Molecular Algorithms Accurately Predict Decreased Susceptibility to Ceftriaxone in *Neisseria gonorrhoeae*

Dear Editor,

We read with great interest the report by Pinto et al.,[1] detailing the first multidrug ceftriaxone-resistant *Neisseria gonorrhoeae* isolate in Portugal. Antimicrobial resistance in *N. gonorrhoeae* is a global health threat, as the pathogen has developed resistance to every class of antibiotic available.[2] Currently, dual therapy with ceftriaxone and azithromycin is recommended by the World Health Organization, although ceftriaxone or cefixime monotherapy is used in some countries.[3,4]

We recently analyzed a global series of publicly available genetic data of *N. gonorrhoeae* isolates through October 15, 2019, and analyzed mutations in genes associated with ceftriaxone resistance.[5] From that analysis, we proposed 4 molecular algorithms to predict decreased susceptibility to ceftriaxone using a cut-point minimum inhibitory concentration (MIC) of 0.064 mg/L.[5] An MIC breakpoint of 0.06 mg/L has been used previously to define decreased susceptibility to ceftriaxone, a midpoint between susceptibility and resistance.[6–8] The proposed algorithms differ in whether they (1) include *penA* or non-*penA* genes (*penB, ponA, mtrR*) and (2) include *penA* mosaicism. Each was estimated to result in either high sensitivity or specificity with moderate complementary specificity and sensitivity.

Antimicrobial susceptibility testing of the strain reported by Pinto et al. showed resistance to ceftriaxone (MIC, 0.19 mg/L) and is therefore considered decreased susceptible according to our breakpoint.[1] Genetic characterization data provided in the report include G542S in a nonmosaic *penA* allele, G120K and A121D in *penB*, and L421P in *ponA*.[1] Based on those results, 2 of our proposed algorithms are applicable.

First, using our algorithm including *penA* and mosaicism (Fig. 1), we estimated that for nonmosaic *penA* strains with no alteration at L447 and at least 1 of G542S, P551L/S, or A501V/T, the sensitivity and specificity for decreased susceptibility were 95% and 62%, respectively.[5] Although not included in their report, analyzing the reported whole-genome sequence of the isolate using PathogenWatch (https://pathogen.watch/) revealed no alteration at L447.

Second, using our algorithm including non-*penA* genes without mosaicism (Fig. 2), having L421P on *ponA* in addition to at least one alteration at G120 or A121 in *penB* is estimated to have a 92% sensitivity and a 61% specificity to detect decreased susceptibility.[5] Furthermore, we reported that the detection of both mutations in nonmosaic strains was estimated to have sensitivity and specificity of 86% and 68%, respectively, which would have also determined the isolate to have decreased susceptibility to ceftriaxone.[5]

Using the data from Pinto et al., we have validated 2 of our proposed molecular algorithms for prediction of decreased susceptibility to ceftriaxone in *N. gonorrhoeae*. Because the reported strain was genetically distinct from all publicly available genomes of other *N. gonorrhoeae* isolates,[1] this validation demonstrates the potential value to using molecular markers and algorithms to predict ceftriaxone decreased susceptibility. In the era of increasing antibiotic resistance in *N. gonorrhoeae*, there is an urgent need to predict resistance to ceftriaxone. Our proposed algorithms could serve as a foundation toward fulfilling that need by simultaneously identifying multiple genetic mutations implicated in decreased susceptibility to ceftriaxone potentially through a variety of diagnostic technologies including targeted next-generation sequencing, clustered regularly interspaced short palindromic repeats (CRISPR), and reverse transcriptase-polymerase chain reaction. Expanded genomic surveillance efforts to monitor genetic loci associated with ceftriaxone resistance on a global scale is critical and will allow for the continued refinement of these algorithms.

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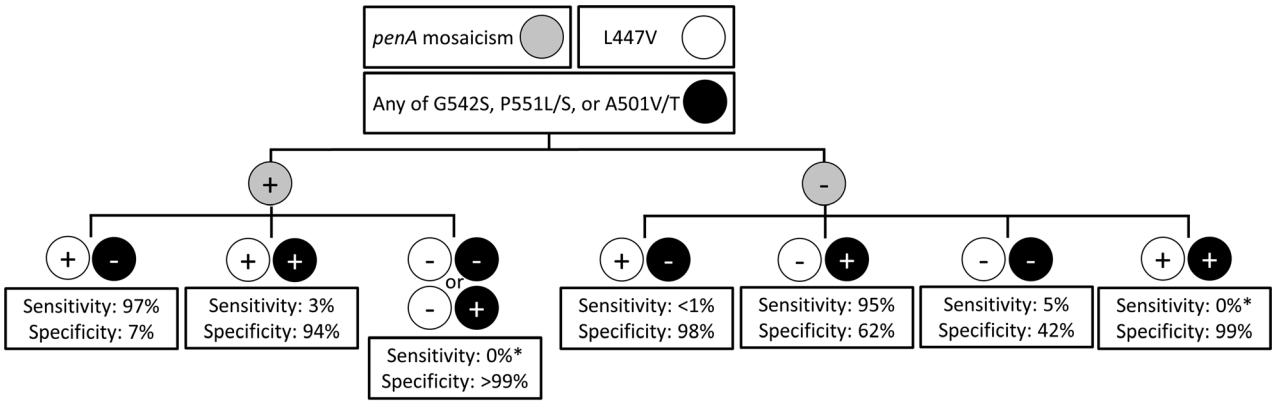

Figure 1. Molecular algorithm previously reported using *penA* amino acid alterations and mosaicism determination. Sensitivity and specificity values are for decreased susceptibility to ceftriaxone. Testing for all genetic loci in these algorithms is intended to be done simultaneously and not necessarily in a stepwise fashion.

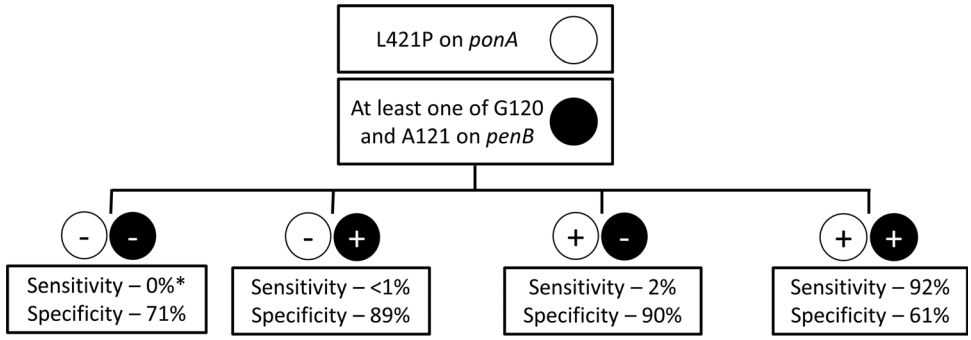

Figure 2. Molecular algorithm previously reported using non-*penA* genes (*penB* and *ponA*) without mosaicism determination. Sensitivity and specificity values are for decreased susceptibility to ceftriaxone. Testing for all genetic loci in these algorithms are intended to be done simultaneously and not necessarily in a stepwise fashion.