam and less activity against cefotaxime, although the opposite may be true in some cases. For example, in SHV and TEM β-lactamases a serine substitution for glycine at amino acid 238 causes decreased hydrolytic activity against ceftazidime but increases activity against cefotaxime.[83] CTX-M–type ESBLs, however, generally have higher hydrolytic activity against cefotaxime than ceftazidime.[94] Multiple β-lactamases conferring resistance to different classes of β-lactam antibiotics can be found within a single bacterial strain. The diversity of cephalosporin susceptibility profiles manifested by different ESBL enzymes makes detection of some ESBL-producing strains a major challenge for the clinical laboratory. These enzymes are not capable of hydrolyzing cephamycins and carbapenems.[95]

Carbapenemase-producing gram-negative organisms can hydrolyze all penicillins, cephalosporins, and carbapenems and they have been reported in several large outbreaks in hospitalized patients since 2001.[96] KPC,[97] a class A plasmid-mediated β-lactamase, has been associated with outbreaks in hospitals in the northeastern regions of the United States,[98–100] and in other countries, such as China, Brazil, and Israel. KPCs are classically associated with *K pneumoniae*; however, outbreaks of KPCs have been reported with *E coli*, *K oxytoca*, *Enterobacter* spp, *Salmonella*,[101] and *Pseudomonas*.[102] Some preliminary evidence demonstrates propagation of KPC-producing organisms in diverse geographic regions in the United States; inside and outside of hospitals; and in community-based health care services, such as nursing homes.

Metallo-β-lactamases are class B β-lactam hydrolyzing enzymes that contain a zinc moiety and have caused several extended outbreaks of nosocomial infections in patients in ICUs and burns units in Europe and Australia.[103] VIM and IMP metallo-β-lactamases are more commonly found in *Pseudomonas* and *Acinetobacter* species. These metallo-β-lactamases can also rapidly hydrolyze cephalosporins and penicillins (but not aztreonam), and can be encoded by integrins, raising concerns regarding horizontal spread of this resistance mechanism. OXA-type carbapenemases are class D β-lactamases that have caused limited outbreaks in the United States.[104] Metallo-β-lactamases are a diverse group of β-lactamases that are active not only against the oxyimino-cephalosporins and cephamycins but also against carbapenems.[105] There are two major groups of metalloid-β-lactamases: the IMP-type carbapenemases and the Verona integron-encoded metallo-β-lactamase (VIM) carbapenemases.[106]

Another mechanism facilitating β-lactamase activity involves loss of porin channels in the outer cellular membrane and upregulation of efflux pumps, decreasing antibiotic concentrations in the periplasmic space and facilitating hydrolysis by β-lactamases. This mechanism often results in increased resistance to cephalosporins, cephamycins, and β-lactamase inhibitors.[83,107,108] Plasmids producing ESBLs often carry resistance to other antibiotics, including aminoglycosides, tetracyclines, chloramphenicol, TMP, and sulfonamides.[83]

Escherichia coli and *Klebsiella* possess other mechanisms of β-lactam antibiotic resistance unrelated to ESBL production. Resistance to extended-spectrum

cephalosporins, cephamycins, oxyimino-β-lactams, and β-lactamase inhibitors in *E coli* and *K pneumoniae* can be mediated by plasmid-mediated β-lactamases similar to those chromosomal AmpC enzymes produced in species such as *E cloacae* and *Serratia marcescens*.[109,110] Additional porin mutations can result in carbapenem resistance.[83] Resistance to β-lactam–β-lactamase inhibitor combinations can occur by alterations in porin channels, TEM and SHV hyperproduction, by the production of inhibitor-resistant TEM enzymes (Bush-Jacoby-Medeiros Group 2br) and in *E coli*, by chromosomal cephalosporinase AmpC production.[83,95,109]

β-lactam–resistant *Klebsiella* and *E coli* strains are often resistant to quinolones and aminoglycosides, leaving few alternatives for treatment.[111] Alterations in DNA gyrase (topoisomerase II and to a lesser extent topoisomerase IV), porin channel mutations, and efflux mechanisms can confer quinolone resistance.[112] Enzymatic modifications can lead to aminoglycoside resistance.[113]

Tigecycline has potent in vitro activity against multidrug-resistant gram-negative bacilli including ESBL-producing organisms.[25] Unfortunately, there are few data from the clinical setting to support the use of tigecycline as a single agent for the treatment of invasive infections caused by multidrug-resistant gram-negative bacilli. A recent study reported failure of tigecycline among patients with serious infections caused by gram-negative bacilli. Of note, these treatment failures occurred among isolates considered to be susceptible to tigecycline.[114]

Pseudomonas and Other Gram-Negative Rods Producing AmpC

AmpC β-lactamase production is another important mechanism of antimicrobial resistance in gram-negative organisms.[115] These hydrolyzing enzymes were discovered in the 1980s and were extensively studied. AmpC enzymes were initially identified only as inducible, chromosome-encoded β-lactamases found in certain species of *Enterobacter*, *Serratia*, *Pseudomonas*, *Providencia*, *Citrobacter*, and indole-positive *Proteus*.[116] Further research into gram-negative resistance also detected AmpC genes on transferrable plasmids in gram-negative bacilli, which do not chromosomally express these types of β-lactamases, including *Klebsiella* spp, *E coli*, or *Salmonella* spp.[117] Plasmid-mediated AmpC hyperproduction and antimicrobial resistance caused by chromosome-encoded AmpC enzymes can substantially complicate the management of gram-negative infections and adversely impact the clinical outcomes of patients.[118]

β-Lactam resistance among gram-negative organisms can also be mediated by two other classes of plasmid-mediated β-lactamases: hydrolyzing carbapenemases and ESBL. The former is briefly discussed in this section, whereas ESBL resistance was covered previously in the section on multidrug-resistant Enterobacteriaceae.

Epidemiology and mechanisms of resistance

The production of AmpC is regulated through a series of complex interactions among chromosomal bacterial genes. Such interactions are influenced by changes in the cytoplasmic concentrations of intermediates of murein peptidoglycan synthesis and degradation.[119] AmpC is usually not produced at high levels initially, but exposure to particular β-lactam antibiotics, including cephalosporins, cephamycins, monobactams, and extended-spectrum penicillins, can ''induce'' or ''derepress'' the production of AmpC enzymes.[120] Additionally, certain genetic mutations lead to constitutive cephalosporinase production.[121] In both of these cases, the increase in AmpC production is regulated by changes in the homeostatic levels of intermediate products of murein synthesis.[119] There is a 20% to 30% risk of clinical failure when a third-generation cephalosporin is used to treat bacteremia secondary to AmpC-producing

Enterobacter. The risk of such failure is much lower in urinary tract infections because of the high local cephalosporin concentrations.[122] AmpC-mediated resistance can be partially overcome by fourth-generation cephalosporins cefepime and cefpirome, which are more stable against AmpC-derepressed strains.[123]

Although AmpC production mediates much of the antibiotic resistance in certain gram-negative organisms, the impermeability of the outer cellular membrane and alterations in the outer membrane often also contribute to β-lactam resistance.[124] Cefepime, a fourth-generation cephalosporin, has neutral charge and a lower affinity for β-lactamases than third-generation cephalosporins, penetrates the outer membrane more effectively, and exhibits increased affinity for some essential PBP.[125] Cefepime often exhibits greater activity against AmpC-producing organisms than other cephalosporins.[126]

Acinetobacter spp

Resistance mechanisms to β-lactam antibiotics in *Acinetobacter* are not clearly understood, but resistance is common. Resistance frequently seems to be related to β-lactamase production, but other mechanisms have been identified. TEM-l and CARB enzymes seem to confer resistance to penicillins and some narrow-spectrum cephalosporins, whereas chromosomally produced cephalosporinases and plasmid-mediated ESBLs are thought to modulate resistance to broader-spectrum cephalosporins.[95,127] Carbapenem resistance is conferred by multiple different mechanisms including carbapenemase production of the IMP- and VIM-type, production of OXA-type β-lactamases, reduced cellular uptake, target mutations, and alterations in the PBP.[128–131] IMP metallo-β-lactamases were first described in a strain of *P aeruginosa* in Japan in 1988. In *Acinetobacter baumannii* IMP metallo-β-lactamases are usually present as part of a class 1 integron. Although metallo-β-lactamases are not the predominant carbapenemases in *A baumannii*, several have been described including IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, and IMP-11.[132] Aminoglycoside resistance is mediated by aminoglycoside-modifying enzymes, and quinolone resistance by mutational changes of topoisomerase IV.[133,134]

Carbapenems are the most reliable therapeutic agents for infections caused by *Acinetobacter*, but resistance has begun to emerge in multiple geographic areas. Recent studies have reported rates of carbapenem resistance in *A baumannii*, the more resistant *Acinetobacter* species, as high as 11%.[135] A recent Centers for Disease Control and Prevention report noted that greater than 25% of *Acinetobacter* strains associated with device-related infections were carbapenem-resistant. A 1999 study reported meropenem resistance at greater than 50% in all isolates of *A baumannii* recovered from 15 hospitals in Brooklyn, New York.[136] A more recent study from the same area reported that rates of carbapenem resistance have exceeded 60% in some hospitals.[137] β-lactam–β-lactamase inhibitor combination antibiotics have good in vitro activity against *Acinetobacter lwoffi* (approximately 15% are resistant) but are less effective against *A baumannii* (20%–30% are resistant to piperacillin-tazobactam).[135] Ampicillin-sulbactam may be active against strains of *Acinetobacter* resistant to all other β-lactam agents, perhaps because of the unique antimicrobial activity of the sulbactam component against some acinetobacters. Ceftazidime and cefepime have modest activity, but approximately 35% of *A lwoffi* and *A baumannii* strains are resistant.[135] Aminoglycoside resistance occurs in approximately 20% to 30% of *A baumannii* isolates.[138] Resistance to quinolones is variable–precluding use of these drugs empirically before the results of susceptibility tests are known. In one study, approximately 80% of isolates tested were found to be ciprofloxacin-resistant.[139] Tigecycline, a relatively new glycylcycline agent, has bacteriostatic activity against

multidrug-resistant *Acinetobacter* species. High-level resistance to tigecycline has been detected among some multidrug-resistant *Acinetobacter* isolates, and there is concern that the organism can rapidly evade this antimicrobial agent by upregulating chromosomally mediated efflux pumps. Given these findings and concern about whether adequate peak serum concentrations can be achieved, currently tigecycline is best reserved for salvage therapy.[140]

Stenotrophomonas Maltophilia

Stenotrophomonas maltophilia (formerly *Xanthomonas maltophilia*) is an aerobic gram-negative rod that causes bacteremia, respiratory tract infection, skin and soft tissue infection, and endocarditis. The virulence factors associated with *S maltophilia* include the production of proteases and elastases and the ability to adhere to synthetic materials. Nosocomial *S maltophilia* pneumonia is associated with adverse outcomes, particularly when the pneumonia is postobstructive or associated with bacteremia. In uncontrolled clinical trials, mortality rates associated with *S maltophilia* bacteremia range from 21% to 69%.[141]

The inducible, chromosomal enzymes *L1* and *L2* in *S maltophilia* confer resistance to β-lactam antibiotics. *L1* is a Bush-Jacoby-Medeiros class 3 enzyme (or metallo-β-lactamase) with broad activity against penicillins, carbapenems, cephalosporins, and β-lactamase inhibitors.[106,142] *L2* is a cephalosporinase (Bush-Jacoby-Medeiros class 2e) active against cephalosporins and monobactams. A TEM-2 β-lactamase encoded on a Tn-1 like transposon was also recently cloned from an *S maltophilia* isolate.[143] Decreased membrane permeability secondary to porin mutations often leads to quinolone resistance.[144] Aminoglycosides generally are not active against *S maltophilia*, probably caused by inactivating enzymes and alterations in the cell surface.[142] Overexpression of the multidrug efflux pump *SmeDEF* in *S maltophilia* may contribute to decreased susceptibility to tetracyclines, erythromycin, quinolones, and chloramphenicol.[145] Recent studies have reported that TMP-SMX resistance is mediated by the sul2 gene, which is associated with production by plasmids and class 1 integrons.[146,147]

TMP-SMX, a bacteriostatic agent, is the treatment of choice for infections caused by *S maltophilia*. Ticarcillin-clavulanate is the only β-lactam–β-lactamase inhibitor combination antibiotic that is reliably effective and may be used in patients who are intolerant of or infected with an isolate resistant to TMP-SMX.[142] Resistance to both of these agents is increasing; studies have reported that 5% of *S maltophilia* in the United States and 10% of isolates in Europe were resistant to TMP-SMX.[148] Ceftazidime does not possess reliable activity and should not be used empirically.[148] Cefepime has greater activity than ceftazidime (susceptibility was 88.7% versus 35.3% of United States bloodstream isolates in one study).[149] Resistance to imipenem approaches 100%.[149] Among the available fluoroquinolones, levofloxacin and moxifloxacin have better in vitro activity than ciprofloxacin. Minocycline has good in vitro activity,[150] but clinical experience is limited.

Tigecycline, a glycylcycline derived from minocycline, is a compound that has demonstrated good in vitro activity against *S maltophilia* strains including TMP-SMX–resistant *S maltophilia*, so it may be considered as a promising therapeutic option for the treatment of nosocomial infections caused by *S maltophilia*.[151] Use of antibiotic combinations, including TMP-SMX plus ticarcillin-clavulanate or a third-generation cephalosporin, and TMP-SMX plus minocycline plus ticarcillin-clavulanate, is being explored for the treatment of serious *S maltophilia* infections.[152] Although aztreonam is usually inactive against *S maltophilia*, one study demonstrated synergistic activity in vitro when combined with ticarcillin-clavulanate.[152] Although

data are limited, resistance to TMP-SMX seems to be emerging, and recent in vitro modeling studies suggest that combination therapies of TMP-SMX plus ciprofloxacin and TMP-SMX plus tobramycin exhibit a greater killing capacity than TMP-SMX alone.[153]

The choice of monotherapy or combination therapy remains controversial. Use of multidrug therapy should be considered in cases of severe infection, particularly if local rates of resistance to TMP-SMX are high.[141]

Salmonella spp

Nontyphoidal species of *Salmonella*, such as *S enteritidis* and *S enterica*, are foodborne pathogens that can asymptomatically colonize the human intestine or cause clinical illnesses, such as gastroenteritis and bacteremia. Resistance to antimicrobial agents used to treat typhoidal and nontyphoidal species has rapidly emerged and disseminated around the world.[154]

Resistance to chloramphenicol in *Salmonella typhi* emerged in the 1970s, and several major outbreaks have been caused by chloramphenicol-resistant strains.[155] Resistance to chloramphenicol is often mediated by a self-transferable plasmid (IncHI) that also mediates resistance to sulfonamides, tetracycline, amoxicillin, TMP-SMX, and streptomycin.[154,156,157]

Resistance to the fluoroquinolones is an emerging problem, particularly in Asia,[158,159] and is usually mediated by chromosomal point mutations in the *gyrA* gene.[160] These quinolone-resistant strains are usually sensitive to ceftriaxone, cefixime, and azithromycin but the clinical response to treatment is usually slower and, with clearance of fever, sometimes exceeding taking 7 days or more. Failure rates are higher (>20%) when infection is caused by quinolone-resistant strains.[161] Resistance to nalidixic acid may predict clinical failure of quinolone therapy, even among isolates with in vitro quinolone susceptibility.[162] Such resistance to nalidixic acid was detected among 23% of *S typhi* isolates identified through the National Antimicrobial Resistance Monitoring System in 2000.[163] Resistance to third-generation cephalosporins (eg, ceftriaxone and cefotaxime) has occurred sporadically.[164]

The quinolones, such as ciprofloxacin, are considered the drugs of choice for empiric treatment of typhoid fever, except in areas of the world where quinolone resistance is common (eg, Asia).[165] So far, resistance to ciprofloxacin is infrequent in the United States, but was found in up to 23% of *S typhi* isolates in the United Kingdom in 1999.[166] In that study, multidrug resistance to chloramphenicol, ampicillin, and TMP was reported in 26% of all *S typhi* isolates.[166] Other potential oral alternatives for treatment of *S typhi* infections include amoxicillin, TMP-SMX, cefixime, azithromycin, and chloramphenicol, as long as these agents possess in vitro activity against a given strain.[154] Third-generation cephalosporins, such as ceftriaxone and cefotaxime, remain active but require intravenous administration.[167]

Resistance among nontyphoidal strains of salmonellae emerged in the 1990s and spread rapidly.[154] Emergence of multiresistance to ampicillin, chloramphenicol, and TMP-SMX is caused in part by the widespread dissemination of *Salmonella typhimurium* definitive phage type 104 (DT 104).[168] This strain contains chromosomal determinants that mediate resistance to ampicillin, chloramphenicol, TMP-SMX, streptomycin, and tetracycline.[169] Resistance to fluoroquinolones has also emerged among nontyphoidal salmonellae in the United Kingdom (including DT104) and in the United States. Resistance to the fluoroquinolones is often mediated by *gyrA* mutations and fortunately this remains uncommon in isolates in the United States.[170] Resistance to broad-spectrum cephalosporins is conferred by

plasmid-mediated AmpC-type cephalosporinase production[171] and sometimes by ESBL production.[172] Resistance to the carbapenems has been reported and is mediated by porin loss; cephalosporinase production; and carbapenemase production (including KPCs).[101,173]

Campylobacter Jejuni

Campylobacter spp are fastidious, curved, seagull-shaped, motile gram-negative bacilli and are the leading cause of foodborne diarrhea and a common cause of travelers' diarrhea.[174] Fluoroquinolone resistance in *Campylobacter* spp has been present since the early 1990s in Asia and Europe, which coincided with the addition of enrofloxacin to animal feed. In Spain, quinolone resistance is well above 50%. Elsewhere in Europe, resistance rates are approximately 10% to 20% and many of the resistant strains are acquired outside the reporting country.[175,176] Thailand experienced a similar increase in the rates of fluoroquinolone resistance in campylobacter during the 1990s (0% in 1990 to 84% in 1995).[177] Rates of fluoroquinolone resistance among *Campylobacter* are lower in the United States, but agricultural use of fluoroquinolones again coincided with increased resistance. In one United States study, resistance increased from 1.3% in 1992 to 10.2% in 1998.[178] Imported isolates can contribute significantly to local resistance patterns.

Resistance to the macrolides is low (<5% in most regions).[179] Most isolates are still susceptible to aminoglycosides, chloramphenicol, clindamycin, nitrofurantoin, and imipenem.[180] A point mutation at codon 86 in the *gyrA* DNA gyrase gene is the most common mutation conferring quinolone resistance.[179] Mutations in the *parC* gene occur less frequently,[181] but the presence of mutations in both regions confers high-level quinolone resistance.[181] Multidrug efflux pumps may also have a role in the development of quinolone resistance.[182] Erythromycin resistance in *Campylobacter* is caused by ribosomal alterations.[179]

Neisseria Gonorrhoeae

Antimicrobial resistance has been a concern with *Neisseria gonorrhoeae* since the 1940s when resistance to sulfonamides was first noted; this was followed by penicillin resistance in the 1950s, tetracycline resistance in the 1980s, and fluoroquinolone resistance in the 1990s.[183,184]

High-level penicillin resistance (MIC $\geq$16 μg/mL) in *N gonorrhoeae* is most often mediated by penicillinase production.[185] Penicillin resistance caused by production of a plasmid-encoded TEM-1 type β-lactamase was first detected in *N gonorrhoeae* in 1976 and has now disseminated worldwide.[186] In the United States, the percentage of penicillinase-producing *N gonorrhoeae* peaked in 1991 at 11% but declined to 0.4% in 2006.[187]

Multiple chromosomal mutations can mediate lower-level penicillin (MIC >2 μg/mL) resistance. Resistance genes typically accumulate in a stepwise fashion, leading to gradually increasing penicillin MICs.[185] These resistance genes include *penA*, which encodes an altered PBP 2; *mtr*, which increases expression of an efflux pump; and *penB*, which decreases antibiotic permeability across the cell membrane through a porin gene mutation.[185,188] Chromosomally mediated resistance to penicillin was present in 1.2% of isolates in a recent United States survey.[187]

Resistance to tetracycline occurs through chromosomally mediated changes in cell membrane porins or by ribosomal protection by the plasmid mediated tetM resistance gene. Additional chromosomal mutations led to resistance to spectinomycin.[189] Macrolide resistance can occur through efflux pumps, *erm* methylases, and changes in the 23S ribosome.[190]

Fluoroquinolone-resistant *N gonorrhoeae* (MIC $\geq$1 μg/mL) has disseminated to many countries,[191] and is widespread in certain parts of Asia. In a recent study, greater than 35% of *N gonorrhoeae* isolates in the Philippines and Vietnam were quinolone resistant.[192] There have also been alarming increases in quinolone resistance reported recently in England and Wales.[191] The overall prevalence of quinolone-resistant gonococci in the United States was 2.2% in 2002, but this increased to greater than 13% by 2005.[183] The rate of quinolone resistance is particularly high among men who have sex with men (29%). Decreased susceptibility to fluoroquinolones is caused by mutations in the *parC* gene of topoisomerase IV and *gyrA* of DNA gyrase.[193,194]

SUMMARY

The emergence of resistance to antimicrobial agents continues to evolve substantially, influencing the evaluation and treatment of infections in nosocomial and health care–associated settings and in the community. Bacteria use several strategies to avoid the effects of antimicrobial agents, and have evolved highly efficient means for clonal spread and for the dissemination of resistance traits. Control of antibiotic-resistant pathogens provides a major challenge for the medical and public health communities and for society. Control of the emergence of resistant pathogens requires adherence to infection control guidelines, such as those issued by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/dhqp/guidelines.html), and physicians, patients, and health care consumers must all understand the need for judicious use of antibiotics (http://www.cdc.gov/drugresistance/healthcare/default.htm).

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