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Tetracycline Antibiotics and Resistance

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Tetracyclines possess many properties considered ideal for antibiotic drugs, including activity against Gram-positive and -negative pathogens, proven clinical safety, acceptable tolerability, and the availability of intravenous (IV) and oral formulations for most members of the class. As with all antibiotic classes, the antimicrobial activities of tetracyclines are subject to both class-specific and intrinsic antibiotic-resistance mechanisms. Since the discovery of the first tetracyclines more than 60 years ago, ongoing optimization of the core scaffold has produced tetracyclines in clinical use and development that are capable of thwarting many of these resistance mechanisms. New chemistry approaches have enabled the creation of synthetic derivatives with improved in vitro potency and in vivo efficacy, ensuring that the full potential of the class can be explored for use against current and emerging multidrug-resistant (MDR) pathogens, including carbapenem-resistant Enterobacteriaceae, MDR *Acinetobacter* species, and *Pseudomonas aeruginosa*.

Tetracycline antibiotics are well known for their broad spectrum of activity, spanning a wide range of Gram-positive and -negative bacteria, spirochetes, obligate intracellular bacteria, as well as protozoan parasites. The first tetracyclines were natural products derived from the fermentations of actinomycetes. Chlortetracycline, produced by *Streptomyces aureofaciens*, and marketed as Aureomycin, was first reported by Benjamin Duggar at Lederle Laboratories in 1948 and approved for clinical use that same year (Duggar 1948). Soon after, Pfizer (New York) scientists isolated oxytetracycline, approved by the U.S. Food and Drug Administration (FDA) in 1950 and marketed as Terramycin (Finlay et al. 1950). Other tetracyclines that followed over the next two decades were also natural products produced by streptomycetes (tetracycline, demethylchlortetracycline) or semisynthetic derivatives with improved antibacterial potency, spectrum, resistance coverage, solubility, and/or oral bioavailability (methacycline, rolitetracycline, lymecycline, doxycycline, and minocycline) (Jarolmen et al. 1970; Cunha et al. 1982; Nelson and Levy 2011). Several of these "legacy" tetracyclines remain in clinical use for the treatment of uncomplicated respiratory, urogenital, gastrointestinal, and other rare and serious infections; however, the dissemination of tetracycline-resistant mechanisms has narrowed their utility, limiting use to only infections with confirmed susceptibility (Fig. 1).

After a long pause in the advancement of the tetracycline class, renewed interest in optimization of tetracyclines during the late 1980s led to the discovery of semisynthetic derivatives with improved potency against difficult-to-treat emerging multidrug-resistant (MDR) Gram-

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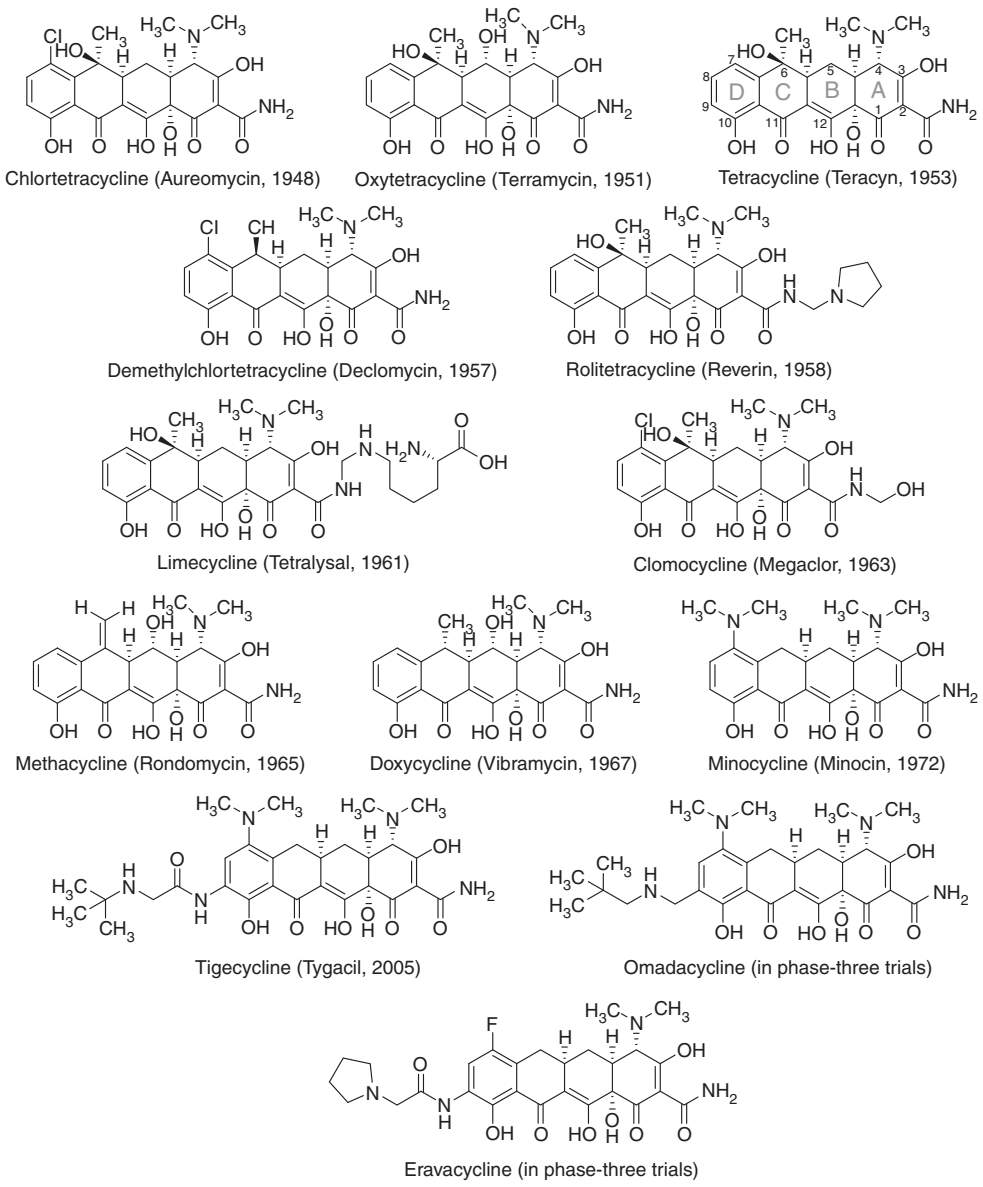

Figure 1. Chemical structures of clinically used tetracyclines and development candidates. Tetracycline structures are labeled with generic names; trade names and year of discovery are indicated within parentheses. The core structure rings (*A*–*D*) and carbons (1–12) are labeled in the chemical structure of tetracycline using the convention for tetracycline carbon numbering and ring letter assignments.

negative and -positive pathogens, including bacteria with tetracycline-specific resistance mechanisms. Tigecycline, a semisynthetic parenteral glycylcycline, was discovered in 1993 by scientists at Lederle (which later became Wyeth, New York), and introduced into clinical use in 2005 (Sum and Petersen 1999; Zhanel et al. 2004). Tigecycline continues to be an important treatment option for serious infections caused by pathogens resistant to other antibiotic classes. In recent years, two new tetracyclines have entered clinical development: omadacycline, a

semisynthetic aminomethylcycline derivative of minocycline discovered at Paratek Pharmaceuticals (Boston, MA) (Draper et al. 2014), and eravacycline, a fully synthetic fluorocycline discovered at Tetraphase Pharmaceuticals (Watertown, MA) (Clark et al. 2012; Xiao et al. 2012). In addition to efficacy against MDR infections, an important feature of these two new antibiotics is their oral formulations. This review will focus on recent developments in the understanding of tetracycline-resistance mechanisms and their potential impact on the clinical utility of tetracycline-class antibiotics.

MECHANISM, UPTAKE, AND TETRACYCLINE-SPECIFIC RESISTANCE

In recent surveillance studies, the prevalence of tetracycline resistance in selected European countries was found to be 66.9% and 44.9% for extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* species (spp.), respectively (Jones et al. 2014), and global tetracycline-resistance percentages were 8.7% and 24.3% for methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumoniae*, respectively (Mendes et al. 2015). Resistance to tetracyclines is usually attributed to one or more of the following: the acquisition of mobile genetic elements carrying tetracycline-specific resistance genes, mutations within the ribosomal binding site, and/or chromosomal mutations leading to increased expression of intrinsic resistance mechanisms. Three general class-specific mechanisms have been well described: efflux, ribosomal protection, and enzymatic inactivation of tetracycline drugs. As there are several recent reviews on the topics of tetracycline-specific resistance determinants and their prevalence in clinical and environmental settings (Roberts 2005, 2011; Jones et al. 2008; Thaker et al. 2010), only a limited discussion of these areas will be covered here.

Uptake and Mechanism of Action

Tetracyclines preferentially bind to bacterial ribosomes and interact with a highly conserved 16S ribosomal RNA (rRNA) target in the 30S ribosomal subunit, arresting translation by sterically interfering with the docking of aminoacyl-transfer RNA (tRNA) during elongation (Maxwell 1967; Brodersen et al. 2000; Pioletti et al. 2001). Tetracyclines are usually considered bacteriostatic antibiotics; however, organism- and isolate-specific bactericidal activity in vitro has been described (Norcia et al. 1999; Petersen et al. 2007; Bantar et al. 2008; Noviello et al. 2008), and, recently, the bactericidal activity of tigecycline against *E. coli* and *K. pneumoniae* in a mouse model suggests that in vitro bactericidal assessments may not necessarily predict in vivo outcomes (Tessier and Nicolau 2013).

The mechanism of tetracycline uptake has been reviewed by Nikaido and Thanassi (1993). Briefly, in Gram-negative cells such as *E. coli*, tetracycline passively diffuses through the outer membrane porins OmpF and OmpC (Mortimer and Piddock 1993; Thanassi et al. 1995), most likely as a Mg^{2+} chelate, and this is consistent with the finding that outer membrane porin mutants show decreased susceptibility to tetracyclines (Pugsley and Schnaitman 1978). Accumulation of tetracycline in the periplasm is driven by the Donnan potential across the outer membrane. The dissociation of tetracycline from Mg^{2+} enables the weakly lipophilic, uncharged form to diffuse through the inner membrane to the cytoplasm where it may be complexed with magnesium and reach its ribosomal target. Uptake into the cytoplasm is partially energy dependent, involving passive diffusion, proton motive force, and phosphate bond hydrolysis (McMurry and Levy 1978; Smith and Chopra 1984; Yamaguchi et al. 1991).

Ribosomal Interactions

Crystallographic studies with the *Thermus thermophilus* 30S ribosomal subunit have revealed at least one high-occupancy tetracycline-binding site (Tet-1) and five other minor binding sites in 16S rRNA (Brodersen et al. 2000; Pioletti et al. 2001). Tetracycline most likely binds complexed with two Mg^{2+} ions at the Tet-1 site located in a pocket formed between helices h34 and h31, near the A-site where aminoacyl-tRNA

docks onto the 30S subunit, consistent with the known mechanism of action (Jenner et al. 2013). The significance of the other five tetracycline-binding sites located elsewhere within the 30S subunit is unclear, and recent crystallographic studies with tigecycline and tetracycline binding to the *T. thermophilus* 70S ribosome (Jenner et al. 2013) and tigecycline binding to the 30S ribosome (Schedlbauer et al. 2015) showed that tigecycline was bound only to the Tet-1 site, and secondary binding sites were not observed (Fig. 2). Additional interactions made between the 9-*tert*-butylglycylamido moiety of tigecycline and C1054 in h34 are consistent with the higher binding affinity and greater antitranslational potency of tigecycline compared with tetracycline (Olson et al. 2006). Interestingly, a different orientation of this tigecycline side chain was observed in the 30S versus the 70S structure (Fig. 2), suggesting that tigecycline must accommodate conformation changes in the primary binding site that occur during decoding (Schedlbauer et al. 2015). Consistent with this recent finding, earlier work by Bauer et al. (2004) showed that tigecycline and tetracycline produced slightly different patterns of Fe^{2+}-mediated RNA cleavage and dimethylsulfate modification, suggesting that both antibiotics bind at the same binding site, but in somewhat different orientations. Ribosome-binding competition experiments with [^{3}H]tetracycline show relative IC_{50} values as follows: eravacycline, 0.22 μM; tigecycline, 0.22 μM; minocycline, 1.63 μM; omadacycline, 1.96 μM; and tetracycline, 4 μM; and results were consistent with these and other novel tetracycline derivatives binding at a single major site (Olson et al. 2006; Grossman et al. 2012; Jenner et al. 2013; Draper et al. 2014).

Binding-Site Mutations

Because most bacteria have multiple rRNA copies, target-based mutations in rRNA conferring tetracycline resistance are usually found in bacteria with low rRNA gene copy numbers. Mutations in 16S rRNA have been reported in *Propionibacterium acnes* (2–3 16S rRNA

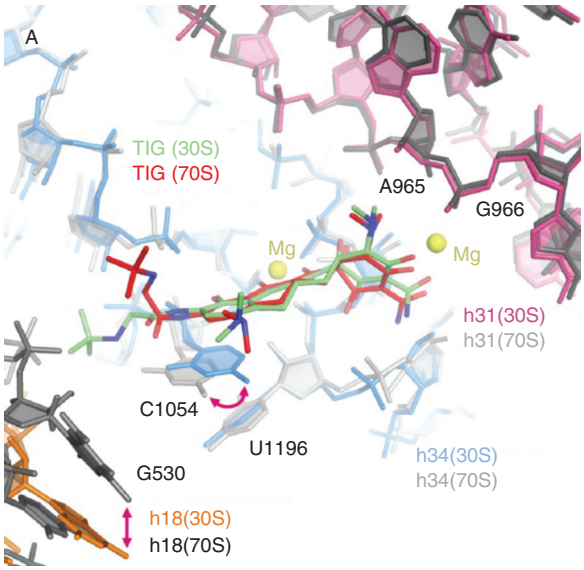

Figure 2. Alternative binding modes of tigecycline at the primary ribosomal-binding site. Alternative tigecycline-binding modes in the 30S (green) and 70S (red) structures are shown, superimposed within the primary tetracycline-binding site. Key nucleotides (G530, A965, G966, C1054, U1196) and helices (h18, h31, h34) are shown in both structures. (From Schedlbauer et al. 2015; reprinted, with permission, from the American Society for Microbiology © 2015.)

copies), *Helicobacter pylori* (1–2 16S rRNA copies), *Mycoplasma bovis* (1–2 16S rRNA copies), and *S. pneumoniae* (4 16S rRNA copies), and the effects of these mutations on tetracycline binding can generally be explained by crystallographic or biophysical data. In *H. pylori*, a triple mutation AGA 965-967 TTC in the h31 loop, and a deletion of G942 (*E. coli* numbering), each conferred tetracycline resistance (Trieber and Taylor 2002). Residues 965–967 are located in the primary, or Tet-1, binding site, whereas G942 is located in the Tet-4 secondary binding site (Brodersen et al. 2000; Pioletti et al. 2001). Mutations in h34 of 16S rRNA were associated with increased tetracycline resistance in *P. acnes* (G1058C) and *M. bovis* (G1058A/C), and tetracycline-resistance mutations A965T, A967T/C, and U1199C (which base pairs with G1058 in h34) were also found in *M. bovis* (Ross et al. 1998; Amram et al. 2015). Although G1058 does not directly interact with tetracycline, mutation to cytosine likely causes a conformational change in the binding site, reducing the affinity of tetracycline for the 30S ribosomal subunit. Preexisting G1058C mutations in *P. acnes* reduced the antibacterial activities of tetracycline, doxycycline, eravacycline, and tigecycline, consistent with all of these tetracycline antibiotics having common interactions with rRNA in bacteria (Grossman et al. 2012). In *S. pneumoniae*, mutations in 16S RNA C1054T and T1062G/A conferred tigecycline resistance when present in the four genomic copies of 16S rRNA (Lupien et al. 2015). Whereas resistance caused by a mutation in C1054 can be explained by the interaction of this residue with tigecycline, a more indirect effect on tigecycline binding may be conferred by mutations in T1062. Nonsense mutations in a gene encoding a 16S rRNA methyltransferase in *S. pneumoniae* were also found to confer reduced tigecycline susceptibility in the study by Lupien et al. (2015). This enzyme methylates position N(2) of G966 in h31 of 16S rRNA in *E. coli* and the alterations in this activity may reduce tigecycline binding to the ribosome.

Unlike rRNA genes, genes encoding ribosomal proteins are single copy and mutations in these genes can confer antibiotic resistance. Mutations in the *rpsJ*, encoding changes or deletions in residues 53–60 in the 30S ribosomal subunit protein S10, have been linked to tetracycline or tigecycline resistance in in vitro studies with Gram-positive bacteria *Bacillus subtilis*, *Enterococcus faecium*, *E. faecalis*, and *S. aureus* (Williams and Smith 1979; Wei and Bechhofer 2002; Beabout et al. 2015a; Cattoir et al. 2015), in clinical isolates of Gram-negative bacteria *Neisseria gonorrhoeae* and *K. pneumoniae* (Hu et al. 2005; Villa et al. 2014), and in in vitro studies with *E. coli* and *Acinetobacter baumannii* (Beabout et al. 2015a). Identification of a tigecycline-resistant *K. pneumoniae* strain with an *rpsJ* mutation encoding Val57Leu in S10 was the first description of tetracycline resistance attributable, at least in part, to a target site mutation in Enterobacteriaeceae (Villa et al. 2014). In the *T. thermophilus* crystal structure, these S10 residues map to a loop projecting toward the aminoacyl-tRNA-binding site in the 30S structure (Brodersen et al. 2000; Carter et al. 2000). Although located ~8.5 Å from tetracycline in the structure, it has been proposed that this region of the S10 protein may alter the interaction of tetracyclines and 16S rRNA in this region (Hu et al. 2005). Mutations in *rpsC* encoding Lys4Arg and His175Asp variations in ribosomal protein S3 were associated with reduced tigecycline susceptibility in *S. pneumoniae* (Lupien et al. 2015). Ribosomal protein S3 has been shown to be important for tetracycline binding to the ribosome (Buck and Cooperman 1990).

Tetracycline-Specific Ribosomal Protection

Tetracycline ribosomal protection proteins (RPPs), originally described in *Campylobacter jejuni* and *Streptococcus* spp., are GTPases with significant sequence and structural similarity to elongation factors EF-G and EF-Tu (Burdett 1986; Taylor et al. 1987; Sanchez-Pescador et al. 1988; Kobayashi et al. 2007). According to a nomenclature list maintained at the University of Washington (faculty.washington.edu/marilynr), there are currently 12 reported ribosomal protection genes. These genes are disseminated through bacterial populations on mobile genetic elements, and many of the genes are

found in both Gram-negative and Gram-positive organisms (Roberts 2011). The most common and best characterized RPPs are Tet(O) and Tet(M), with 75% sequence similarity to each other. These proteins catalyze the GTP-dependent release of tetracycline from the ribosome (Connell et al. 2003a,b). Cryoelectron microscopic structural studies indicate that RPPs compete with EF-G for an overlapping binding site, and it is thought that RPPs dissociate tetracycline from its binding site by directly interfering with the stacking interaction of the tetracycline D-ring and 16S rRNA base C1054 within h34 (Donhofer et al. 2012; Li et al. 2013). Conformational changes induced by RPPs promote rapid binding of the EF-Tu • GTP • aa-tRNA ternary complex, enabling translation to continue in the presence of tetracycline (Donhofer et al. 2012). RPP mechanisms confer resistance to tetracycline, minocycline, and doxycycline; however, other tetracyclines containing side chains at the C-9 position of the D-ring, such as tigecycline and other glycylcyclines, eravacycline and other fluorocyclines, and omadacycline, generally retain translational inhibitory and antibacterial activities in the presence of RPPs (Table 1) (Rasmussen et al. 1994; Bergeron et al. 1996; Grossman et al. 2012; Jenner et al. 2013). The 9-*t*-butylglycylamido moiety at the C-9 position in tigecycline was shown to improve binding affinity and translational inhibition by >100-fold and 20-fold, respectively, over that of tetracycline; however, the mechanism of RPP evasion could not be fully explained (Olson et al. 2006). Recently, using a set of novel synthetic tetracycline derivatives containing C-9 side chains with different degrees of bulkiness, Jenner et al. (2013) showed that, in addition to conferring enhanced interactions with C1054 (Schedlbauer et al. 2015), steric interference by the bulk of the C-9 side chain is also a significant factor in maintaining ribosome binding in the presence of RPPs. Although earlier reports have shown the relative immunity of tigecycline to RPP mechanisms, a recent study by Beabout et al. (2015b) has linked *Tn*916-associated constituitive overexpression and increased copy number of *tet*(M) to tigecycline resistance in *E. faecalis*.

Tetracycline-Specific Efflux

The most common tetracycline-specific efflux pumps are members of the major facilitator superfamily (MFS) of transporters (Chopra and Roberts 2001); however, there have been rare reports of non-MFS pumps (Teo et al. 2002; Warburton et al. 2013). The latest tally shows that 30 distinct tetracycline-specific efflux pumps reported in bacteria (faculty.washington.edu/marilynr; updated August 6, 2015). These pumps extrude tetracycline antibiotics from the inside of cells at the expense of a proton, and have been assigned to seven different groups according to amino acid sequence

Table 1. The activities of tetracyclines against recombinant *E. coli* expressing major tetracycline-specific resistance mechanisms

	MIC (μg/mL)					
	E. coli lacZ	*E. coli tet*(M)	*E. coli tet*(K)	*E. coli tet*(A)	*E. coli tet*(B)	*E. coli tet*(X)
Eravacycline	0.063	0.063	0.031	0.25	0.063	4
Tigecycline	0.063	0.13	0.063	1	0.063	2
Doxycycline	2	64	4	32	32	16
Minocycline	0.5	64	1	8	16	4
Tetracycline	2	128	128	>128	>128	128
Ceftriaxone	0.063	0.13	0.063	0.13	0.13	0.13

Genes were overexpressed in *E. coli* DH10B from a recombinant expression vector under the control of an arabinose promoter. Standardized MIC assays were performed according to CLSI methodology as previously described.

MIC, minimal inhibitory concentration; *tet*(M), ribosomal protection; *tet*(K), Gram-positive tetracycline efflux; *tet*(A) and *tet*(B), Gram-negative efflux; *tet*(X), flavin-dependent monooxygenase.

Data is reprinted, with permission, from Grossman et al. (2012).

similarities and the number of times they traverse the inner membrane (9–14 times) (Guillaume et al. 2004; Thaker et al. 2010). The most clinically prevalent pumps are members of either group 1 or group 2. The group 1 drug $-H^+$ antiporters contain 12 transmembrane segments organized into α and β domains connected by a large interdomain cytoplasmic loop. This group includes Tet(A) and Tet(B), the most commonly found tetracycline pumps in Gram-negative clinical isolates. The group 2 pumps possess 14 transmembrane segments and include Tet(K) and Tet(L), the most common tetracycline-specific efflux pumps in Gram-positive clinical isolates. In addition to their role in conferring tetracycline resistance, group 2 pumps are also monovalent cation $-H^+$ antiporters, and may play a role in coping with sodium stress, alkali stress, and potassium insufficiency (Guay et al. 1993; Krulwich et al. 2001). Pumps assigned to group 3–7 include pumps that are less prevalent clinically (Guillaume et al. 2004).

The order of substrate preference across all tetracycline efflux pump types can be shown in recombinant *E. coli* strains overexpressing representative pumps in an isogenic background: tetracycline > minocycline, doxycycline > tigecycline, eravacycline (Table 1) (Grossman et al. 2012). It should be noted, however, that it is likely that multiple strain-specific factors in clinical isolates affecting uptake and intrinsic efflux systems, in addition to the level of expression of tetracycline-specific pumps, play a coordinated role in the overall susceptibility to tetracyclines. Tet(A), Tet(B), and Tet(K) pumps are all able to recognize tetracycline, minocycline, and doxycycline. Whereas Tet(B) and Tet(K) overexpression had no effect on tigecycline and eravacycline, overexpression of Tet(A) produced a fourfold increase in eravacycline minimal inhibitory concentration (MIC) and a 16-fold increase in tigecycline MIC versus the negative control strain, indicating that these newer tetracyclines are recognized to differing extents by the Tet(A) pump (Table 1) (Grossman et al. 2012). Earlier characterizations of the substrate specificity of Tet pumps in nonisogenic strain backgrounds led to the conclusion that tigecycline was not a substrate for Tet(A) (Petersen et al. 1999), and that a naturally occurring amino acid sequence variation (Ser-Phe-Val→Ala-Ser-Phe) in the interdomain loop sequence at residues 201–203 affected recognition of tigecycline and minocycline (Tuckman et al. 2000). More recent work has shown that recombinant expression of either Tet(A) pump variation in *E. coli* produced similar tigecycline and minocycline susceptibility, confirming that these amino acid residues do not appear to be involved in substrate recognition (Fyfe et al. 2013). The notion that mutations in tetracycline pumps can alter substrate specificity is, however, supported by studies with *tet*(B) in which mutations encoding residues in transmembrane domains had opposing effects on tetracycline versus glycylcycline susceptibility (Guay et al. 1994), and site-directed mutations in the interdomain loop had opposing effects on tetracycline versus minocycline and doxycycline susceptibility (Sapunaric and Levy 2005). These studies suggest the possibility that tetracyclines could select for resistance mutations within tetracycline pump genes during clinical use; however, this has not yet been reported in clinical isolates.

Enzymatic Inactivation of Tetracyclines

Evidence of a tetracycline-modifying enzyme mechanism was first described as an activity encoded by a *Bacteroides* plasmid expressed in *E. coli* (Speer and Salyers 1988, 1989). This activity was subsequently characterized as a flavin-dependent monooxygenase, encoded by an expanding family of *tet*(X) orthologs, capable of covalently inactivating all tetracyclines with the addition of a hydroxyl group to the C-11a position located between the C and B rings of the tetracycline core (Fig. 1) (Speer et al. 1991; Yang et al. 2004; Moore et al. 2005; Grossman et al. 2012; Aminov 2013). Because *Bacteroides* species are obligate aerobes, it is not surprising that the oxidoreductases encoded by *tet*(X) and its orthologs *tetX1* and *tetX2* do not confer resistance in the isolates in which they were originally found (Whittle et al. 2001). The environmental origin of *tet*(X) is suggested by its iden-

tification in *Sphinogbacterium* spp., a Gram-negative soil bacterium that expresses a functional Tet(X) (Ghosh et al. 2009). Further, the presence of *tet*(X) and genes encoding similar tetracycline-inactivating activities, also known as "tetracycline destructases," in agricultural and aquacultural bacteria ensures the persistence of this resistance mechanism in the food chain, facilitating crossover into human pathogens (Aminov 2013; Forsberg et al. 2015). Because of the conjugative nature of *tet*(X)-containing plasmids and transposons, recent reports of *tet*(X) in Enterobacteriaceae and Pseudomonadaceae hospital urinary tract infection (UTI) isolates in Sierra Leone, and *tet*(X) in *A. baumannii* in a Chinese hospital, are of concern with regard to the spread of this mechanism (Leski et al. 2013; Deng et al. 2014).

Other less well-characterized tetracycline-modifying mechanisms have also been described. An NADP-requiring tetracycline-modifying activity similar to that of Tet(X) was expressed from the metagenomic DNA of uncultivatable oral microflora; however, there is no homology between the deduced amino acid sequence of Tet(37) from the oral metagenome and Tet(X) from *Bacteroides* (Diaz-Torres et al. 2003). Another gene, *tet*(34), has been cloned from the chromosome of *Vibrio* spp. and encodes a xanthine-guanine phosphoribosyltransferase capable of conferring resistance to oxytetracycline (Nonaka and Suzuki 2002). The clinical relevance of these two tetracycline-modifying enzymes remains to be determined.

INTRINSIC MULTIDRUG-RESISTANCE MECHANISMS AFFECTING TETRACYCLINES

Complex intrinsic regulatory networks in bacteria modulate the uptake and intracellular accumulation of most antibiotics, including tetracyclines. Mutations affecting expression and/or function of one or more key repressor, activator, pump, or porin can simultaneously impact the susceptibility to a broad range of antibiotic classes (Fig. 2).

AraC Transcriptional Activators in Gram-Negative Bacteria

MarA, RamA, SoxS, RobA, and the newly described RarA are members of the "AraC-family" of bacterial transcriptional activators that enable Gram-negative bacteria to respond to different types of environmental stress, including antibiotic exposure (Fig. 3) (Martin and Rosner 2001; Grkovic et al. 2002; De Majumdar et al. 2013). Each activator regulates a set of genes in response to a specific type of stress (Martin et al. 2008; Martin and Rosner 2011); for instance, MarA regulates more than 60 genes collectively referred to as the "*mar* regulon," for multiple antibiotic resistance (Barbosa and Levy 2000). AraC-family activators bind to a consensus 20 base pair sequence via two helix–turn–helix motifs that comprise the DNA-binding domain. The DNA-binding site is known as the "box" and is located in the promoter region of stress-responsive genes (i.e., "marbox" for MarA, etc.). Further, AraC-family activators can bind their own promoters and autoactivate their own expression (Alekshun and Levy 1997; Rosenblum et al. 2011). Mutations promoting constituitive expression of AraC-family regulons are now known to be common mechanisms contributing to multidrug resistance.

The first description of *mar* in *E. coli* by George and Levy in 1983 showed that amplifiable resistance to tetracyclines, as well as structurally and mechanistically unrelated antibiotics, including chloramphenicol, penicillins, cephalosporins, puromycin, nalidixic acid, and rifampin, was caused by an energy-dependent efflux system (George and Levy 1983a,b). The Mar regulon is now known to be widespread in enteric Gram-negative species, including *E. coli*, *K. pneumoniae*, *Salmonella* spp., *Shigella* spp., *Citrobacter* spp., *Enterobacter* spp., and *Yersinia* spp. (Cohen et al. 1993; Alekshun and Levy 1997). The *mar* locus encodes two divergent operons regulated by a repressor MarR that binds to an operator MarO (Martin and Rosner 1995; Seoane and Levy 1995). Induction of MarR triggers the expression of *marC* in one direction and *marRAB* in the other direction. Whereas tetracycline has been shown to be an

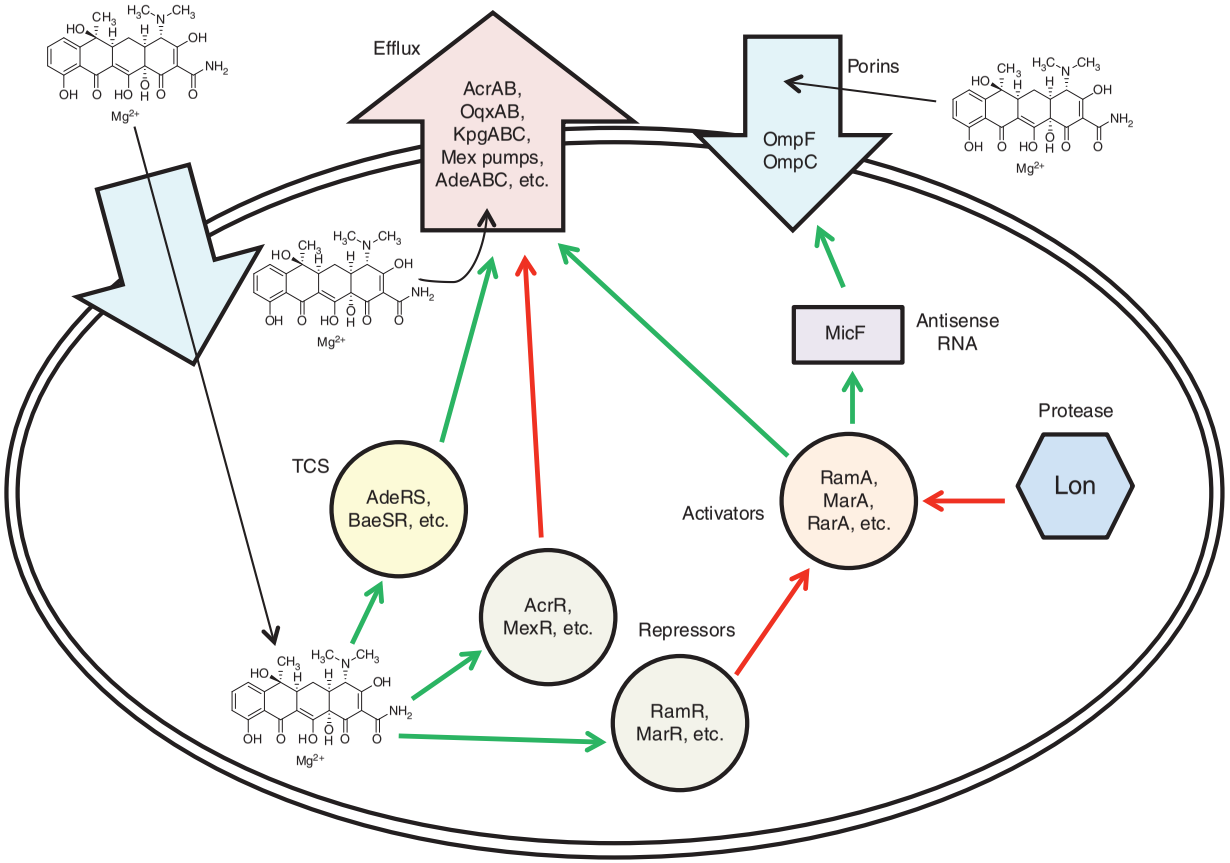

Figure 3. Regulation of expression of Gram-negative intrinsic multidrug-resistance mechanisms affecting tetracyclines. A summary of known regulatory mechanisms affecting tetracycline susceptibility are shown. Green arrows indicate interactions in which tetracycline resistance is "increased," and red arrows indicate interactions in which tetracycline resistance is "reduced." See the text for details. TCS, Two-component signal transduction system.

inducer of *marA* expression (Hachler et al. 1991), induction appears to be via an indirect mechanism because direct binding of tetracycline to MarR could not be shown (Martin and Rosner 1995). MarA is a key activator of stress-responsive genes, and its role in promoting overexpression of the major multidrug efflux pump, AcrAB (Li and Nikaido 2009), is central to conferring an MDR phentoype in enteric bacteria (Gambino et al. 1993; Alekshun and Levy 1997). MarA also controls the expression of the major Gram-negative porin OmpF through the up-regulation of *micF*. MicF is an antisense RNA regulator of *ompF* expression, acting by reducing the levels of *ompF* mRNA (Cohen et al. 1988; Andersen and Delihas 1990; Gambino et al. 1993). Reduction in *ompF* expression contributes to reduced accumulation of tetracycline and other antibiotics (Mortimer and Piddock 1993; Thanassi et al. 1995). The roles of MarC and MarB in multidrug resistance are less well defined; MarC has been shown to encode a periplasmic protein, which appears to indirectly affect the transcription of *marA* (Vinue et al. 2013). Whereas first-step *mar* mutants may not confer clinically relevant resistance to some classes of antibiotics, it is possible that first-step mutants can achieve clinically relevant resistance to tetracycline (George and Levy 1983b); however, this has not yet been shown in clinical isolates. Reduced susceptibility to tigecycline in *E. coli* clinical isolates has been attributed to increased overexpression of AcrAB correlating with mutations in *marR* and increased transcription of *marA* (Keeney et al. 2008). In a study by Linkevicius et al. (2013), targeted sequencing of loci suspected to be involved in tigecycline resistance found a deletion in *marR* in one of eight *E. coli* clinical isolates with reduced tigecycline susceptibility; however, MICs were still well below the resistance breakpoint (MIC = 0.19 μg/mL).

RamA, another AraC-family activator, was first identified in *K. pneumoniae* showing reduced susceptibility to a range of unrelated antibiotics, including tetracycline (George et al. 1995). Expression of the *ramA* gene is repressed by RamR, encoded by the *ramR* gene that is divergently transcribed from the nearby *ramA* gene. Similar to MarR, tetracycline does not directly bind RamR; thus, induction of *ramA* appears indirect (Yamasaki et al. 2013). Analogous to regulation by *marA* in *E. coli*, overexpression of *ramA* was shown to also reduce porin expression and up-regulate AcrAB efflux in *K. pneumoniae* (George et al. 1995; Ruzin et al. 2005b), *S. enterica* (van der Straaten et al. 2004a,b; Nikaido et al. 2008), and *Enterobacter cloacae* (Keeney et al. 2007). RamA function appears to be independent of MarA, as RamA-mediated increases in AcrAB expression were not associated with increases in MarA expression (Ruzin et al. 2005b). Although, heterologously expressed *ramA* from bacteria, including *K. pneumoniae*, *Salmonella*, *Citrobacter*, and *Enterobacter* spp., is functional in *E. coli* (Chollet et al. 2004; van der Straaten et al. 2004b; Ruzin et al. 2005b; Reinhardt 2014), a *ramA* gene has not been identified in several enteric species, notably *E. coli* and *Shigella* spp.

In *K. pneumoniae*, a survey of recent literature suggests an emerging theme that AraC-family activators, especially *ramA*, play a prominent role in clinically relevant resistance to tetracycline antibiotics. A study by Bratu et al. (2009) showed that reduced tigecycline susceptibility in *K. pneumoniae* clinical isolates from New York City correlated with *ramA* and *soxS* expression, but not with *marA* or *acrAB* expression (Bratu et al. 2009). However, in the same study, *K. pneumoniae* mutants selected in vitro for reduced tigecycline susceptibility showed increases in *marA* and *acrB* expression, but not *soxS* and *ramA*, so the interplay of regulators appears complicated. In another study, analysis of 72 demographically and geographically diverse *K. pneumoniae* clinical isolates from the tigecycline phase 3 clinical trials showed that isolates with tigecycline MIC values >2 μg/mL had a statistically significant correlation with elevated expression of *ramA* and a less significant trend with *acrA* expression (Ruzin et al. 2008). The association of tigecycline resistance with mutations in genes encoding MDR repressors (*ramR*, *acrR*) and/or increased expression of genes encoding AraC-family activators (*rarA*, *marA*, *ramA*) and efflux pump subunits (*acrB*,

oqxB) has been described in several studies with geographically diverse isolates from Germany (Hentschke et al. 2010); Turkey, Singapore, Chile, and Pakistan (Rosenblum et al. 2011); Italy (Villa et al. 2014); and China (Bratu et al. 2009; Sheng et al. 2014; Zhong et al. 2014; He et al. 2015).

Additional pathways to tigecycline resistance in *K. pneumoniae* are suggested by the identification of tigecycline-resistant *K. pneumoniae* clinical isolates that do not overexpress *ramA* (Rosenblum et al. 2011) and by the isolation of low-level tigecycline-resistant strains from a *K. pneumoniae ramA* deletion mutant (Veleba and Schneiders 2012). Bioinformatic scanning of the *K. pneumoniae* genome for new AraC-family regulators identified *rarA* (Veleba et al. 2012). Expression of *rarA* and the nearby operon *oqxAB* encoding an MDR efflux pump were found to be elevated in geographically diverse *K. pneumoniae* MDR clinical isolates (Veleba et al. 2012) and *E. cloacae* isolates (Veleba et al. 2013) with reduced tigecycline susceptibility. The presence of *rarA* was confirmed in the genomes of *Enterobacter* and *Serratia* spp., and similar to other AraC-family regulators, overexpression of *rarA* produced a low-level MDR phenotype, including tigecycline resistance in *K. pneumoniae* and *E. cloacae* (Veleba et al. 2012, 2013). RarA is thought to be the activator of *oqxAB* (Kim et al. 2009), and has been linked to the regulation of *acrAB* and *ompF* expression (De Majumdar et al. 2013).

Two-Component Systems

Two-component signal transduction systems (TCSs) in bacteria are the most common form of bacterial signal transduction, and are generally composed of a membrane-bound histidine kinase and a response regulator, to which a phoshoryl group is transferred, allowing it to function as a transcription factor affecting the expression of responsive genes (Bem et al. 2015). Several TCSs have been implicated in modulating susceptibility to tetracycline-class antibiotics in Gram-negative and -positive bacteria, presumably by affecting permeability and/or expression of intrinsic multidrug efflux systems.

In *A. baumannii*, the AdeRS TCS controls expression of the major multidrug efflux pump AdeABC (Marchand et al. 2004). Mutations in the AdeR regulator and/or AdeS sensor affecting the normal phosphotransfer process can lead to the constituitive expression of the AdeABC efflux pump (Marchand et al. 2004). Ruzin et al. (2007) were the first to show that elevated tigecycline MICs (4 μg/mL) were associated with constituitive overexpression of AdeABC in two clinical isolates, and coincided with an insertion element in the *adeS* gene in both isolates. In more recent studies, characterizing 81 genetically diverse XDR and 38 carbapenem-resistant MDR *A. baumannii* clinical isolates from Taiwan (Sun et al. 2012, 2014b) reported that tigecycline resistance (MIC $\geq$8 μg/mL) correlated with overexpression of AdeABC in the majority of isolates, likely resulting from mutations in *adeR* and *adeS* encoding changes in conserved amino acid residues, or an insertion sequence (IS) in *adeS* producing a truncated constituitively "on" form of AdeS. Although a more detailed understanding of how these mutations impact AdeRS signaling and AdeABC expression remains to be elucidated, the recurrence of genetic alterations in *adeR* and *adeS* genes strongly implicates the involvement of AdeRS and the AdeABC efflux system in tigecycline resistance. The existence of multiple mechanisms affecting tigecycline susceptibility in *A. baumannii* is suggested by cases of tigecycline-resistant isolates in which either no mutations in *adeR* and *adeS* were found (Hornsey et al. 2010c; Yoon et al. 2013; Sun et al. 2014b) or additional mutations in *rpsJ*, *rrf*, *msbA*, and *gna* were associated with increasing the level of tigecycline resistance in *adeS* mutants; the possible roles of *rrf*, *msbA*, and *gna* in tigecycline resistance remains to be shown (Hammerstrom et al. 2015). Interestingly, reduced susceptibility to tigecycline, minocycline, and doxycycline was associated with a deletion in the *trm* (tigecycline-related methyltransferase) gene encoding an *S*-adenosyl-L-methionine-dependent methyltransferase in an *A. baumannii* isolate; this newly identified mechanism may

be responsible for some resistance not attributable to AdeABC (Chen et al. 2014; Lomovskaya et al. 2015).

Other TCSs that have been associated with resistance to tetracyclines include BaeSR in *A. baumannii* (Lin et al. 2014), PhoBR in *K. pneumoniae* (Srinivasan et al. 2012), and RprXY in *Bacteroides fragilis* (Rasmussen and Kovacs 1993); however, the relevance of these systems in conferring clinical resistance to tetracyclines is not yet understood.

Lon Protease

Induction of multidrug resistance through AraC-family regulators in Gram-negative bacteria is posttranslationally regulated by the cytoplasmic ATP-dependent serine protease, Lon, which is involved in the degradation of unstable or misfolded proteins (Tsilibaris et al. 2006). In the absence of environmental stress, Lon promotes rapid reversion of stress phenotypes by binding at amino-terminal residues of activators MarA, RamA, SoxS and proteolytically degrading them (Griffith et al. 2004; Nicoloff et al. 2006; Ricci et al. 2014). It follows that mutations in *lon* may prolong the stability of these stress-responsive activators, increasing expression of *acrAB* and other resistance genes, leading to antibiotic resistance or reduced susceptibility.

The involvement of Lon protease in the development of antibiotic resistance was shown in a series of 13 *E. coli* cultures derived from a single inoculum in which a significant subpopulation ($\sim 3.7 \times 10^{-4}$) contained a *lon*::IS*186* mutation, or deletion in *lon*, and was capable of growing in low-level tetracycline and chloramphenicol (Nicoloff et al. 2007). Most mutants characterized in this study also contained IS elements in *marR* or *acrR*, or tandem amplifications of the *acrAB* region, and the antibiotic-resistance phenotype, at least in part, could be attributed to these mutations. Because Lon protease is also involved in the stability of transposase enzymes from IS elements and transposons, *E. coli lon* mutant strains show higher transposition rates and greater genome instability (Derbyshire et al. 1990; Nagy and Chandler 2004; Rouquette et al. 2004). Further, the *lon* gene itself is a hotspot for IS insertions (SaiSree et al. 2001). Thus, the potential to select for early steps in drug resistance in vitro appears to be much higher in *lon* mutants, and this is supported by the finding that genomic duplications of the region encoding the major efflux pump, AcrAB, can be readily isolated in an *E. coli lon* mutant (Nicoloff et al. 2006; Nicoloff and Andersson 2013).

Whether *lon* mutations increase the potential to select for resistance to tetracyclines, or any other antibiotic class, in clinical isolates during infection is not entirely clear (Butler et al. 2006). There has been at least one report of an *E. coli acrR* (A191V), *lon*::IS*186* mutant isolated from a UTI, and this mutant showed reduced susceptibility to tigecycline (MIC = 0.25 μg/mL), but still did not reach a level considered clinically significant (Linkevicius et al. 2013). A tigecycline-resistant *K. pneumoniae* clinical isolate containing a frameshift within the coding region of *lon* was reported by Fyfe et al. (2015); however, this mutant also had a deletion in *ramR*, which presumably also contributed to the tigecycline-resistant phenotype (MIC = 8 μg/mL). In this same study, *K. pneumoniae lon* mutants generated by transposon mutagenesis showed 8- to 32-fold increases in the parental tigecycline MIC (0.5 μg/mL), suggesting that mutation in *lon* can contribute to clinically significant resistance levels. Additional studies are needed to clarify the contribution of *lon* to the development of resistance to tetracyclines and other antibiotics in clinical isolates.

Intrinsic Efflux of Tetracyclines

A large component of the intrinsic antibiotic-resistance response in bacteria is due to increased expression of intrinsic efflux pumps (Piddock 2006; Li et al. 2015). As described earlier, expression of these genes can be modulated by locally or distally encoded negative and positive regulators, and mutations up-regulating or down-regulating expression of the regulators themselves, or the efflux pumps they regulate, can impact antibiotic susceptibility. Susceptibility to tetracycline-class antibiotics has been

linked to a wide variety of intrinsic efflux systems in Gram-negative and -positive bacteria summarized in Table 2.

Overexpression of AcrAB, the major pump found in Enterobacteriaceae, and a member of the resistance-nodulation-division (RND) superfamily, has been implicated in resistance to tigecycline, in *E. coli* (Hirata et al. 2004), *Enterobacter* spp. (Keeney et al. 2007), *K. pneumoniae* (Ruzin et al. 2005b), *Morganella morganii* (Ruzin et al. 2005a), and *Proteus mirabilis* (Visalli et al. 2003). Two recently identified pumps, OqxAB and KpgABC, in *K. pneumoniae* also appear to have some association with tigecycline resistance, but their clinical significance is uncertain (Nielsen et al. 2014; Bialek-Davenet et al. 2015; He et al. 2015). In *Serratia marcescens*, pumps with specificity for tetracycline and/or tigecycline include SdeXY-HasF and SmdAB (Chen et al. 2003; Matsuo et al. 2008; Hornsey et al. 2010a).

RND-type pumps are also implicated in conferring reduced susceptibility in nonfermenter and anaerobic Gram-negative bacteria. In *A. baumannii* clinical isolates, as discussed earlier, reduced susceptibility to tigecycline and eravacycline has been correlated with AdeABC pump expression (Ruzin et al. 2010; Abdallah et al. 2015). Interestingly, the AdeABC pump appears to show some selectivity among the tetracyclines, as minocycline is reported to be a weaker substrate than other tetracyclines (Coyne et al. 2011; Lomovskaya et al. 2014). *Pseudomonas aeruginosa* strains are generally less susceptible to tetracycline antibiotics, including tigecycline, and this is largely because of expression of the MexAB-OprM, MexCD-OprJ, MexXY-OprM pumps (Dean et al. 2003). In *Stenotrophomonas maltophilia*, SmrA and SmeDEF (Alonso and Martinez 2001; Zhang et al. 2001; Al-Hamad et al. 2009), and in *B. fragilis*, BmeABC (Pumbwe et al. 2006), have been reported to recognize tetracycline, but their clinical significance remains to be shown.

Much less is known about the regulation of intrinsic resistance to tetracyclines in Gram-positive bacteria. The best-characterized intrinsic Gram-positive pump with demonstrated specificity for tetracyclines is the multidrug and toxic compound extrusion (MATE)-family pump, MepA, in *S. aureus*. Although this pump does not appear to recognize tetracycline as a substrate, fourfold and 64-fold increases in MIC for eravacycline and tigecycline, respectively, were observed for a MepA overexpressing strain versus the isogenic parent, indicating a distinct tetracycline substrate specificity for this pump (McAleese et al. 2005; Sutcliffe et al. 2013). The NorB pump, negatively regulated by MgrA in *S. aureus*, has also been reported to recognize tetracycline (Truong-Bolduc et al. 2005).

THE PRESENT AND FUTURE FOR TETRACYCLINES

Minocycline

Historically, minocycline has been available in both oral and intravenous dosage formulations. As options for the treatment of MDR *A. baumannii* are limited, the recent approval of a new intravenous (IV) formulation, Minocin IV, for treatment of *Acinetobacter* spp. and other difficult-to-treat Gram-positive and -negative pathogens, is a valuable repurposing of an old antibiotic for targeted use (The Medicines Company 2015). In the 2004–2013 Tigecycline Evaluation and Surveillance Trial (TEST) report, the highest level of in vitro susceptibility against *A. baumannii* isolates was reported for minocycline (84.5%), and 70.3% susceptibility was observed against MDR *A. baumannii* (Hoban et al. 2015). In the global 2007–2011 SENTRY surveillance program, minocycline was the second most active antibiotic against *A. baumannii* (79.1% susceptible) (Castanheira et al. 2014). This might be explained to some extent by the ability of minocycline to thwart AdeABC efflux, and a lower rate of minocycline-resistance development in *A. baumannii* (Lomovskaya et al. 2014). Clinical responses to Minocin IV used as a monotherapy or in combination for the treatment of MDR *A. baumannii* infections appear encouraging (Goff et al. 2014; Ritchie and Garavaglia-Wilson 2014; Falagas et al. 2015), but this therapy will likely be a stop-gap as the spread of RPPs should increase minocycline resistance.

Table 2. Intrinsic bacterial multidrug efflux mechanisms conferring resistance to tetracycline drugs

Pathogen	Efflux pump family	Known tetracycline specificity	References
A. baumannii	RND	AdeABC: tetracycline, tigecycline, minocycline,[a] doxycycline[a] AdeDE: tetracycline AdeFGH: tetracycline, minocycline, tigecycline AdeIJK: tetracycline, minocycline, doxycycline, tigecycline	Chau et al. 2004; Ruzin et al. 2007; Damier-Piolle et al. 2008; Coyne et al. 2010, 2011; Ruzin et al. 2010; Lomovskaya et al. 2014, 2015
B. fragilis	RND	BmeABC: tetracycline	Pumbwe et al. 2006
E. coli	RND	AcrAB: tetracycline, tigecycline, minocycline, doxycycline AcrEF: tetracycline, tigecycline, minocycline, doxycycline	Hirata et al. 2004
Enterobacter spp.	RND	AcrAB: tetracycline, tigecycline, minocycline OqxAB: tigecycline	Keeney et al. 2007; Hornsey et al. 2010b; Veleba et al. 2013
E. faecalis	ABC	EfrAB: doxycycline (not tetracycline)	Lee et al. 2003
K. pneumoniae	RND	AcrAB: tetracycline, tigecycline, minocycline OqxAB: tigecycline, tetracycline KpgABC: tigecycline	Ruzin et al. 2005b; Ruzin et al. 2008; Veleba and Schneiders 2012; Nielsen et al. 2014; Zhong et al. 2014; He et al. 2015
P. aeruginosa	RND	MexAB-OprM: tetracycline, minocycline, doxycycline, chlortetracycline, oxytetracycline, tigecycline MexCD-OprJ: tetracycline, chlortetracycline, oxytetracycline, tigecycline MexJK: tetracycline MexXY-OprM: tetracycline, minocycline, doxycycline, chlortetracycline, oxytetracycline, tigecycline	Masuda et al. 2000; Morita et al. 2001; Chuanchuen et al. 2002; Dean et al. 2003; Schweizer 2003
P. mirabilis	RND	AcrAB: tigecycline, minocycline	Visalli et al. 2003
S. aureus	MATE	MepA: tigecycline, eravacycline (not tetracycline) NorB: tetracycline (not minocycline)	McAleese et al. 2005; Truong-Bolduc et al. 2005; Sutcliffe et al. 2013
Stenotrophomonas maltophilia	ABC RND	SmrA: tetracycline SmeDEF: tetracycline, doxycycline, minocycline, tigecycline	Alonso and Martinez 2001; Zhang et al. 2001; Al-Hamad et al. 2009
Serratia marcescens	RND ABC	SdeXY-HasF: tetracycline, tigecycline SmdAB: tetracycline	Chen et al. 2003; Matsuo et al. 2008; Hornsey et al. 2010a

[a]Poorer substrates for AdeABC as compared with other tetracycline drugs.

RND, Resistance-nodulation-division superfamily; MATE, multidrug and toxic compound extrusion family; ABC, ATP-binding cassette transporter family.

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Tigecycline

Tigecycline has a broad spectrum of coverage, including activity against MRSA, vancomycin-resistant *Enterococcus* spp. (VRE), MDR *A. baumannii*, and ESBL-producing and carbapenem-resistant Enterobacteriaceae (CRE), supporting the currently approved indications of complicated skin and skin structure infections, complicated intra-abdominal infections (cIAI), and community-acquired bacterial pneumonia (CABP) (Stein and Babinchak 2013; Wyeth Pharmaceuticals 2016). The administration of tigecycline is limited to IV only. Given its activity against MDR pathogens, tigecycline was evaluated for use in hospital-acquired pneumonia, ventilator-associated pneumonia, and diabetic foot infections; however, clinical studies showed lower cure rates versus comparator drugs (Wyeth Pharmaceuticals 2016). Broader usage and alternative dosing regimens for serious infections continue to be explored in clinical studies (Ramirez et al. 2013; Stein and Babinchak 2013). Based on meta-analyses of clinical trial data, the FDA issued a safety alert in 2010 and a black box warning in 2013 because of an observed increase in mortality risk in patients treated with tigecycline, as compared with other drugs (U.S. Food and Drug Administration 2010, 2013). Whereas the cause of death during tigecycline treatment remains uncertain, mortality appeared to occur in patients with complicated worsening infections or underlying medical conditions.

Since its approval in 2005, tigecycline maintains high levels of susceptibility in global surveillance studies despite sporadic reports of resistance during use: *E. coli* and *K. pneumoniae* in the United Kingdom (Stone et al. 2011; Spanu et al. 2012); *K. pneumoniae* in Greece (Neonakis et al. 2011), Saudi Arabia (Al-Qadheeb et al. 2010), Spain (Rodriguez-Avial et al. 2012), the United States (Nigo et al. 2013); *E. hormaechei* in France (Daurel et al. 2009); *A. baumannii* in the United States (Peleg et al. 2007; Reid et al. 2007; Anthony et al. 2008); *E. faecalis* in the United Kingdom (Cordina et al. 2012) and Germany (Werner et al. 2008); and *B. fragilis* in the United States (Sherwood et al. 2011). There are also reports in which tigecycline resistance actually predated the use of tigecycline in institutions in which resistance was detected (Rosenblum et al. 2011; Zhong et al. 2014) or arose during treatment with another antibiotic (Hornsey et al. 2010b).

In the 2004–2013 TEST report, among the Enterobacteriaceae ($n = 118{,}899$), enterococci ($n = 20{,}782$), methicillin-resistant *S. aureus* ($n = 14{,}647$), and *S. pneumoniae* ($n = 14{,}562$), susceptibility to tigecycline was 97%, >99%, ≥99.9%, and ≥99.9%, respectively (Hoban et al. 2015). Against MDR Enterobacteriaceae ($n = 9372$), defined as resistant to more than three different classes of antibiotics, susceptibility to tigecycline was 83.2%. And, among the carbapenem-resistant *Enterobacter* spp. ($n = 578$), *E. coli* ($n = 181$), and *K. pneumoniae* ($n = 1330$), susceptibility to tigecycline was 83%, 97.2%, and 92%, respectively. Although there are no breakpoints available against *A. baumannii*, tigecycline maintained an MIC_{90} (MIC inhibiting 90% of the isolates) of 2 μg/mL against all *A. baumannii* ($n = 16{,}778$), as well as an MDR subset ($n = 6743$). The gap in coverage of *P. aeruginosa* is evident by an MIC_{90} of 16 μg/mL against all collected isolates ($n = 28{,}413$) and ≥32 μg/mL against the MDR subset ($n = 3496$). Similar findings were observed in recent reports from the SENTRY (Sader et al. 2013, 2014), Regional Resistance Surveillance (Jones et al. 2014), and CANWARD (Zhanel et al. 2013) programs.

Omadacycline

The aminomethylcycline derivative of minocycline, omadacycline, has completed a phase 2 trial for safety and efficacy in skin and skin structure infections (SSSI) and is being developed for use in SSSI, CABP, and UTIs with IV and oral formulations (Noel et al. 2012). Omadacycline was shown to have MIC_{90} values against MRSA ($n = 39$), VRE ($n = 19$), *Streptococcus pyogenes* ($n = 30$), penicillin-resistant *S. pneumoniae* ($n = 23$), and *Haemophilus influenzae* ($n = 53$) of 0.5, 0.5, 0.25, ≤0.06, and 2 μg/mL, respectively (Macone et al. 2014). Against *E. coli* ($n = 23$) and *K. pneumo-*

niae ($n = 14$), MIC_{90} values were 2 μg/mL and 4 μg/mL, respectively. The 9-alkylaminomethyl modification of minocycline endows omadacycline with activity against ribosomal protection mechanisms (Draper et al. 2014).

Eravacycline

Eravacycline, a broad spectrum, fully synthetic fluorocycline with novel C-9 pyrrolidinoacetamido and C-7 fluoro modifications, completed a phase 2 trial for cIAI and has completed pivotal phase 3 trials for cIAI and complicated UTI (Solomkin et al. 2014), with future indications expected to include other serious infections. Both IV and oral formulations are in development. In evaluations against large panels of aerobic and anaerobic Gram-negative and -positive bacteria, eravacycline showed MIC_{90} values ranging from $\leq$0.008 to 2 μg/mL for all species, except *P. aeruginosa* and *Burkholderia cenocepacia* (MIC_{90} values of 16–32 μg/mL) (Sutcliffe et al. 2013; McDermott et al. 2015; Morrissey et al. 2015a,b,c). In the study by Sutcliffe et al. (2013), eravacycline showed activity against tetracycline-resistant *E. coli* ($MIC_{50/90} = 0.25/0.5$ μg/mL; $n = 157$), *E. cloacae* ($MIC_{50/90} = 2/4$ μg/mL; $n = 25$), and *P. mirabilis* ($MIC_{50/90} = 1/2$ μg/mL; $n = 109$). In a recent study with more than 4000 contemporary Gram-negative pathogens from New York City hospitals, eravacycline $MIC_{50/90}$ values were 0.12/0.5 μg/mL for *E. coli*, 0.25/1 μg/mL for *K. pneumoniae*, 0.25/1 μg/mL for *Enterobacter aerogenes*, 0.5/1 μg/mL for *E. cloacae*, and 0.5/1 μg/mL for *A. baumannii* (Abdallah et al. 2015). Eravacycline also shows good activity against MDR bacteria, including Enterobacteriaceae and *A. baumannii* expressing extended spectrum β-lactamases, carbapenem resistance, and mechanisms conferring resistance to other antibiotic classes (Sutcliffe et al. 2013; Grossman et al. 2014b; Abdallah et al. 2015).

CONCLUSION

Tetracycline-class antibiotics have treated serious life-threatening infections for more than 60 years; however, as with every other antibiotic class, use has led to resistance development. Historically, potency, spectrum, and tetracycline-resistance hurdles have been addressed semisynthetically with chemical modifications of earlier natural product derivatives. The most successful examples of this approach include minocycline, doxycycline, tigecycline, and omadacycline. More recently, a fully synthetic chemistry approach has led to the discovery of eravacycline, which shows promise in the treatment of serious infections caused by a broad range of bacterial pathogens. Ongoing exploration of synthetic tetracycline derivatives has enabled improvements in potency against *P. aeruginosa* and other difficult-to-treat MDR Gram-negative pathogens (Deng et al. 2012; O'Brien et al. 2012; Xiao et al. 2013; Grossman et al. 2014a; Sun et al. 2014a, 2015). The ability to synthesize completely novel, "unnatural" tetracyclines opens new opportunities to more fully explore the potential of this familiar and clinically validated antibiotic class.

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