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MAJOR ARTICLE

Establishing Novel Molecular Algorithms to Predict Decreased Susceptibility to Ceftriaxone in *Neisseria gonorrhoeae* Strains

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Background: Globally, decreased susceptibility to ceftriaxone in *Neisseria gonorrhoeae* is rising. We aimed to compile a global collection of *N. gonorrhoeae* strains and assess the genetic characteristics associated with decreased susceptibility to ceftriaxone.

Methods: We performed a literature review of all published reports of *N. gonorrhoeae* strains with decreased susceptibility to ceftriaxone (>0.064 mg/L minimum inhibitory concentration) through October 2019. Genetic mutations in *N. gonorrhoeae* genes (*penA, penB*, *mtrR*, and *ponA*), including determination of *penA* mosaicism, were compiled and evaluated for predicting decreased susceptibility to ceftriaxone.

Results: There were 3821 *N. gonorrhoeae* strains identified from 23 countries and 684 (18%) had decreased susceptibility to ceftriaxone. High sensitivities or specificities (>95%) were found for specific genetic mutations in *penA, penB, mtrR,* and *ponA*, both with and without determination of *penA* mosaicism. Four algorithms to predict ceftriaxone susceptibility were proposed based on *penA* mosaicism determination and *penA* or non-*penA* genetic mutations, with sensitivity and specificity combinations up to 95% and 62%, respectively.

Conclusion: Molecular algorithms based on genetic mutations were proposed to predict decreased susceptibility to ceftriaxone in *N. gonorrhoeae*. Those algorithms can serve as a foundation for the development of future assays predicting ceftriaxone decreased susceptibility within *N. gonorrhoeae* globally.

Keywords. *Neisseria gonorrhoeae*; gonorrhea; ceftriaxone; resistance; decreased susceptibility; algorithms.

Neisseria gonorrhoeae is the second most common bacterial sexually transmitted infection in the world [1]. Antimicrobial resistance in *N. gonorrhoeae* is a major concern, as the organism has developed resistance to every class of antibiotics used for treatment [2]. Currently, dual therapy with ceftriaxone and azithromycin is widely recommended for gonorrhea treatment, although ceftriaxone monotherapy is recommended in some countries [3, 4].

In the past 2 decades, *N. gonorrhoeae* strains with either decreased susceptibility or resistance to the extended-spectrum cephalosporins (ESCs) have emerged [2]. Although *N. gonorrhoeae* uses a number of mechanisms to develop resistance to antibiotics, there are primarily 4 genes that contribute to decreased susceptibility to ESCs—*penA*, *mtrR*, *penB*, and *ponA* [5, 6]. *penA* and *ponA* are involved in catalyzing the cross-linking of bacterial cell walls [7], *penB* in the permeability of porins to antimicrobials [8], and *mtrR* in transcription of the efflux pump that transports antimicrobials [9]. There is considerable variability in the amino acid alterations within those genes [6], leading to difficulty in developing molecular assays to predict susceptibility to ESCs.

Prior research describing the variability within the genetic loci important for decreased susceptibility to ESCs in *N. gonorrhoeae* have focused on isolates localized to specific countries or regions [5, 10–16]. There are very few reports that aggregate isolates from multiple countries or regions and none that provide a global aggregate of isolates with resistance or decreased susceptibility to ceftriaxone [17–19]. Many reports have found associations between various genetic loci and ceftriaxone resistance, but those data are limited by small sample sizes, narrow geographic regions, and focus on different gene loci.

Here, we performed a global analysis of all available genetically characterized strains of *N. gonorrhoeae* with decreased susceptibility to ceftriaxone. Based on that analysis, we identified specific genetic alterations in *penA, mtrR, penB,* and *ponA* with high sensitivity or specificity to predict decreased susceptibility to ceftriaxone, and proposed parsimonious molecular algorithms for prediction of *N. gonorrhoeae* strains with decreased susceptibility to ceftriaxone (CRO-DS strains).

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MATERIALS AND METHODS

Definition of Resistance and Decreased Susceptibility

There are 2 different minimum inhibitory concentration (MIC) break points used to define resistance to ceftriaxone in *N. gonorrhoeae*. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines an MIC of ≤0.125 mg/L as susceptible and an MIC of >0.125 mg/L as resistant [20], while the Clinical and Laboratory Standards Institute (CLSI) defines an MIC ≤0.25 mg/L as susceptible and >0.25 mg/L as nonsusceptible [21]. Over time, decreased susceptibility was established to monitor the emergence of resistance as a middle point between susceptibility and resistance or nonsusceptibility. Although not specified by EUCAST or CLSI, investigators have classified decreased susceptibility as >0.064 mg/L [22]. In our report, we also defined decreased susceptibility as MIC values >0.064 mg/L.

Literature and Genetic Analysis

One author (E. Y. L.) performed a literature search of all articles published in PubMed up to 15 October 2019 under the search terms "*Neisseria gonorrhoeae*" and "ceftriaxone." All reports (N = 939) were reviewed for information about ceftriaxone MIC and genetic characterization of *N. gonorrhoeae*. Of the 939 reports, there were 169 unique reports that included genetic information and MIC data, of which 111 contained information about specific genetic alterations in *N. gonorrhoeae*. All genetically characterized *N. gonorrhoeae* strains with respect to *penA*, both susceptible and with decreased susceptibility to ceftriaxone, were recorded. Using information in those reports, or by directly contacting the authors, we also recorded genetic alterations with respect to *penB*, *ponA*, and *mtrR*.

Examining *penA* Amino Acid Alterations

penA is a gene that codes for the penicillin-binding protein (PBP) 2 and is important for the binding of ESCs [23]. Mutant *penA* in *N. gonorrhoeae* has been associated with resistance to ceftriaxone [5]. There are currently 83 specific amino acid positions in *penA* associated with decreased susceptibility to ceftriaxone [2]; different combinations of alterations at those sites have been classified as Ohnishi sequences, with each assigned a specific roman numeral. In this report, *penA* amino acid alterations of each genetically characterized strain were obtained from either the Ohnishi sequences or the whole-genome sequences. Ohnishi sequences are defined on the NG-STAR Canadian Web site (https://ng.star.canada) or within the report detailing the site's development [24]. Ohnishi sequences that were unpublished were obtained by contacting authors.

Whole-genome sequences were accessed through the National Center for Biotechnology Information (https://ncbi.nlm.nih.gov), using the studies' project codes. The amino acid sites of interest were located, and alterations were recorded.

Previous reports have documented specific amino acid alterations associated with resistance for both mosaic and nonmosaic *penA* alleles in *N. gonorrhoeae*, where "mosaic" *penA* alleles contain up to 62 mutations with respect to wild type, and "nonmosaic" alleles contain only point mutations [24]. Alterations in mosaic strains include A311V (alanine to valine at position 311), I312M, V316T/P, T483P/S, A501V/P/T, N512Y, G545S, and P551L/S [2, 6], while alterations in nonmosaic strains include insD345 (aspartate insertion following position 345), A501V/T, G542S, and P551L/S [5, 6, 22]. In this report, all strains compiled were searched for *penA* amino acid alterations associated with resistance and separated by mosaic status. Mosaicism was determined if alterations in amino acids 375–377 were found, as detailed by Deng et al [25].

Examining non-*penA* Amino Acid Alterations

penB (*porB1b)* is an allele of *porB*, an outer membrane porin associated with ceftriaxone resistance in *N. gonorrhoeae* through alterations at G120 and A121 [17], amino acids located within the constriction loop of porin. Alterations at those sites lead to decreased permeability of this porin for antimicrobials [8]. Therefore, any alterations in G120 and A121 of *penB* were recorded. *The ponA* gene encodes for PBP1 and, like *penA*, has a role in β-lactam resistance [7]. However, the effect is considered minor; PBP1 has a 10-fold lower affinity for penicillin than PBP2 [26]. The L421P alteration in *ponA* has been associated with contributing to ceftriaxone resistance [17], sometimes with respect to mosaic strains specifically [17]. All instances of L421P were recorded. *mtrR* encodes a major transcriptional repressor of the *mtr* gene locus. The most common alteration on the *mtrR* gene associated with increased ceftriaxone resistance is the deletion of an adenine residue from the 13–base pair inverted repeat contained within the promoter region (*mtrR* delA) [10], resulting in a net increased production of the MtrC-MtrD-MtrE efflux pump, which increases antimicrobial efflux [9]. Because the majority of reports excluded alterations in the *mtrR* coding region, we only included information on *mtrR* delA.

Estimation of Algorithm Sensitivity and Specificity

We developed algorithms targeting *penA* alterations, including mosaicism and nonmosaicism, and non-*penA* (*penB*, *mtrR*, and *ponA*) gene alterations to predict decreased susceptibility to ceftriaxone in *N. gonorrhoeae* strains identified in our literature review. Algorithms were developed by using different combinations of the individual genetic alterations with high sensitivities or specificities; we reported the combinations resulting in the highest sensitivities or specificities. Sensitivity was calculated by dividing the number of CRO-DS isolates with the targeted genetic modification by the total number of CRO-DS isolates. Specificity was calculated by dividing the number of ceftriaxone-susceptible (CRO-S) isolates without the targeted genetic modification by the total number of CRO-S isolates.

RESULTS

In total, we compiled 3821 sequenced *N. gonorrhoeae* strains from 23 countries. Five countries accounted for 75% of all characterized strains: the United States (30%), Russia (14%), Canada (12%), New Zealand (11%), and China (8%). (Figure 1) Among all identified strains, 684 (18%) were CRO-DS, originating from 20 countries, of which China (37%), Canada (23%), United States (14%), South Korea (8%), and Japan (6%) were the most frequent. For each strain, we report the specific genetic mutations associated with decreased susceptibility to ceftriaxone in *penA*, *penB*, *ponA*, and *mtrR* (see Supplementary Data).

Susceptibility Analysis in *penA* Genes

No *penA* amino acid mutation or combination of mutations yielded both high sensitivity and specificity for decreased susceptibility to ceftriaxone. The *penA* amino acid mutations that yielded the highest sensitivities or specificities are listed in Table 1. Among all mosaic CRO-DS strains, the L447V mutation was found in every strain (100% sensitivity, 0.8% specificity). Each of the mutations I312M, V316T/P, H541N, F504L, and A510V yielded similar sensitivities (>95%) and specificities (<5%). In contrast, A311V and T483P/S were not found in any CRO-S mosaic strains, corresponding to 100% specificity, but had sensitivities <10%.

While individual genetic mutations resulted in specificities or sensitivities of ≥95%, they were associated with sensitivity or specificity values of <10%. As a result, we screened various combinations of genetic alterations, and substantial improvements were found for the nonmosaic *penA* strains. Among those strains, identification of ≥1 of certain amino acid alterations—G542S, P551L/S, or A501V/T—resulted in 94.7% sensitivity and 45% specificity for predicting CRO-DS strains; isolated detection of A501V/T resulted in sensitivity of 66.1% and specificity of 91.9%. Finally, detecting only A510V in nonmosaic strains resulted in similar sensitivity (100%) and specificity (2.7%) to that seen in mosaic strains.

Among all *N. gonorrhoeae* strains, alterations at F504L or A510V each produced high sensitivities (98.1%). In contrast, detection of A501V/T resulted in a sensitivity of 36.9% and specificity of 93.6%, while detection of A311V or T483P/S resulted in <4% sensitivities but >99.0% specificities (Table 1).

Susceptibility Analysis in Non-*penA* Genes

Similarly, no non-*penA* amino acid mutation or combination of mutations yielded both high sensitivities and specificities. As a result, as done with *penA*, different combinations of non-*penA* alterations were screened. The non-*penA* amino acid mutations that yielded the highest sensitivities or specificities are listed in Table 2. Among mosaic strains, detecting *ponA* L421P, either *penB* G120 or A121, or both *penB* alterations all yielded sensitivities >97% and specificities of approximately 10%. In contrast, nonmosaic strains yielded much higher specificity/sensitivity combinations. Detecting *mtrR* delA resulted in 95% sensitivity and 61% specificity, while additional detection of L421P in *ponA* slightly decreased sensitivity to 89% but notably increased specificity to 72%. Alternatively, detecting only L421P resulted in a higher rate capture rate of CRO-DS strains (99.6% sensitivity, 45.4% specificity). Among all *penA* strains, detection of the L421P mutation resulted in 99.6% sensitivity and 45.4% specificity; additional detection of ≥1 of the G120/A121 alterations in *penB* increased specificity to 60.7% (92.2% sensitivity). (Table 2)

Algorithms to Predict Decreased Susceptibility to Ceftriaxone

The sensitivity and specificity results above were used to create several testing algorithms to predict CRO-DS *N. gonorrhoeae* strains. We present 4 primary algorithms: (1) *penA* detection, (2) *penA* detection with mosaicism determination, (3) non-*penA* detection, and (4) non-*penA* detection with mosaicism determination. (Figures 2 and 3) Testing for all genetic loci in these algorithms are intended to be done simultaneously.

In strategy 1, detection of A510V and A311V in *penA* yielded 4% sensitivity and 100% specificity for predicting CRO-DS strains, while detection of A510V without A311V yielded 94% sensitivity and 25% specificity (Figure 2A). In strategy 2, the *penA* algorithm is first stratified by mosaic strain status, with the results separated according to the presence or absence of mosaic alterations. Mosaic strains with the presence of L447V and ≥1 of G542S, P551L/S, or A501V/T was associated with 97% sensitivity and 7% specificity for predicting CRO-DS strains, while L447V without any of the 3 alterations was associated with 3% sensitivity and 94% specificity. In nonmosaic strains, detection of L447V without G542S, P551L/S, and A501V/T resulted in <1% sensitivity and 98% specificity, and detection of ≥1 of the 3 alterations without L447V resulted in 95% sensitivity and 62% specificity (Figure 2B).

In strategy 3, detection of *ponA* L421P and ≥1 of G120 or A121 in *penB* resulted in 92% sensitivity and 61% specificity for CRO-DS. However, without either alteration in *penB*, the sensitivity and specificity dropped to 2% and 90%, respectively (Figure 3A). In strategy 4, similar to strategy 2, the algorithm is first stratified by mosaic and nonmosaic strain status. Detection of both *ponA* L421P and *mtrR* delA in mosaic strains resulted in 95% sensitivity and 31% specificity for CRO-DS strains, while detection of only *ponA* L421P resulted in 2% sensitivity and 96% specificity. In nonmosaic strains, sensitivity and specificity were 89% and 72% respectively when both alterations were present. However, absence of *mtrR* delA resulted in substantial reduction in sensitivity (4%) but increased specificity (85%) (Figure 3B).

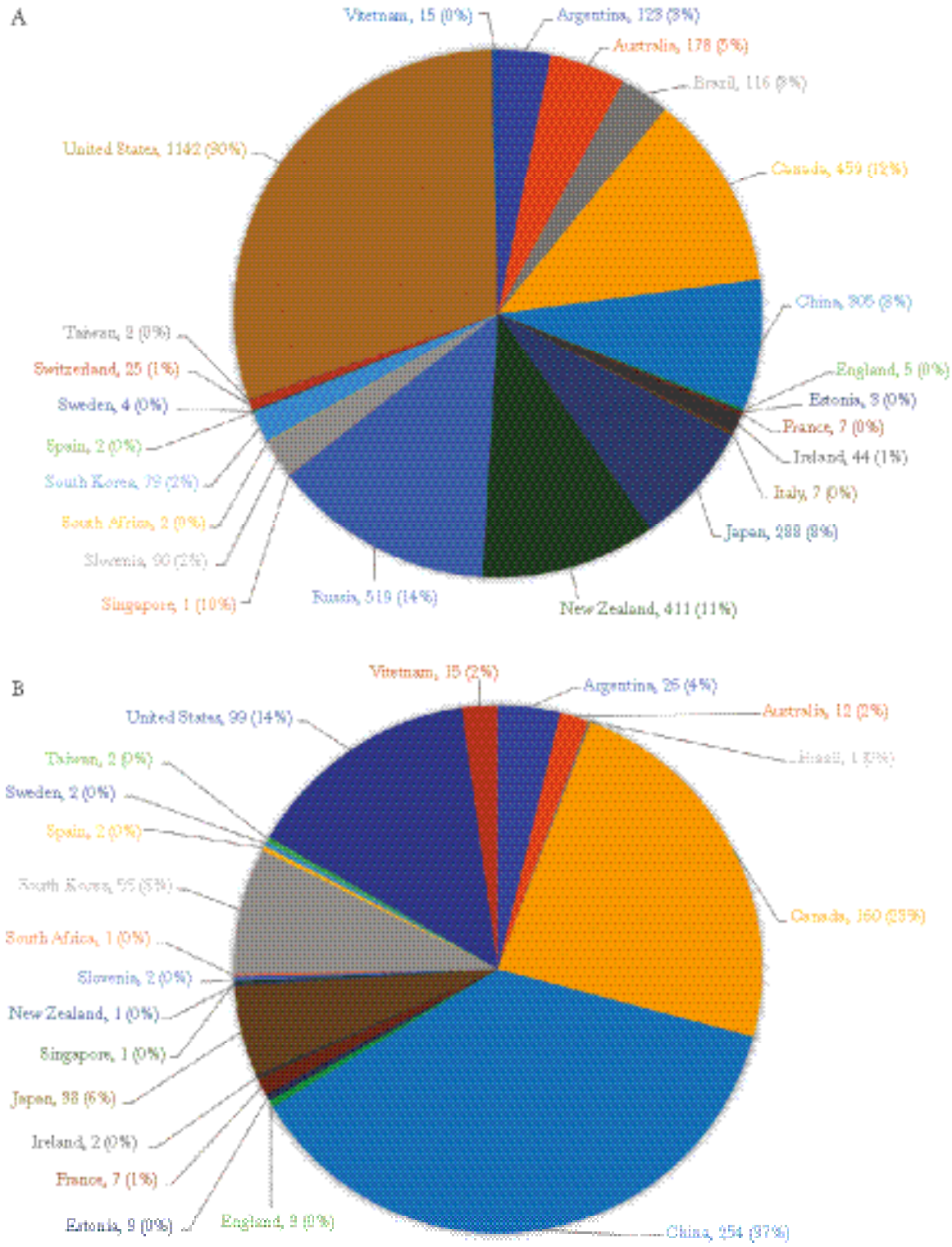

Figure 1. Geographic distribution of *Neisseria gonorrhoeae* strains with minimum inhibitory concentration data, by country (N = 3821). *A,* All strains. *B,* Strains with decreased susceptibility.

DISCUSSION

We present findings on 3821 sequenced *N. gonorrhoeae* strains from around the world with reported MICs to ceftriaxone. For each strain, we reported the genetic mutations associated with decreased susceptibility to ceftriaxone in the *penA*, *penB*, *ponA*, and *mtrR* genes. We compiled and analyzed the genetic mutations associated with decreased susceptibility to ceftriaxone from a global collection of isolates. Through our analysis, we

Table 1. Amino Acid Alterations and Combinations in the *penA* Gene of *Neisseria gonorrhoeae* Strains Associated With the Highest Sensitivity and Specificity for Decreased Susceptibility to Ceftriaxone

	Mosaic *penA*		Nonmosaic *penA*		All Strains	
penA Alterations	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
L447V	1	0.008	0.003	0.963	0.447	0.742
A311V	0.092	1	0	0.999	0.039	0.999
I312M	0.98	0.032	0	0.997	0.429	0.781
T483P/S	0.082	1	0	0.997	0.036	0.998
V316T/P	0.962	0.043	0	0.997	0.416	0.793
H541N	0.957	0.017	0.068	0.907	0.464	0.699
F504L	0.957	0.012	1	0.027	0.981	0.023
A510V	0.957	0·012	1	0.027	0.981	0.023
≥1 of G542S, P551L/S, A501V/T	0.032	0.972	0.947	0.450	0.583	0.623
A501V/T	0.007	0.997	0.661	0.919	0.369	0.936
≥1 of G542S, P551L/S, I566V	0.359	0.810	0.953	0.328	0.716	0.487
≥1 of G542S, P551L/S, A574N	0.359	0.582	0.950	0.207	0.723	0.276
≥1 of P551L/S and I566V	0.360	0.582	0.908	0.386	0.690	0.422

report the sensitivities and specificities associated with the detection of those genetic determinants. We proposed 4 algorithms for detection of decreased susceptibility to ceftriaxone in *N. gonorrhoeae* that can guide development of future diagnostic assays.

Our analysis of all CRO-DS strains found 380 mosaic and 305 nonmosaic strains. Previous reports have suggested that a primary mechanism by which *N. gonorrhoeae* achieves resistance is through acquisition of a mosaic *penA* allele [23]. However, we found that using mosaic *penA* to predict CRO-DS strains would result in missing a substantial number of strains with decreased susceptibility. Our findings are consistent with other reports that have found mosaicism insufficient for predicting ceftriaxone resistance and highlight the importance of reviewing isolates from various parts of the world [11].

Our analysis identified high specificities (>86%) associated with detection of A311V, A501P/T/V, and G542S when analyzed among all *N. gonorrhoeae* strains, suggesting they are viable predictors of decreased susceptibility and higher MIC values. Similarly, Demczuk et al [27] were able to predict (R^2 = 0.721) MICs to ceftriaxone in *N. gonorrhoeae* strains using 5 genetic loci in *penA*: A311V, A501P/T/V, N512Y, A516G, and G542S. However, our data suggest that N512Y and A516G were associated with lower specificities and might be less important within a global collection of strains. (Supplementary Table 4) The differences in our findings likely reflect the global variability of these alterations, because their analysis was limited to isolates from 4 countries. In addition, we used a categorical classification of decreased susceptibility, whereas their report used MIC values [27].

For mosaic *penA* strains, we found high sensitivities but very low specificities for CRO-DS strains among alterations I312M, V316T/P, N512Y, and G545S, signifying that while the majority of CRO-DS strains have these alterations, these loci are not key drivers of decreased susceptibility. However, this does not preclude them from influencing ceftriaxone susceptibility. Tomberg et al [28] showed that V316T, G545S, and I312M display epistasis, such that reverting these alterations in mosaic *penA* completely abolished resistance, while introducing them into wild-type strains had minor effects on MIC. That report also demonstrated decreased MICs after reverting N512Y to wild type in mosaic strains [28]. The mosaic amino acid alterations A311V, T483P/S, A501V/P/T, and P551S all have low sensitivities and high specificities. They are in close proximity to the active site of PBP2 acylation and have been associated with higher ceftriaxone MICs [6, 12, 28, 29]. While rare, they are highly associated with resistance, and are strong candidates to be used in molecular assays to predict CRO-DS strains.

For nonmosaic *penA* strains, we found A501V/T to be strongly associated with decreased susceptibility to ceftriaxone. The A501V/T residue is located on the β3-β4 loop that has mutations critical for penicillin resistance, with bulkier side chains potentially producing steric clash with the R1 group of ESCs [28]. In addition, introducing A501V/T in vitro increased MIC to ceftriaxone [28]. In contrast, the roles G542S and P551L/S have in contributing to resistance are inconclusive with nondiscriminatory sensitivities and specificities (Supplementary Table 3). However, G542S and P551L/S were previously shown to be associated with nonmosaic CRO-DS strains [22]. Monitoring for those alterations as more genetic and antibiotic susceptibility data emerge will be important. Finally, despite being found in all nonmosaic strains, insD345 does not appear to be linked to decreased ceftriaxone susceptibility [13].

In our report, *penB* G120/A121, *ponA* L421P, and *mtrR* delA were all shown to be important in contributing to *N. gonorrhoeae* decreased susceptibility to ceftriaxone, with high sensitivities and specificities. Our findings are consistent with the literature identifying the importance of *penB*, *ponA*, and *mtrR* in contributing to decreased susceptibility to ceftriaxone in *N. gonorrhoeae* [17]. Demczuk et al [27] also found that all 3 of those alterations are important in predicting ceftriaxone MICs in *N. gonorrhoeae*. The specificities of *penB* G120/A121, *ponA* L421P, and *mtrR* delA all decreased substantially when detected in mosaic strains but increased in nonmosaic *penA* strains, suggesting interactions between these 3 loci and nonmosaic *penA* alleles in the development of decreased susceptibility to ceftriaxone.

The molecular algorithms to predict CRO-DS strains are proposed using a global genetic analysis. The primary approach in establishing the molecular algorithms was aimed at maximizing either sensitivity or specificity. High sensitivity ensures that

Table 2. Amino Acid Alterations and Combinations in Non-*penA* Genes of *Neisseria gonorrhoeae* Strains Associated With the Highest Sensitivity and Specificity for Decreased Susceptibility to Ceftriaxone

	Mosaic *penA*		Nonmosaic *penA*		All Strains	
Non-*penA* Alterations	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
ponA L421P	1	0.108	0.991	0.521	0.996	0.454
mtrR delA	0.954	0.172	0.952	0.609	0.953	0.528
mtrR delA + *ponA* L421P	0.919	0.239	0.893	0.717	0.906	0.64
≥1 *penB* G120, A121 alteration(s)	0.988	0.111	0.945	0.486	0.966	0.421
mtrR delA + *penB* G120 +A121 alteration(s)	0.971	0.38	0.852	0.721	0.918	0.649
penB G120 + A121 alterations	0.984	0.126	0.902	0.607	0.943	0.523
ponA L421P + and ≥1 of *penB* G120, A121 alteration(s)	0.977	0.127	0.862	0.684	0.922	0.607

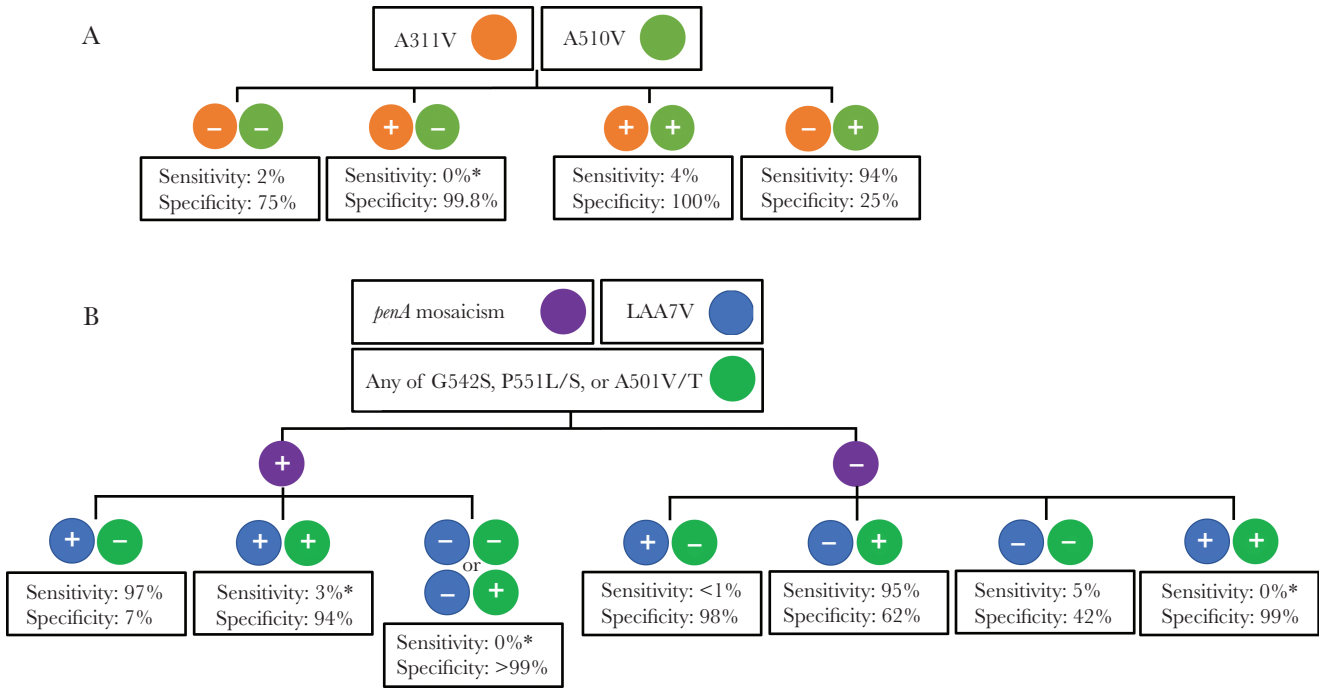

Figure 2. Two sets of molecular algorithms using *penA*. *A,* Algorithm that excludes mosaicism determination. *B,* Algorithm that includes mosaicism determination, which can be done by screening *penA* amino acid positions 375–377. Sensitivity and specificity values are for decreased susceptibility to ceftriaxone. Testing for all genetic loci in these algorithms are intended to be done simultaneously, and not necessarily in a stepwise fashion. Asterisks denote combinations of amino acid alterations for which no strains with decreased susceptibility have been reported.

nearly all CRO-DS strains are captured, while high specificity ensures that if detected, the strain will likely have decreased susceptibility. From this approach, we have established targets for a molecular assay that could be powerful and versatile in predicting CRO-DS strains. The proposed algorithms could achieve sensitivities or specificities >95%, with certain sensitivities accompanied by specificities >60%. Those algorithms lay the foundation for the development of molecular assays that could be used in a global population of *N. gonorrhoeae* strains to predict decreased susceptibility to ceftriaxone. Moreover, by proposing 4 different testing strategies based on 4 different genes (*penA*, *penB*, *mtrR*, and *ponA*), we present pathways that have the potential to be tailored based on local *N. gonorrhoeae* epidemiology.

The use of the proposed algorithms in practice will depend on the frequency and distribution of strains with decreased susceptibility in a target population. Based on our reported sensitivities and specificities, positive and negative predictive values could be estimated. For example, using a theoretical 5% and 30% prevalence of CRO-DS strains in a specific population, a testing strategy detecting *ponA* L421P mutations in nonmosaic strains (99.1% sensitivity and 52.1% specificity), the positive predictive value for decreased susceptibility would be 10% and 47%, respectively. Owing to high sensitivities, the negative predictive value would be >99% in that testing strategy at both 5% and 30% prevalence of decreased susceptibility. The prevalence of mosaic strains is low, comprising 8.6% and 2.8% of the *N. gonorrhoeae* strains in the United States and China,

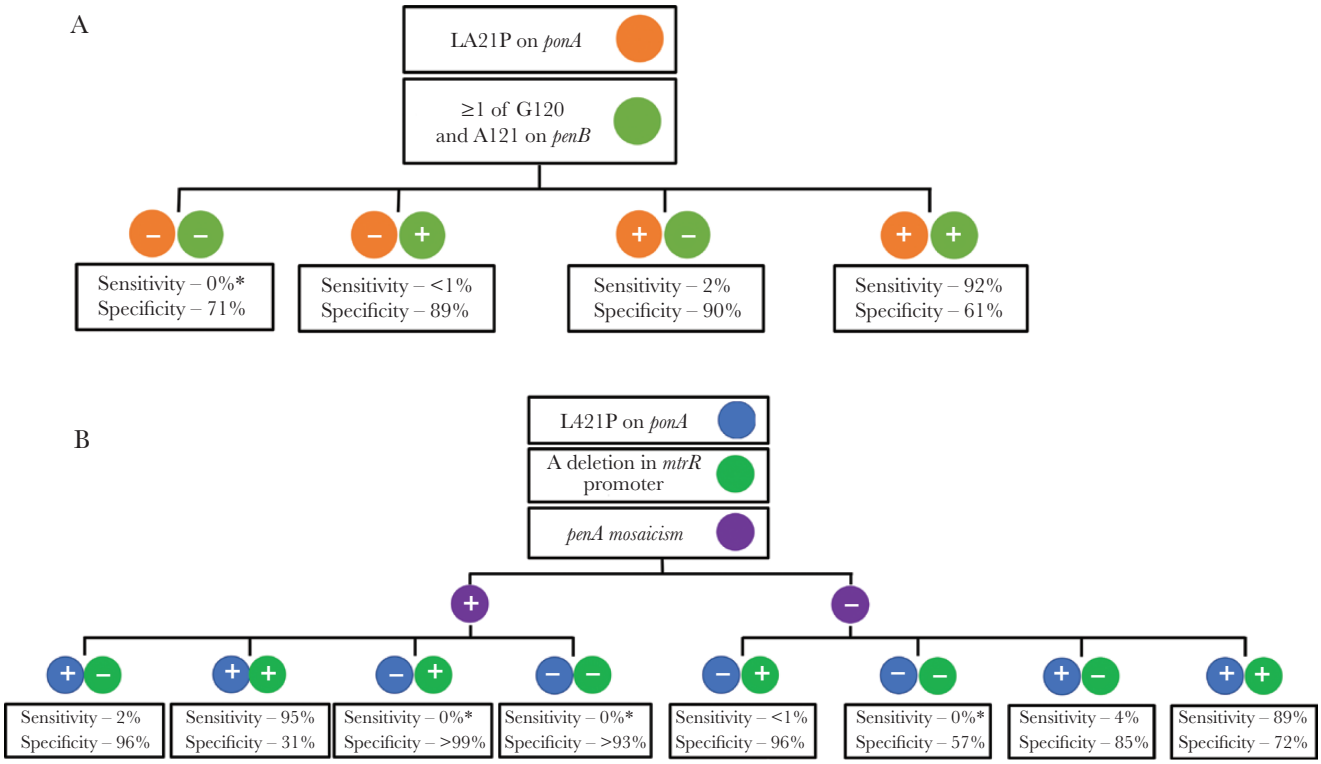

Figure 3. Two sets of molecular algorithms using non-*penA* genes. *A,* Algorithm that excludes mosaicism determination. *B,* Algorithm that includes mosaicism determination, which can be done by screening of *penA* amino acid positions 375–377. Sensitivity and specificity values are for decreased susceptibility to ceftriaxone. Testing for all genetic loci in these algorithms are intended to be done simultaneously, and not necessarily in a stepwise fashion. Asterisks denote combinations of amino acid alterations for which no strains with decreased susceptibility have been reported.

respectively [14, 15], and as such, false-positives among mosaic strains would have minimal overall impact. Ultimately, the goals of the algorithms are to detect *N. gonorrhoeae* strains with decreased susceptibility to ceftriaxone, and this depends on the population that is being tested; as prevalence increases, so too does the positive predictive value and the need for a highly sensitive test. While the proposed molecular algorithms are preliminary and will require validation, they can be used as a framework to guide future research aimed at assessing the clinical performance in different populations.

Other molecular algorithms to predict *N. gonorrhoeae* CRO-DS strains have been reported. Peterson et al [30] reported a molecular assay to predict decreased susceptibility to cephalosporins, including ceftriaxone. Our algorithms achieved similar sensitivities with regard to *ponA*, *mtrR*, and *penB* (>95%), although with lower specificities. However, Peterson et al used only samples from Canada, which might limit generalizability. Doná et al [31] developed a molecular algorithm to predict resistance to ESCs based on screening for the Asp345 deletion (delD345), which is equivalent to a lack of insD345, and G545S in mosaic *penA* alleles X and XXXIV. Our results agree that detection G545S and delD345 are highly sensitive among mosaic strains (93.8% and 97.6%, respectively) but differ in the extremely low specificity (Supplementary Table 2). However, the algorithm was evaluated only in isolates from Switzerland and focused only on *penA* mosaic X and XXXIV alleles, which comprise approximately 18.5% and 31.9% of all globally reported mosaic alleles. Thus, by performing our analysis on a global database of isolates, our report expands generalizability and can guide the development of future molecular assays predicting decreased susceptibility to ceftriaxone.

Our report has several limitations. First, we are limited by the availability of genomic data for both CRO-DS and CRO-S *N. gonorrhoeae* strains; strains from several countries were reported but not sequenced [10, 16]. Second, our whole-genome sequencing (WGS) analysis focused on the 83 *penA* amino acid alterations of the Ohnishi sequences only. This method was chosen to encapsulate the results of all studies with genetic characterizations, because most studies have either not performed WGS or have not published their results. We attempted to reach all authors that did not publish their WGS results but did not always receive a reply. Although we used prevalent alterations found in *penA*, it is possible that other alterations are on the rise and associated with decreased susceptibility to ceftriaxone. Finally, of the 4 proposed diagnostic algorithms, we cannot determine which would be optimal for detection of CRO-S infections, because our data set contains an overrepresentation of CRO-DS strains. Identifying such an algorithm is key, as there

are currently limited options available for treatment of CRO-DS infections. Further research on the application of those diagnostic algorithms is therefore needed.

We identified, compiled, and analyzed findings from 3821 global isolates of *N. gonorrhoeae* with reported MICs to ceftriaxone. We analyzed genetic information for all *N. gonorrhoeae* isolates with decreased susceptibility to ceftriaxone and generated preliminary molecular algorithms to predict decreased susceptibility to ceftriaxone. The identified genetic targets can be used to guide the development of molecular assays to predict CRO-DS strains, with utility in a variety of global settings.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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