

TITLE: Disc diffusion as a strategy to screen azithromycin resistance in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is the etiological agent of gonorrhea, a sexually transmitted disease. This microorganism is highly efficient in acquiring and developing antimicrobial resistance. Currently, the CDC recommended therapy is based in the combination of ceftriaxone (250 mg) and azithromycin (1 g), both considered last resource drugs against this microorganism. Individuals allergic to beta-lactams may use azithromycin (2 g). In this context, azithromycin resistance is an important emergent phenotype in *N. gonorrhoeae* worldwide. However, detection of such strains is complicated by divergent azithromycin resistance breakpoints in EUCAST and CLSI, expressed in minimal inhibitory concentrations (MIC), which must be determined by agar dilution or MIC test strips. EUCAST defines *N. gonorrhoeae* strains with azithromycin MIC ≥ 0.5 $\mu\text{g/mL}$ as resistant. CLSI establishes the epidemiological cutoff value (ECV) of MIC ≥ 2 $\mu\text{g/mL}$ for non-wild-type *N. gonorrhoeae*. Indeed, there are evidences that strains with mutations in the 23S rRNA, which is the most impacting azithromycin resistance mechanism in *N. gonorrhoeae*, present MIC ≥ 2 $\mu\text{g/mL}$. The aim of the present study was to investigate the feasibility of disc diffusion (DD) as a strategy to screen *N. gonorrhoeae* strains presenting MIC \geq ECV. A total of 165 isolates with previously determined DD results, obtained in Rio de Janeiro between 2006 and 2015 were included in the study. Among them, 42 isolates with azithromycin DD ≤ 33 mm (suggestive for MIC 0.5 $\mu\text{g/mL}$ according to Mehaffey *et al.*, 1996) were selected for a new test. After inoculated in GC agar plates according to CLSI recommendations, such strains were exposed simultaneously to azithromycin discs (Cefar) and E-test azithromycin strips (Biomerrieux). Data obtained were plotted in a scatter diagram in order to evaluate correlations between MIC and DD inhibition zones. Our results indicated that isolates with azithromycin MIC between 0.094 $\mu\text{g/mL}$ and 1.5 $\mu\text{g/mL}$ presented DD values varying from 30 to 40 mm with poor correlation between the data ($R^2=0.0722$). In contrast, all isolates with azithromycin MIC ≥ 2 $\mu\text{g/mL}$ presented DD ≤ 26 mm and linear correlation among MIC and DD values of $R^2=0.6645$. Therefore, our data indicate that inhibition zones ≤ 26 mm may be related to isolates with MIC ≥ 2 $\mu\text{g/mL}$, which is the ECV defined by CLSI. The use of this breakpoint might represent an easy and inexpensive way to screen azithromycin resistant *N. gonorrhoeae* isolates.

Keywords: *Neisseria gonorrhoeae*, resistance, azithromycin, minimal inhibitory concentrations

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