Macrolide-Inducible Resistance to Clindamycin and the D-Test

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Clindamycin has been used to treat serious infections caused by susceptible *Staphylococcus aureus* strains in children for more than 30 years. It remains effective for many infections caused by community-acquired methicillin-resistant *S. aureus* (CA-MRSA). Clindamycin also is useful for selected infections caused by pneumococci, group A streptococci, and a number of other microbes. Absorption after oral administration is nearly complete, yielding serum concentrations that approximate those from intravenous (IV) administration. This permits early transition to outpatient management of susceptible infections without the complications of continued IV access.

Clindamycin resistance is common among health care-associated MRSA strains. Most CA-MRSA remain susceptible to date, but resistance rates vary by region. Pneumococcal resistance to clindamycin may exceed 30% in some areas of the US, while that from the relapse was resistant to both. This led to abandonment of clindamycin for treatment of endocarditis.

The mechanism by which resistance to clindamycin can emerge during therapy, the D-test used to detect it, and the clinical implications are discussed in this review.

**INDUCIBLE MACROLIDE RESISTANCE**

The clinical implications of a positive D-test begin with an understanding of cross-resistance for 3 antibiotic families that share a common binding site—macrolides (eg, erythromycin, clarithromycin, azithromycin), lincosamides (eg, clindamycin), and group B streptococci (eg, quinupristin). This cross-resistance, called the MLSB phenotype, results from enzymatic dimethylation of an adenine residue in these antibiotics’ binding site in the 23S rRNA component of the 50S ribosomal subunit. The methylase enzyme is encoded by a multiallele plasmid-borne gene *erm*, which occurs predominantly as variants *erm(C)* or *erm(A)* in staphylococci (other variants occur in pneumococci, hemolytic streptococci, and enterococci). When the MLSB phenotype is the result of constitutive (“always on”) *erm* expression, initial in vitro susceptibility tests show resistance to all 3 antibiotic classes. When the inducible genotype (iMLS*) is present, in vitro tests show resistance to 14- and 15-membered ring macrolides listed above, but susceptibility to clindamycin and 16-membered ring macrolides (available in some countries other than the US) is retained—these agents do not induce resistance to themselves.

The inducible methylase system is regulated at the level of mRNA translation (translational attenuation) rather than gene transcription. The gene is transcribed to mRNA constitutively, but the mRNA cannot be read into protein without the presence of a translational inducer. In the inducible *erm* cassette, the open reading frame of the methylase gene and its dedicated ribosomal binding site is preceded by DNA sequences that encode another ribosomal binding site, a short amino-acid peptide with a stop codon, and an additional sequence that includes 2 inverted repeats (IRs).

In the attenuated state, the secondary structure of the transcribed mRNA has 2 stem-loops (Fig. 1 panel A). The first involves the last 8 codons of the short peptide (functionally, IR1) and an adjacent IR2. The second consists of an IR3 sequence (which follows IR2) that binds to the sequences encoding the ribosomal binding site and start codon for the methylase gene (functionally, IR4). This effectively “hides” the ribosomal binding site and start codon for the methylase in the second (IR3–IR4) stem-loop, such that the methylase enzyme cannot be translated.

When macrolide molecule capable of inducing the methylase translation binds to its target site in a ribosome, the “inhibited” steps of translation are blocked, and translation can resume only after ATP has hydrolyzed the inducer–methylase complex.
ribosomes is still able to bind to and start translation of the mRNA sequence of the short peptide. The ribosome stalls after reading several codons but remains complexed with the mRNA strand (macrolides work by inhibiting elongation of peptide chains beyond 3 or 4 residues). This attached stalling leads to a conformational change in the secondary structure of the mRNA: the inducible-state stem loops are opened up, a new secondary structure of the mRNA: the inducible methylase cassette in the absence of macrolides. Two stem loops are formed by the interactions of inverted repeat 1 (IR1) with IR2 and IR3 with IR4. IR4 consists of the ribosomal binding site and initial codons of the methylase mRNA. Ribosomes are unable to complex with the methylase binding site in this conformation. The peptide and IR2 are deleted, and all mature ribosomes are methylated. Adapted from Mol Cell. 2008;30:190–202.

These initial steps occur at very low macrolide concentrations (10–100 ng/mL of erythromycin, below the MIC for many susceptible microbes), such as may occur shortly after a macrolide dose is administered to a patient but before accumulation of MIC-equivalent concentrations at the site of infection. At this early stage, macrolide molecules are bound to only a small fraction of the mature ribosomes extant in the microbial cytoplasm. Most ribosomes remain macrolide-free (uninhibited) and readily able to associate with the newly exposed erm ribosomal binding site. The mRNA then is translated, producing sufficient methylase to modify the MLSB binding sites of the ribosomes that remain uninhibited. The microbe translation—clindamycin does not induce resistance to itself, and in vitro testing of isolates with the iMLSB genotype demonstrates clindamycin susceptibility. However, the macrolide-inducible DNA sequences that precede the erm(C) methylase open reading frame undergo mutations, substitutions or deletions that generate readily translatable (now constitutive MLSB) secondary mRNA structures in about 1 in 2 million replications (Fig. 1 panel C). Many infections, especially when purulent collections are present, have microbial burdens that are exceed the denominator of this mutation rate by 10-fold or more, such that small numbers of clindamycin-resistant microbes are likely common in infections caused by such iMLSB strains.

During the past 10 years, treatment failure has reported in handfuls of adult and pediatric cases when clindamycin was used for MRSA infections caused by strains that initially appeared susceptible to clindamycin but resistant to macrolides. Clindamycin resistance was evident upon retesting of the recurring isolates. The common theme of these cases has been initial improvement on clindamycin with subsequent recrudescence days to weeks into or after completion of therapy. This is consistent with the above low frequency mutation rate from iMLSB genotype to constitutive MLSB erm expression, where a resistant subpopulation survives the innate and early adaptive host immune responses and emerges to cause new or worsening of existing signs and symptoms.

THE D-TEST

The combination of resistance to erythromycin with susceptibility to clindamycin in S. aureus (and other gram-positive microbes) can be due to the iMLSB genotype or efflux pumps that remove macrolides but not clindamycin from the microbe. The D-test, based on disk diffusion susceptibility testing, is recommended to determine if the iMLSB genotype is present. In the D-test, disks containing erythromycin (15 μg) and clindamycin (2 μg) are placed 15 to 20 mm apart on an agar plate that has been inoculated with the clinical isolate (Fig. 2). A clindamycin-susceptible, erythromycin-resistant isolate should have a zone of inhibition ≥21 mm in diameter, with minimal if any inhibition of growth around the erythromycin disk.

The round zones of erythromycin and clindamycin that diffuse outward from the disks partially overlap in this configuration. Erythromycin molecules reach the outer region of the clindamycin zone prior to clindamycin molecules. When the iMLSB genotype is present, this leads to methylase translation, permitting microbial growth in this region despite subsequent diffusion of resistance relevant to clindamycin? Clindamycin binding to the same 23S rRNA subunit does not lead to induction of methylase translation.
inhibitory concentrations of clindamycin. This growth blunts the expected round zone of growth-inhibition around the clindamycin disk into a D-shape facing the erythromycin disk (Fig. 2), which indicates a positive D-test. A negative D-test (round zone) indicates efflux-mediated macrolide resistance with retained clindamycin susceptibility.

CLINICAL IMPLICATIONS OF A POSITIVE D-TEST

A positive D-test indicates the presence of iMLSB$_B$ genotype. This means that it is possible, but far from certain, that a subpopulation of microbes resistant to clindamycin may emerge and lead to clinical failure or recrudescence. Infections caused by $S$. $aureus$ strains that carry the iMLSB$_B$ genotype often respond to clindamycin therapy without relapse$^3$—resistant subpopulations either do not develop or are eliminated by host responses before progression of infection can occur. However, because of the clinical reports of clindamycin failure associated with D-test$^+$ strains, the Clinical and Laboratory Standards Institute recommends that laboratories report D-test$^+$ isolates as resistant to clindamycin.$^{20}$

Clinical and Laboratory Standards Institute recommendations also suggest inclusion of a comment that “this isolate is presumed to be resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients.” This is an important allowance, as relatively minor infections such as cellulitis caused by D-test$^+$ $S$. $aureus$ strains (which can be MRSA or MSSA) will respond adequately. These minor infections are far more common than serious invasive infections, and change to other antimicrobial agents based simply on the D-test$^+$ result in these cases may serve only to increase sensitization risks to the new agent or increase cost of therapy. Consequences of recrudescence in most initially minor episodes also are unlikely to be severe, especially if recognized early on.

For sepsis, pneumonia, osteomyelitis, and other serious invasive $S$. $aureus$ infections, even the small risk of emergence of resistance indicated by a positive D-test result generally should lead to avoidance of clindamycin, or prompt change from clindamycin to another agent to which the isolate is susceptible.$^9$ When starting or continuing clindamycin for less severe infections caused by D-test$^+$ strains, close follow-up for potential failure and late relapse is needed. Patients and/or their caregivers should be counseled regarding potential relapse and to seek care early if they have concerns of recurrence.

At the community level, relative frequency of constitutive MLS$_B$-related versus iMLSB$_B$-related clindamycin resistance should be monitored to provide appropriate guidance for empiric therapy for clinical scenarios where community-acquired $S$. $aureus$ infection is suspected. Reporting aggregate clindamycin resistance statistics without separating out the proportion deemed resistant on the basis of a positive D-test could lead to premature abandonment of clindamycin as an empiric option in clinical situations for which this may still be an appropriate agent. The risk of failure in the first hours to days of empiric clindamycin therapy prior to availability of D-test results seems exceedingly small based on clinical reports to date—but ongoing vigilance is required.

Clindamycin suppresses production of Panton-Valentine leukocidin, alpha-hemolysin, and toxic-shock syndrome toxin 1 by $S$. $aureus$ in vitro at the translational (ribosomal) level.$^{21}$ Improved outcomes for severe group A streptococcal infections treated with clindamycin versus beta-lactam agents have been demonstrated in a mouse myositis model$^{22}$ and in a clinical case series in children.$^{23}$ This is the basis for use of clindamycin for toxin suppression in addition to a cell wall-active agent for treatment of life-threatening streptococcal and staphylococcal infections (eg, toxic shock syndrome, necrotizing fasciitis).

Toxin production by isolates resistant to clindamycin via constitutive methylase expression will not be impacted by “adjunctive” clindamycin. However, for infection by strains with the iMLSB$_B$ genotype, suppression of toxin production by clindamycin can reasonably be expected, at least during the critical first hours to days of therapy when this effect is most likely to be clinically important. A miniscule fraction at most...
(<0.01%) of the total progeny of such a strain might be impervious to clindamycin early on, due to the spontaneous mutations that can confer constitutive expression. As with empiric use of clindamycin for suspected S. aureus infections above, the decision to switch from empiric use of clindamycin for toxin suppression to another ribosomally active agent such as linezolid can be based on local rates of constitutive resistance among clinical isolates of S. aureus and group A streptococci.

REFERENCES