Azithromycin Breakpoint for *Neisseria gonorrhoeae*

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1 Foreword

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Using the CLSI voluntary consensus process, the Subcommittee on Antimicrobial Susceptibility Testing develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting. The subcommittee reviews data from various sources and studies (eg, *in vitro*, pharmacokinetic-pharmacodynamic, and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and quality control (QC) ranges.

The details of the necessary and recommended data for selecting appropriate breakpoints and QC ranges, and how the data are presented for evaluation, are described in CLSI document M23.[1] CLSI antibacterial breakpoints are provided in CLSI documents M100[2] and M45.[3]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods, QC parameters, and the manner in which breakpoints are established may be refined to ensure more accurate results. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should always be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment. For more information, visit www.clsi.org.

This CLSI rationale document is based on CLSI agenda items submitted by the Working Group on Azithromycin.

2 Introduction

Treatment of *Neisseria gonorrhoeae* infections is a significant challenge because resistance has emerged to nearly all therapeutic options. Currently, the Centers for Disease Control and Prevention (CDC) and the World Health Organization recommend dual therapy with ceftriaxone (250 mg, intramuscular) and azithromycin (1 g, oral) for treatment of uncomplicated gonorrhea.[4] This regimen is hoped to preserve the effectiveness of ceftriaxone because a strain is unlikely to be resistant to both ceftriaxone and

azithromycin. *N. gonorrhoeae* strains with resistance to a single agent may be effectively eradicated when immediately treated with two drugs, if the strain is susceptible to the second agent. Globally, only isolated case reports from the United Kingdom and Australia have identified isolates with dual high-level ceftriaxone and azithromycin minimal inhibitory concentrations (MICs) (ie, resistance).[5,6]

Susceptibility testing of individual strains to direct treatment choice is not routinely performed because most cases are identified through use of nucleic acid amplification tests (NAATs), not culture. Culture and susceptibility testing are recommended for all cases of treatment failure, and surveillance cultures with susceptibility testing are critical to informing international treatment guidelines. The absence of azithromycin breakpoints precludes the possibility of US Food and Drug Administration (FDA)–cleared devices to test azithromycin in the medical laboratory, and differing cutoffs may be used when surveillance data are evaluated.

Azithromycin is a macrolide. The mechanism of antibacterial action is binding to 23S ribosomal RNA (rRNA), blocking protein synthesis. Mutations in genes encoding 23S rRNA have been associated with treatment failure during azithromycin monotherapy for *N. gonorrhoeae*, particularly when all four alleles of the *N. gonorrhoeae* 23S rRNA gene are affected. Reports have identified treatment failures for isolates with MICs of 4 or > 256 µg/mL, associated with the 23S rRNA C2611T and A2059G mutations, respectively.[7,8] Other genetic mutations (eg, meningococcal-like [mosaic] *mtrR*) can also increase azithromycin MICs, but only to < 16 µg/mL. The association with treatment failure for these mutations is not established.

For the current azithromycin breakpoint for *N. gonorrhoeae*, see Table 1.

Table 1. Current CLSI Azithromycin Breakpoint*

Organism Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			
		S	SDD	I	R
N. gonorrhoeae	Azithromycin	≤ 1	–	–	–

* Last reviewed June 2018; first published in CLSI document M100, 29th ed.[2]
Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

3 Standard Dosages and Pharmacokinetic Data

After oral administration, azithromycin rapidly leaves the circulation to enter tissues, achieving high and prolonged drug concentrations in peripheral sites including genital sites.

A single 500-mg oral dose of azithromycin in healthy adult volunteers is associated with the pharmacokinetic parameters shown in Table 2.

Table 2. Pharmacokinetic Parameters for 500 mg Azithromycin[9]

Pharmacokinetic Parameters (Mean)	Total *N* = 12	
	Day 1	Day 5
C_{max} (µg/mL)	0.41	0.24
T_{max} (h)	2.5	3.2
AUC_{0-24} (µg • h/mL)	2.6	2.1
C_{min} (µg/mL)	0.05	0.05
Urinary excretion (% dose)	4.5	6.5

Abbreviations: AUC_{0-24}, area under the concentration time curve from 0 to 24 hours; C_{max}, maximum concentration of drug in serum; C_{min}, minimum concentration of drug in serum; h, hours; T_{max}, time to maximum serum concentration.

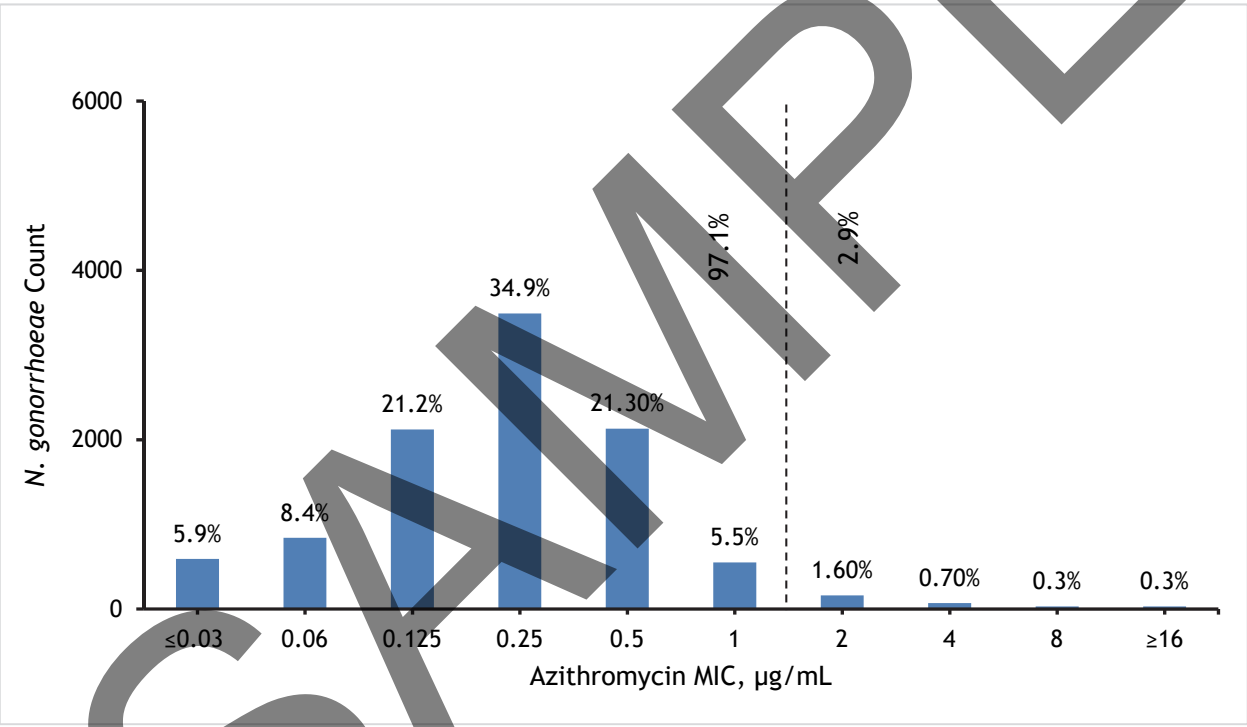
Median azithromycin exposure (AUC_{0-288}) in polymorphonuclear leukocytes is 800-fold greater than in serum following a three-day regimen.[9]

At 19 hours, azithromycin concentration in the cervix is 2.8 µg/g, 70-fold higher than in plasma. At 10 to 12 and nine to 18 hours, sputum and tonsil azithromycin concentrations are 2.9 µg/mL and 4.5 µg/g, 30- and > 100-fold greater than in serum or plasma.[9]

For a single 1-g oral dose of azithromycin in healthy men ($N = 10$), the median plasma concentration at two hours was 1.1 µg/mL (0.1 to 1.4 µg/mL), and rectal tissue concentration peaked between two hours and four days (median 24 hours) with a median C_{max} of 132.6 µg/g (12.7 to 2695.8 µg/g).[10,11] For rectal tissue concentration, the estimated AUC_{0-96} and $AUC_{0-\infty}$ were 3644 and 13 103 (µg/g) • hr, respectively. Azithromycin elimination was biphasic with a median initial half-life of 24.2 hours (time zero to 96 hours) and the total median elimination half-life (time zero to day 14) of 86.6 hours. The elimination rate constant was 0.008/hour.

4 Minimal Inhibitory Concentration Distribution Data

US national surveillance data from the CDC Gonococcal Isolate Surveillance Project (GISP) were reviewed for 2014, 2015, and 2016. Figure 1 shows the azithromycin MIC distribution of 15 496 isolates from 2014 to 2016. The mode and MIC_{50} were 0.25 µg/mL. Notably, in this systematic sentinel site surveillance method, there were few isolates (2.9%) with azithromycin MICs > 1 µg/mL. The epidemiological cutoff value (ECV) was calculated as 1 µg/mL.



Abbreviations: GISP, Gonococcal Isolate Surveillance Project; MIC, minimal inhibitory concentration.

Figure 1. Azithromycin MIC Distribution for *N. gonorrhoeae* Isolates Collected by GISP (2014-2016)[12]

A total of 723 GISP isolates from 2013 to 2015 underwent whole genome sequencing. "High-level resistance" was defined as an azithromycin MIC > 16 µg/mL. The data are shown in Figure 2. Isolates with A2059G and C2611T were identified. Isolates with four of four alleles harboring the mutations had MICs ≥ 16 µg/mL. Isolates with one to two alleles mutated had MICs of 0.5 or 1 µg/mL.