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# Genomic Analysis of *Neisseria gonorrhoeae* WHO_A_2024 and Molecular Docking and ADMET Evaluation of Quercetin against Penicillin-Binding Protein 2 (PBP2): An in *Silico* Study

**Mohammed Torki Hammood[1], Methaq Hussein Abbas[2]**

[1, 2] Department of biotechnology, College of science, University of Anbar, Ramadi, 31001, Iraq

[*]**Corresponding author:** Mohammed Torki Hammood**;** **mohammed.torki@uoanbar.edu.iq**

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## Abstract

Even though Neisseria gonorrhoeae is still one of the most significant bacterial sexually transmitted infections (STIs), growing antimicrobial resistance (AMR) is a significant barrier to treatment and disease control. This study was designed to characterize the genomic profile and antimicrobial resistance of N. gonorrhoeae WHO_A_2024 and to assess the in silico interaction and ADMET properties of Quercetin with penicillin-binding protein 2 (PBP2). The complete genome sequence of the WHO_A_2024 genome (GCF_040404645.1) was used for multilocus sequence typing (MLST), identification of antimicrobial resistance genes, and CRISPR–Cas-associated sequences. Molecular docking was used to evaluate the binding between quercetin and PBP2 (PDB ID: 6P54), and then computational ADMET analysis was performed. The genome was a 2,308,468 bp chromosome having 52% GC content. Seven-locus matches were observed between **ST10316** and WHO_A_2024 by MLST. The only determinant associated with a predicted resistant phenotype was for rpsE T24P substitution, is a low-level spectinomycin resistance. Extended-spectrum cephalosporins, fluoroquinolones, tetracyclines, and macrolides were not expected to show resistance. Four short and low-confidence CRISPR and one General-Class1 Cas-associated cluster were found. The hydrogen-bond, van der Waals, and π-mediated interactions resulted in a predicted PBP2 binding affinity of −6.707 kcal mol−1 for quercetin. The drug-likeness was moderate, and intestinal absorption was predicted by ADMET analysis, but some limitations in permeability and bioavailability and some metabolic and toxicity alerts were found. Integrated genomic and computational analyses identified WHO_A_2024 and suggested quercetin as a possible candidate for binding to PBP2. These computational findings need to be validated by experimental antimicrobial, pharmacokinetic, target-engagement, and safety studies.

**Keywords:** *Neisseria gonorrhoeae*; whole-genome analysis; antimicrobial resistance; PBP2; quercetin; molecular docking.

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## 1. Introduction

Neisseria gonorrhoeae is one of the most significant sexually transmitted bacterial pathogens globally and is a concern due to antimicrobial resistance (AMR) in the bacteria. In 2020, gonococcal infections were estimated to be about 82.4 million in the population of adults aged 15–49 years across the world, based on the estimates provided by the World Health Organization (WHO). The clinical importance of gonorrhoea is not limited to acute urogenital infection and may contribute to developing pelvic inflammatory disease, which in

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turn can result in infertility, adverse reproductive outcomes, and affect one's susceptibility to human immunodeficiency virus infection [1]. The emergence or acquisition of gonococcal resistance to several classes of antimicrobial agents (sulfonamides, penicillins, tetracyclines, fluoroquinolones, and macrolides) has made effective control of gonorrhoea a greater challenge. More importantly, decreased susceptibility and resistance to extended-spectrum cephalosporins have also been reported, which warrant concerns about the sustainability of currently available therapeutic options [2,3].

A complex interplay of genetic recombination, antimicrobial resistance genes, and chromosomal mutations confers resistance to N. gonorrhoeae. Resistance or decreased antimicrobial susceptibility has been linked to several loci such as penA, ponA, porB, mtrR, gyrA, parC, and 23S rRNA, as well as other genes or regulatory regions that play a role in antimicrobial targets, membrane permeability, and drug efflux [4]. PenA, which encodes penicillin-binding protein 2 (PBP2), an essential transpeptidase in peptidoglycan biosynthesis, is one of these determinants and is especially crucial. Mosaic alleles or mutated alleles of penA have been shown to alter susceptibility to extended-spectrum cephalosporins and are an important molecular mechanism that can be associated with decreased susceptibility to cefixime and ceftriaxone [5]. Structural studies of gonococcal PBP2 have also shown that conformational changes are important during β-lactam recognition and acylation, which has led to the conclusion that PBP2 is a relevant target for structure-based investigation in the context of the biology of the gonococci [6].

Microbial studies of gonococcal populations and resistance mechanisms have now been facilitated by significant improvements in whole genome sequencing (WGS). WGS can simultaneously be used for strain typing, characterizing the structure of a population, detecting known AMR determinants, determining evolutionary relationships, and for surveillance of emerging AMR lineages [7,8,9]. Conventional phenotypic antimicrobial susceptibility testing is thus a complement to genomic surveillance. Nevertheless, genomic predictions should be viewed “with great care” and should not be used as a substitute for the phenotypic prediction of susceptibility, especially when resistance mechanisms are unknown or not fully understood [10]. The complete genome sequences then offer an excellent platform to integrate genome-wide analysis of resistance determinants and other genomic adaptations with molecular typing.

Another aspect of bacterial genome organization that has the potential to affect their interaction with mobile genetic elements and their genome evolution is CRISPR–Cas systems. These adaptive immune systems recognize and target foreign nucleic acids via proteins that are part of the CRISPR system and guide RNAs derived from the spacers [11]. The distribution and structure of CRISPR–Cas systems, however, are highly dynamic across Neisseria species. While functional CRISPR–Cas systems were described in some strains, endogenous CRISPR activity is not regarded as being present in all strains of N. gonorrhoeae [12]. Thus, it is advisable to take with a grain of salt the presence of CRISPR-like arrays or Cas-associated genes found in gonococcal genomes by computational methods. They may have biological significance, but should be considered based on the architecture of the arrays, the composition of the spacers in the array, the organization of the Cas genes in the genome, and the predicted confidence of the array's computational prediction, not necessarily as proof of a functional CRISPR–Cas immune system.

In addition to the genomic methods, structure-based computational methods offer valuable tools to explore the interaction of relevant bacterial proteins with possible antimicrobial agents. In the early stages of drug discovery, molecular docking has been applied to predict possible modes of binding for

a ligand molecule to a specific protein in a certain region, and to evaluate the binding affinity by calculating the relative binding energy, which is helpful for hypothesis generation [13]. In addition, computational assessment of absorption, distribution, metabolism, excretion, and toxicity (ADMET) may yield some preliminary information on the pharmacokinetic behavior, drug-like properties, and potential safety concerns of candidate compounds. However, the experimental confirmation of both docking and ADMET predictions is necessary to make biological or therapeutic conclusions [14].

Quercetin is a natural polyphenolic flavonoid, distributed in plant-derived foods, and has been noted for its reported biological activities, which have attracted great interest. The antibacterial activity of quercetin has been previously documented in different experimental studies and reviews in relation to various Gram-negative and Gram-positive bacteria. Some proposed mechanisms include disruption of the function of bacterial membranes, inhibition of nucleic acid and protein synthesis, modulation of virulence-associated processes, and inhibition of bacterial biofilm formation [15,16]. The results give support to the use of computational methods to study the association of quercetin with specific molecular targets of bacteria. However, the ability to predict molecular binding does not necessarily imply antibacterial activity or direct inhibition of the selected target and needs to be suitably validated experimentally.

The present study thus used an integrated in silico approach to characterize the whole genome of N. gonorrhoeae WHO_A_2024 (RefSeq accession GCF_040404645.1), which includes a chromosome of about 2.31 Mb. Genomic analysis was performed to include molecular typing, antimicrobial resistance determinants, and CRISPR–Cas-associated features. The results of these analyses were then combined with a structure-based study of PBP2 encoded by penA, where the interaction between quercetin and PBP2 was predicted with molecular docking. Furthermore, the physicochemical, pharmacokinetic, and toxicity characteristics of quercetin were evaluated using the computational ADMET analysis. This approach of going from genome to target was integrated to create a genomic framework for WHO_A_2024 and to test a computational hypothesis for an interaction between quercetin and PBP2 that could be tested in the future.

## 2. Materials and Methods

### 2.1. Genome retrieval and annotation

Neisseria gonorrhoeae WHO_A_2024 was retrieved from the National Center for Biotechnology Information (NCBI) RefSeq database (assembly accession: GCF_040404645.1, GenBank accession: GCA_040404645.1). The genome assembly consists of one circular chromosome of 2,308,468 bp and has an overall GC content of 52% and a reported sequencing depth of 150×. The deposited genome sequence was sequenced using both short-read and long-read sequencing platforms (Illumina and PacBio Sequel, respectively) and assembled using Unicycler v0.5.0. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) was used to annotate the genome. The entire chromosome is available in RefSeq accession NZ_CP145090.1, which is also available in GenBank accession CP145090.1. The deposited annotation lists 2,429 annotated genes, including 2,105 protein-coding genes. These data were employed to perform further molecular typing and in silico analysis of antimicrobial resistance and other genomic features.

### 2.2. Multilocus sequence typing

Neisseria gonorrhoeae WHO_A_2024 was subjected to multilocus sequence typing (MLST) using MLSTFinder v1.0.1 [17] which was based on whole-genome sequence-based MLST. Allelic profiles were assigned using the pubMLST-2024_public database and the seven-locus Neisseria MLST scheme that consists of housekeeping genes (abcZ, adk, aroE, fumC, gdh, pdhC, and pgm). The sequence identity and sequence coverage were used

to evaluate matches with the corresponding reference allele sequences for each locus. The sequence type (ST) was then determined by the allelic profile at the seven MLST loci.

### 2.3. Antimicrobial resistance analysis

Genomic AMR determinants for Neisseria gonorrhoeae WHO_A_2024 were predicted using ResFinder/PointFinder, allowing the determination of acquired resistance genes and chromosomal resistance mutations, as well as the antimicrobial phenotype prediction that corresponds to them [18]. The identification concentrated on genetic determinants that correspond to clinically important classes of antimicrobials, especially on penA, ponA, porB, gyrA, parC, pilQ, and rpsE, and on 23S rRNA and regulatory regions of mtrC, mtrR, and macAB. Variants were annotated based on resistance phenotype assignments in ResFinder/PointFinder. Variations that lacked resistance phenotype assignments were annotated as genomic variants, although not recognized as resistance determinants independently. Such an approach was applied to make a distinction between resistance mutations and sequence polymorphisms of unclear phenotypic significance.

### 2.4. CRISPR–Cas analysis

Neisseria gonorrhoeae WHO_A_2024 CRISPR arrays and associated CRISPR-associated (Cas) genes were characterized using CRISPRCasFinder, which is an online tool that allows the analysis of CRISPR repeat-spacer arrays and associated Cas protein sequences [19]. Chromosome NZ_CP145090.1 was screened, and features such as array locations, repeat consensus sequence, number of spacers, evidence level, Cas genes, and predicted system type were noted. The credibility of the identified CRISPR arrays was determined on the basis of evidence levels provided by CRISPRCasFinder. All arrays identified in the chromosome were classified as evidence level 1, meaning they were low-confidence predictions, and were thus regarded as potential CRISPRs rather than actual CRISPR-Cas systems to be safe in case these arrays were merely genomic features.

### 2.5. Target protein and ligand preparation

Penicillin-binding protein 2 (PBP2), which is coded by the penA gene, was chosen as the molecular target due to its important function in peptidoglycan biosynthesis in bacteria and β-lactams' action and resistance in N. gonorrhoeae. The experimentally solved 3D structure of the penicillin-binding protein 2 was downloaded from the Protein Data Bank (ID code: 6P54). Quercetin was chosen as a ligand for the molecular docking experiment, and its chemical structure was taken from the PubChem database (CID: 5280343). Before the molecular docking experiment, the structure of PBP2 was processed by removing the unnecessary components of the crystalline structure and adding polar hydrogen atoms and partial charges. The quercetin structure was prepared for conformational analysis, and its rotatable bonds were defined.

### 2.6. Molecular docking

A molecular docking analysis was carried out using AutoDock Vina, which employs an empirical scoring function and an optimized algorithm to calculate the binding of the ligands and the binding affinity of the receptor [20]. Quercetin was docked in the binding site of penicillin-binding protein 2 (PBP2; PDB ID: 6P54). In the docking experiment, the receptor was kept rigid while the flexible bonds of quercetin were subjected to conformational changes for better exploration of possible binding modes. The docking poses were scored based on their Vina binding affinities in kcal $mol^{-1}$, and the highest-ranked biologically realistic conformation was chosen for further study on protein-ligand interactions. The docked complex was analyzed for interactions between the molecules, including hydrogen bonds, van der Waals interactions, π-interactions, and unfavorable interactions.

### 2.7. ADMET and drug-likeness prediction

In silico ADMET analysis of quercetin was performed through ADMET-AI v1.3.1, a machine-learning-based tool for predicting physicochemical, pharmacokinetic, and toxicity properties of chemical entities [21]. Physicochemical parameters examined included molecular weight, LogP, number of hydrogen bond donors and acceptors, topological polar surface area (TPSA), and quantitative estimate of drug-likeness (QED). Absorption parameters predicted included human intestinal absorption, oral bioavailability, aqueous solubility, Caco-2 permeability, parallel artificial membrane permeability assay (PAMPA) permeability, and P-glycoprotein inhibition. Distribution-related parameters included blood-brain barrier penetration, plasma protein binding, and volume of distribution at steady state. Metabolism characteristics were examined via prediction of probabilities of quercetin functioning as an inhibitor or substrate of major isoforms of cytochrome P450 (CYP450). Elimination characteristics were evaluated based on prediction of half-life and clearance parameters. Toxicological properties were examined through prediction of hERG channel blockade, mutagenicity, drug-induced liver injury, carcinogenicity, clinical toxicity, and skin reaction. All described ADMET parameters were interpreted only as computer-generated predictions and not as experimentally proven data concerning the pharmacokinetics, metabolism, and toxicology of quercetin.

## 3. Results

### 3.1. Multilocus sequence typing of Neisseria gonorrhoeae WHO_A_2024

The Neisseria gonorrhoeae WHO_A_2024 sequence type is ST10316 from multilocus sequence typing (MLST) analysis. All of the housekeeping seven genes, abcZ-59, adk-39, aroE-67, fumC-157, gdh-149, pdhC-71 and pgm-65, showed complete matches. Full allelic profiles were obtained, with 100% sequence identity at each locus and 100% coverage of the reference alleles, leading to the complete allelic profile 59–39–67–157–149–71–65. No partial or ambiguous allele matches were found, and WHO_A_2024 was confirmed to be assigned to ST10316. This sequence-type designation sets the background of the strain and gives a certain genome background for further analysis of antimicrobial resistance determinants and other genome properties, as shown in Table 1.

The predicted antimicrobial resistance profile suggested by whole-genome sequencing analysis was predominantly susceptible, with only resistance predicted for spectinomycin. The rpsE T24P substitution (ACC→CCC) in ribosomal protein S5 was annotated as a determinant of low-level spectinomycin resistance, and this was the prediction. Additionally, an acquired sequence with 88.74% nucleotide identity was identified, but not associated with a phenotype of β-lactam resistance through the prediction platform used in this study.

A number of other chromosomal variants were detected in antimicrobial resistance-associated loci, such as gyrA I250M; parC V384I, V421A, V435A, V596I, and V646A; pilQ S341N and N648S; and porB G213D, T215A, G255Q, A256T, and M257I. But none of these substitutions were used in ResFinder/PointFinder to assign a resistance phenotype. Similarly, no resistance phenotype assignments were found for the mtrC promoter region, mtrR promoter region, or multiple copies of 23S rRNA. Overall, rpsE T24P was the major resistance-associated determinant with an explicitly predicted phenotype, while no resistance was predicted in the major therapeutic categories of antimicrobial drugs (such as the fluoroquinolones, extended-spectrum cephalosporins, carbapenems, tetracyclines, and macrolides). These results differentiate the single phenotype-associated resistance determinant from other genomic variants that were found but not predicted to be associated with resistance, as shown in Table 2.

**Table 1. Multilocus sequence typing characteristics of *Neisseria gonorrhoeae* WHO_A_2024**

| Isolate | MLST scheme | Sequence type | Locus | Allele | Identity (%) | Coverage (%) | Description |
|---|---|---|---|---|---|---|---|
| | | | *abcZ* | 59 | 100.00 | 100.00 | Complete match to *abcZ*_59 |
| | | | *adk* | 39 | 100.00 | 100.00 | Complete match to *adk*_39 |
| | | | *aroE* | 67 | 100.00 | 100.00 | Complete match to *aroE*_67 |
| | | | *fumC* | 157 | 100.00 | 100.00 | Complete match to *fumC*_157 |
| **WHO_A_2024** | ***Neisseria*** | **ST10316** | *gdh* | 149 | 100.00 | 100.00 | Complete match to *gdh*_149 |
| | | | *pdhC* | 71 | 100.00 | 100.00 | Complete match to *pdhC*_71 |
| | | | *pgm* | 65 | 100.00 | 100.00 | Complete match to *pgm*_65 |
| | | | **Seven-locus profile** | **59–39–67–157–149–71–65** | **100.00** | **100.00** | **Complete seven-locus MLST profile supporting assignment to ST10316** |

**Table 2. Genomic antimicrobial-resistance profile of *Neisseria gonorrhoeae* WHO_A_2024**

| Antimicrobial / resistance category | Gene or locus | Genomic determinant | WGS-predicted phenotype | Interpretation |
|---|---|---|---|---|
| **Spectinomycin** | **rpsE** | **T24P (ACC→CCC)** | **Resistant** | Only antimicrobial specifically predicted as resistant; mutation associated with low-level spectinomycin resistance |
| β-lactams | *penA* | Acquired-gene hit; 88.74% identity | No resistance predicted* | *penA* detected, but no specific β-lactam-resistant phenotype was assigned |
| β-lactams | *penA* | Chromosomal locus; 99.83% identity, 100% coverage | No resistance predicted | No resistance-associated amino-acid substitution reported in the output |
| β-lactams | *ponA* | 99.96% identity, 100% coverage | No resistance predicted | Locus detected without a reported resistance-associated substitution |
| β-lactams / permeability | *porB* | G213D, T215A, G255Q, A256T, M257I | No resistance predicted | Multiple substitutions detected, but no phenotype was assigned to these variants |
| Quinolones | *gyrA* | I250M | No resistance predicted | Variant detected without a resistance phenotype assignment |
| Quinolones | *parC* | V384I, V421A, V435A, V596I, V646A | No resistance predicted | Multiple variants detected without a resistance phenotype assignment |
| Outer-membrane transport | *pilQ* | S341N, N648S | No resistance predicted | Variants detected without an assigned antimicrobial phenotype |
| Macrolide/efflux regulation | *mtrC* promoter | Promoter detected; 100% identity | No resistance predicted | No resistance-associated promoter alteration reported |

| | | | | |
|---|---|---|---|---|
| Macrolide/efflux regulation | *mtrR* promoter | Promoter detected; 99.14% identity | No resistance predicted | No resistance phenotype assigned |
| Macrolide/efflux regulation | *macAB* promoter | Promoter detected; 97.37% identity | No resistance predicted | No resistance phenotype assigned |
| 23S rRNA | 23S rRNA alleles | Multiple copies detected | No resistance predicted | No macrolide-resistance mutation reported |
| Fluoroquinolones | — | No resistance determinant assigned | No resistance | Ciprofloxacin/fluoroquinolone resistance not predicted |
| Extended-spectrum cephalosporins | — | No resistance determinant assigned | No resistance | Ceftriaxone, cefixime and cefotaxime resistance not predicted |
| Carbapenems | — | No resistance determinant assigned | No resistance | Imipenem, meropenem and ertapenem resistance not predicted |
| Tetracyclines | — | No resistance determinant assigned | No resistance | Tetracycline, doxycycline and minocycline resistance not predicted |
| Macrolides | — | No resistance determinant assigned | No resistance | Azithromycin and erythromycin resistance not predicted |
| **Overall WGS-AMR profile** | — | **rpsE T24P was the only determinant linked by the analysis to a resistant phenotype** | **Spectinomycin resistance predicted** | Genome otherwise lacked a ResFinder-predicted resistant phenotype for the major antimicrobial classes examined |

### 3.2. CRISPR profile

Table 3 shows the Neisseria gonorrhoeae WHO_A_2024 (NZ_CP145090.1) chromosome was submitted to CRISPR CasFinder, and four putative CRISPR arrays and one Cas-associated cluster were identified. One spacer was found in each of the predicted CRISPR arrays, and they were all rated as level 1, low-confidence CRISPR. The four arrays were located at genomic positions 51,314–51,413, 1,576,470–1,576,584, 2,028,893–2,028,992, and 2,187,104–2,187,203 bp, respectively. The repeat consensus sequences were

compared, and arrays 1, 3, and 4 have close repeats, while array 2 has a different repeat, indicating that the repeat sequences are not completely homogeneous.

Moreover, a Cas-associated region was identified ranging from 159,160 bp to 991,567 bp and classified as General-Class1. Four predicted Cas-gene annotations were found in this region: two Cas3 annotations and Cas4 and Cas7. In general, the genome contained a small number of CRISPR spacers, with just four located in the four arrays predicted. All the arrays were assigned evidence level 1, and the Cas-associated region was not classified beyond the general Class 1 level, so these results were conservatively interpreted as possible CRISPR-like loci and Cas components of Class 1. The computational predictions do not confirm the existence of a complete, subtype-resolved or functionally active CRISPR–Cas system in WHO_A_2024.

**Table 3. Genomic characteristics of putative CRISPR arrays and Cas-associated genes identified in *Neisseria gonorrhoeae* WHO_A_2024**

| Element | CRISPR ID/classification | Genomic coordinates (bp) | Spacers/genes | Repeat consensus / Cas genes | Evidence level |
|---|---|---|---|---|---|
| CRISPR array 1 | NZ_CP145090.1_1 | 51,314–51,413 | 1 spacer | GATTCCCGCCTGTGCGGGAATGACGG | 1 |
| Cas cluster | General-Class1 | 159,160–991,567 | 4 genes | *cas3* ×2, *cas4*, *cas7* | — |
| CRISPR array 2 | NZ_CP145090.1_2 | 1,576,470–1,576,584 | 1 spacer | CGGCTTGTTGCCCTCTCTCTAACTCTCTCCC | 1 |
| CRISPR array 3 | NZ_CP145090.1_3 | 2,028,893–2,028,992 | 1 spacer | GATTCCCGCCTGCGCGGGAATGACGG | 1 |
| CRISPR array 4 | NZ_CP145090.1_4 | 2,187,104–2,187,203 | 1 spacer | GGATTCCCACTTTCGCGGGAATGACG | 1 |
| **Overall profile** | **NZ_CP145090.1** | **2,308,468-bp chromosome** | **4 CRISPR-like loci; 4 spacers; 1 Cas cluster** | **General-Class1-associated Cas genes** | **All arrays: 1** |

### 3.3. Molecular interaction of quercetin with PBP2.

The molecular docking analysis was performed to assess the predicted interactions between quercetin (PubChem CID: 5280343) and penicillin-binding protein 2 (PBP2; PDB ID: 6P54), which is found in the penA locus analysed during the genomic analysis of Neisseria gonorrhoeae WHO_A_2024. Quercetin is bound to the PBP2 pocket in a well-defined manner, with a predicted binding energy of −6.707 kcal mol−1. The intermolecular interactions present in the selected docking pose were analysed and showed a wide variety of conventional hydrogen bonds, van der Waals contacts, and π-mediated interactions with the aromatic scaffold of quercetin. The hydroxyl groups of quercetin allowed multiple possible interactions with neighboring amino acid residues, and the aromatic rings in the molecule that were conjugated allowed additional π–cation and π–alkyl interactions in the binding site. An unfavourable contact was also noticed, indicating that the proposed complex was not completely sterically or electrostatically complementary. In general, the results from the docking studies suggest that quercetin is able to fit into the PBP2-ligand binding pocket in a computationally favourable way and can form multiple stabilizing interactions with the residues surrounding the binding pocket. However, the binding affinity predicted to be −6.707 kcal mol−1 is a computational estimation of the binding favourability, which cannot be considered an independent criterion of high PBP2 binding affinity or antibacterial activity. Predicted interactions are therefore validated by experimental binding and functional assays to assess the biological relevance, as shown in Figure 1.

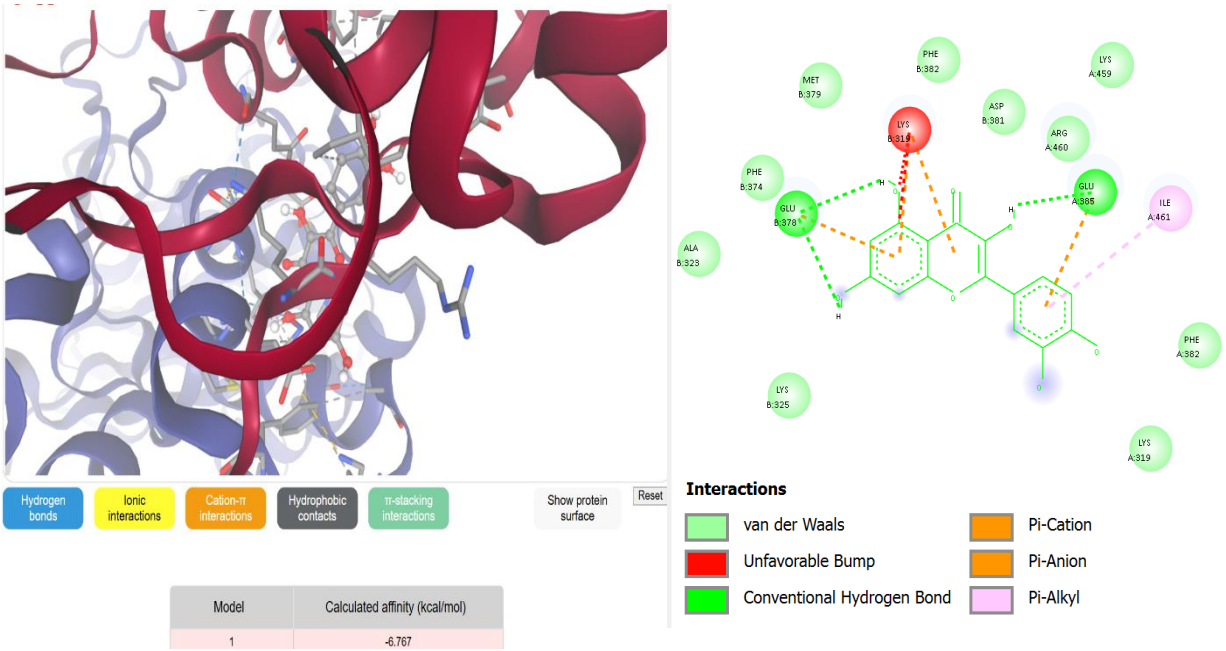



**Figure 1. Molecular docking and interaction profile of quercetin with penicillin-binding protein 2 (PBP2) of *Neisseria gonorrhoeae* WHO_A_2024.**

### 3.4. The ADMET profile of quercetin

**Table** 4 and Figure 2 show the in silico ADMET analysis of quercetin (PubChem CID: 5280343), which showed a moderate LogP (1.99), a relatively high topological polar surface area (TPSA = 131.36 $Å^2$), and a molecular weight of 302.24 Da. Quercetin has a considerable hydrogen-bond-accepting and donating ability with seven hydrogen-bond acceptors and five hydrogen-bond donors. However, it is somewhat polar, which may restrict passive membrane diffusion. The quantitative evaluation of drug-likeness (QED = 0.43) revealed moderate overall drug-like properties.

The predicted absorption profile indicated that the drug is highly likely to be absorbed by humans in the intestine with a high probability of human intestinal absorption (1.00), while the oral bioavailability was relatively low (0.37). Quercetin also had a predicted aqueous solubility of −3.09 log mol $L^{-1}$, and predicted an effective permeability of −5.43 log($10^{-6}$ cm $s^{-1}$) across the Caco-2 cell monolayer. These results indicate that there may be a permeation/bioavailability limitation even with the good intestinal absorption prediction. The potential for inhibition of P-glycoprotein was fairly low (0.22).

The distribution parameters of quercetin showed a very low predicted probability of blood–brain barrier penetration (0.05) and relatively high plasma protein binding (88.13%), with a predicted steady-state volume of distribution of 1.79 L $kg^{-1}$. LIMITED CENTRAL NERVOUS SYSTEM PENETRATION AND SIGNIFICANT PLASMA PROTEIN ASSOCIATION are suggested by this profile.

The major predicted cytochrome P450 interaction was inhibition of CYP1A2 (0.88), followed by CYP3A4 inhibition (0.66). Lower inhibition probabilities were predicted for CYP2C19 (0.24), CYP2C9 (0.31), and CYP2D6 (0.22). The probabilities of quercetin being a CYP2C9 and CYP2D6 substrate were low (0.05 for both), while the probability of being a CYP3A4 substrate was intermediate (0.49). The results suggest enzyme inhibition and/or substrate activity that might pose a metabolic interaction risk, such as with CYP1A2 and CYP3A4, although they are not experimental evidence of enzyme inhibition or substrate activity.

The hepatocyte clearance was predicted to be 75.31 µL $min^{-1}$ per $10^6$ cells, the microsomal clearance was predicted to be 0.08 µL $min^{-1}$ $mg^{-1}$, and the half-life was predicted to be 10.03 h. The predicted clinical toxicity probabilities (0.03) and carcinogenicity probabilities (0.03) were relatively low, using toxicity modeling. By contrast, higher model outputs were found with models for drug-induced liver injury (0.93) and skin reaction (0.89), and intermediate model outputs were derived from models for mutagenicity (0.56) and hERG blockade (0.45). The predictions for toxicity should be read as alerts to experiment with the calculation and not as proof of toxicity.

Overall, the predicted ADMET profile of quercetin suggests moderate drug-like properties and good predicted intestinal absorption, but also presents some potential limitations such as high polarity, low cellular permeability, moderate predicted oral bioavailability, high plasma protein binding, potential CYP1A2/CYP3A4 interactions, and selected toxicity alerts. These results, combined with the molecular docking results, provide a rationale for further evaluation of quercetin as an in silico PBP2-binding compound and highlight the importance of experimental studies regarding the antibacterial activity, pharmacokinetics, target engagement, and safety of quercetin.

**Table 4. Predicted physicochemical and ADMET properties of quercetin (PubChem CID 5280343)**

| Category | Parameter | Predicted value | Description / interpretation |
|---|---|---|---|
| **Physicochemical** | Molecular weight | **302.24 Da** | Moderate molecular size within conventional small-molecule space |
| | LogP | **1.99** | Moderate lipophilicity |
| | H-bond acceptors | **7** | Multiple potential hydrogen-bond acceptor sites |
| | H-bond donors | **5** | High hydrogen-bonding capacity |
| | TPSA | **131.36 Å²** | High polarity that may limit passive membrane permeability |
| | QED | **0.43** | Moderate predicted drug-likeness |
| **Absorption** | Human intestinal absorption | **1.00** | High predicted intestinal absorption |
| | Oral bioavailability | **0.37** | Limited-to-moderate predicted oral bioavailability |
| | Aqueous solubility | **−3.09 log(mol/L)** | Limited predicted aqueous solubility |
| | Caco-2 permeability | **−5.43 log($10^{-6}$ cm/s)** | Relatively limited predicted cellular permeability |
| | PAMPA permeability | **0.55** | Intermediate predicted passive permeability |
| | P-glycoprotein inhibition | **0.22** | Low predicted probability of P-gp inhibition |
| **Distribution** | BBB penetration | **0.05** | Very low predicted blood–brain barrier penetration |
| | Plasma protein binding | **88.13%** | Substantial predicted plasma protein binding |
| | Volume of distribution | **1.79 L/kg** | Appreciable predicted tissue distribution |
| **Metabolism** | CYP1A2 inhibition | **0.88** | High predicted inhibition probability |
| | CYP2C19 inhibition | **0.24** | Low predicted inhibition probability |
| | CYP2C9 inhibition | **0.31** | Low-to-moderate predicted inhibition probability |
| | CYP2D6 inhibition | **0.22** | Low predicted inhibition probability |
| | CYP3A4 inhibition | **0.66** | Moderate-to-high predicted inhibition probability |
| | CYP2C9 substrate | **0.05** | Low predicted substrate probability |
| | CYP2D6 substrate | **0.05** | Low predicted substrate probability |

| | | | |
|---|---|---|---|
| | CYP3A4 substrate | **0.49** | Intermediate predicted substrate probability |
| **Excretion** | Half-life | **10.03 h** | Moderate predicted elimination half-life |
| | Hepatocyte clearance | **75.31 µL/min/10⁶ cells** | Relatively high predicted hepatocyte clearance |
| | Microsomal clearance | **0.08 µL/min/mg** | Low predicted microsomal clearance |
| **Toxicity** | hERG blocking | **0.45** | Intermediate predicted hERG-blocking probability |
| | Clinical toxicity | **0.03** | Low predicted clinical-toxicity probability |
| | Mutagenicity | **0.56** | Intermediate predicted mutagenicity signal |
| | Drug-induced liver injury | **0.93** | High predicted DILI probability |
| | Carcinogenicity | **0.03** | Low predicted carcinogenicity probability |
| | Skin reaction | **0.89** | High predicted skin-reaction signal |
| **Overall profile** | ADMET assessment | — | Moderate physicochemical drug-likeness with predicted intestinal absorption, but limited permeability/bioavailability and notable CYP1A2, DILI, and skin-reaction alerts requiring experimental validation |

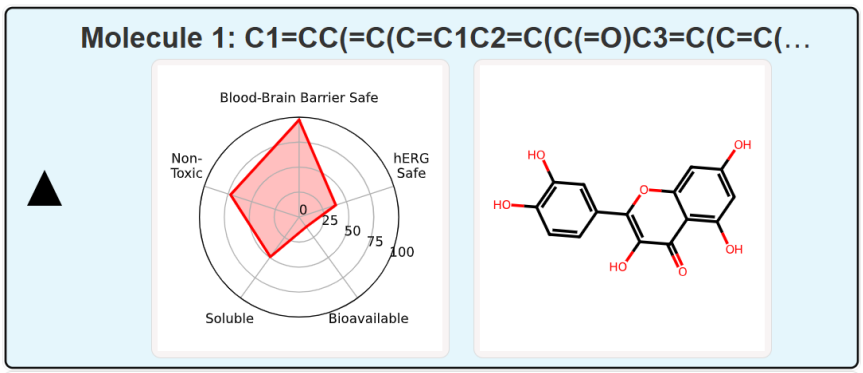



**Figure 2. Chemical structure and predicted ADMET profile of quercetin (PubChem CID: 5280343).**

## 4. Discussion

The Neisseria gonorrhoeae (NG) WHO_A_2024 was identified by whole-genome analysis and matched with ST10316 with 100% sequence identity and coverage for all seven of the loci (abcZ, adk, aroE, fumC, gdh, pdhC, and pgm). MLST has been developed as a standardized and repeatable method to define the genetic background of any N. gonorrhoeae isolate and to explore the population structure and relationships of N. gonorrhoeae. Gonococcal genomes do have a lot of recombination, however, and an MLST sequence type does not provide the whole story on the background of the genome and should not be viewed as a full indicator of genome characteristics [22]. It should therefore be understood that WHO_A_2024 has been assigned, and not as a representation of an antimicrobial resistance phenotype.

The results of the antimicrobial resistance analysis gave some functional information about the genomic characteristics of WHO_A_2024. Of the determinants found, rpsE T24P was the only one specifically linked to a predicted resistant phenotype, which was low-level spectinomycin resistance. This forecast is biologically realistic and would be consistent with past experimental results. Ilina et al. showed that association of the Thr24Pro substitution in the ribosomal protein S5 (rpsE) with spectinomycin resistance occurs in N. gonorrhoeae. Additionally, the substitution in the mutant rpsE allele was found to be compatible with the recipient gonococcal strains, and the transfer of the mutant rpsE gene was shown to confer the observed resistance pattern [23]. The finding of the same T24P substitution in WHO_A_2024 is thus supportive of the expected low-level spectinomycin resistance.

A total of 11 other sequence variants were found in loci commonly associated with gonococcal antimicrobial resistance: gyrA I250M; parC V384I, V421A, V435A, V596I and V646A; pilQ S341N and N648S; and porB G213D, T215A, G255Q, A256T and M257I. However, none of these substitutions were given a resistance phenotype by ResFinder/PointFinder. Likewise, in the present case, multiple copies of the 23S rRNA, the macAB promoter region, and the mtrC promoter region were found, but with no resistance prediction. This is important, as a change in the sequence at an AMR locus does not necessarily indicate a change that confers resistance. Numerous mutations and regulatory changes are associated with gonococcal resistance; either multiple determinants for resistance are required, or the interactions between them and the overall genomic environment contribute to resistance [3]. Variants with confirmed genotype–phenotype association should not be considered AMR determinants, but rather genomic polymorphisms of unknown resistance significance.

A consideration of this is especially significant for the fluoroquinolone-associated sites. DNA gyrase (encoded by gyrA) and topoisomerase IV (encoded by parC) are well-known targets of mutations in N. gonorrhoeae that cause fluoroquinolone resistance [5]. The substitutions found in WHO_A_2024, however, were not defined as resistance-conferring in the genomic prediction system used. Therefore, their detection does not warrant the ciprofloxacin-resistant phenotype. The discovery underscores the need to consider that an AMR-associated gene may contain sequence variation, but that a validated resistance-conferring mutation is not necessarily associated with it.

The findings for β-lactams are similar. However, an acquired penA hit was identified with 88.74% nucleotide identity, but no specific β-lactam-resistant phenotype was assigned. PenA is the gene that encodes an essential transpeptidase, PBP2, which is involved in peptidoglycan biosynthesis and is a primary target of β-lactam antibiotics. Specific changes in PBP2, including mosaic penA alleles, and corresponding resistance-associated amino-acid substitutions, can lead to lower levels of acylation of PBP2 by the extended-spectrum cephalosporins

(ESCs) cefixime and ceftriaxone and may be associated with lower susceptibility to the ESCs [5]. Detection of penA sequence similarity or divergence, however, is not enough to imply cephalosporin resistance if there is not a validated resistance-associated configuration. This was corroborated by the absence of resistance prediction for ceftriaxone, cefixime, and cefotaxime.

In general, the spectrum of resistance predicted by WGS of WHO_A_2024 was relatively narrow, with a single phenotype of resistance against spectinomycin being explicitly assigned. For the commonly used classes of antimicrobials tested, no genomic resistance was predicted, such as fluoroquinolones, extended-spectrum cephalosporins, carbapenems, tetracyclines, and macrolides. This is significant as N. gonorrhoeae is a species that can gain antimicrobial resistance through chromosomal mutation, recombination, and horizontal acquisition of genes [4]. However, the "no resistance" designation must be taken with a grain of salt because the prediction of susceptibility is only as accurate as the resistance databases and knowledge of genotype–phenotype relationships.

This sort of integrated genomic research is best carried out within the boundaries of a WHO gonococcal reference genome. WHO gonococcal reference strains have been well characterized and developed for quality assurance, antimicrobial resistance monitoring, molecular epidemiology, and comparative genome studies. In past WHO panels, molecular characterization of resistance determinants and phenotypic antimicrobial susceptibility testing have been both used and interdependent, with both complements being used [24]. Thus, genomic characterization of WHO_A_2024 allows for the study of its molecular and resistance-associated characteristics, and phenotypic antimicrobial susceptibility testing continues to be crucial for determining clinically relevant resistance profiles.

One key constraint of the current analysis is that the antimicrobial resistance profile was computed solely based on genomic prediction and was not included in the present computational pipeline with experimental minimum inhibitory concentration (MIC) data. The predicted low level of spectinomycin resistance due to rpsE T24P should be phenotypically confirmed. Likewise, the other chromosomal variants that are not assigned resistance should not be considered as resistance determinants in the absence of experimental and/or epidemiological evidence. Additional genomic variants found in WHO_A_2024 could have biological significance if future investigations involve using standardized antimicrobial susceptibility testing in conjunction with comparative whole-genome analysis, which would allow for more powerful genotype–phenotype correlation and better understanding of their potential biological significance.

## 5. Conclusion

A genome and structure-based characterization of Neisseria gonorrhoeae WHO_A_2024 is presented. Strain typing via whole-genome analysis identified the strain as ST10316, and the profile of the predicted antimicrobial resistance was relatively limited, with the only determinant explicitly associated with a resistant phenotype being rpsE T24P (which is predicted to be associated with low-level spectinomycin resistance). Sequence variants found in known AMR-associated loci that are other than the identified ones above were not associated with the developed resistance phenotypes and should not be considered as resistance-conferring until validated by phenotype. There were four low-confidence CRISPR-like arrays identified and a General-Class1 Cas-associated region by the CRISPRCasFinder analysis, but these are not enough to conclude the presence of an entire or a functional CRISPR–Cas system. Based on the 3D structure, it was found that quercetin can bind to the investigated PBP2 pocket with a plausible binding

orientation, hydrogen-bond and van der Waals interactions, and a binding affinity of −6.707 kcal $mol^{-1}$. However, the above predicted interaction is not direct evidence of PBP2 inhibition or of antibacterial activity. Additionally, in silico ADMET properties showed moderate drug-like properties as well as some limitations in terms of membrane permeability, oral bioavailability, metabolic interactions, and some toxicity alerts. Together, these results provide a genomic mapping of the genes in the WHiB2 genome and suggest that the interaction of quercetin with PBP2 is a computational hypothesis that could be tested. These computational results need to be further validated phenotypically by measuring antimicrobial susceptibility, determining the antibacterial minimum inhibitory concentration, PBP2 inhibition and target-engagement assay, molecular dynamics simulation and experimental pharmacokinetic and toxicological studies to better understand the biological and potential therapeutic implications.

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