REVIEW ARTICLE

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Resistance genes of *neisseria gonorrhoeae* to cefixime and azithromycin

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ABSTRACT

Gonorrhea is the second most common sexually transmitted bacterial infection (STI), following Chlamydia. *Neisseria gonorrhoeae* resistant to antibiotics are increasing globally in the world. In recent years, many studies have reported reduced susceptibility of *N.gonorrhoeae* to almost all clinically useful antibiotics and also reported cases of multi-resistance. Resistance mechanisms for *N. gonorrhoeae* can occur through genetic and non-genetic changes. Resistance to cefixime and azithromycin as first-line antibiotics for monotherapy recommended by the World Health Organization (WHO) has been reported from several countries. Genetic changes were reported as the main cause of *N.gonorrhoeae* resistance to cefixime and azithromycin. Based on the WHO and the United States Centers for Disease Control and Prevention recommendations, countries are increasingly using a combination of cephalosporin and azithromycin for the treatment of gonorrhea. The aim of this review is to analyze genetic variation of *N.gonorrhoeae* resistance to cefixime and azithromycin. Articles published in English in the last 12 years (from 2010 to 2021) were retrieved from Science Direct, PubMed, Springerlink, Oxford and Nature using relevant searching terms. Mutants of cefixime-resistant *N.gonorrhoeae* are mediated by mosaic and non-mosaic *pen*A genes encoding penicillin binding protein 2. In addition, mutations in the repressor and promoter genes of *mtr*R were also found that caused overexpression of the microbial efflux pump. Meanwhile, *N. gonorrhoeae* resistance to azithromycin reportedly occurs through two strategies, namely overexpression of the efflux pump (mutation of the *mtr*R codon region) and decreased affinity for antibiotics (single base mutation in the 23S rRNA gene). With the limited choice of antibiotics for the management of *N.gonorrhoeae*, it is necessary to do regular surveillance for monitoring drug resistance. By understanding the mechanism of resistance, the use of these antibiotics can be rationally optimized.

Keywords : *Neisseria gonorrhoeae*, resistance, cefixime, azithromycin

Abbreviations:
mtrR = multiple transferable resistance repressor; PBP2 = Penicillin-binding protein 2; 23S rRNA = 23S ribosomal RNA

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INTRODUCTION

Treatment of gonorrhea in the late 1930s was started using sulfonamide antibiotics.[1] However, several years later clinical isolates were found to be resistant to sulfonamides resulting in decreased effectivity of these antibiotics.[2] Currently, five classes of antibiotics are used to treat *N.gonorrhoeae*, namely penicillins, cephalosporins, tetracyclines, fluoroquinolones, and macrolides.[1,3] The increasing resistance of *N.gonorrhoeae* to these antibiotics is reportedly associated with the accumulation of *N.gonorrhoeae* resistance genes.[4,5] Treatment failure persists even though the drug dose is increased.[6] Failure of this treatment can lead to increased spread of multiresistant *N. gonorrhoeae* and potentially serious complications in patients such as infertility, abortion and fetal death.[7,8]

Varied mechanisms of resistance in *N.gonorrhoeae* cause decreased sensitivity or increased resistance to antibiotics, including target modification of antibiotics, overexpression of the efflux pumps, decreased influx of antibiotics through changes in the permeability of the porin proteins and enzymatic destruction of antibiotics. Combinations of these mechanisms are most commonly found in *N. gonorrhoeae* resistant to cefixime and azithromycin, such as horizontal transfer of genetic material or spontaneous mutations that alter *N.gonorrhoeae* structural and non-structural genes.[9]

In this review paper, a total of 56 articles published in English in the last 12 years (from 2010 to 2021) were retrieved from Science Direct, PubMed, Springerlink, Oxford Academic and Nature databases using the following keywords: *Neisseria gonorrhoeae* AND mutation AND cefixime AND azithromycin AND sexually transmitted infections.

Initially, 192 articles were found to match the inclusion criteria, but in the end 136 articles were removed due to duplication, failure of access, and irrelevant topic (shown in Figure 1). Finally, the writing of this review was carried out using the 56 articles that met the inclusion criteria to be written into a full paper.

Neisseria gonorrhoeae pathogenesis

Neisseria gonorrhoeae is a Gram-negative diplococcus that is pathogenic in humans.

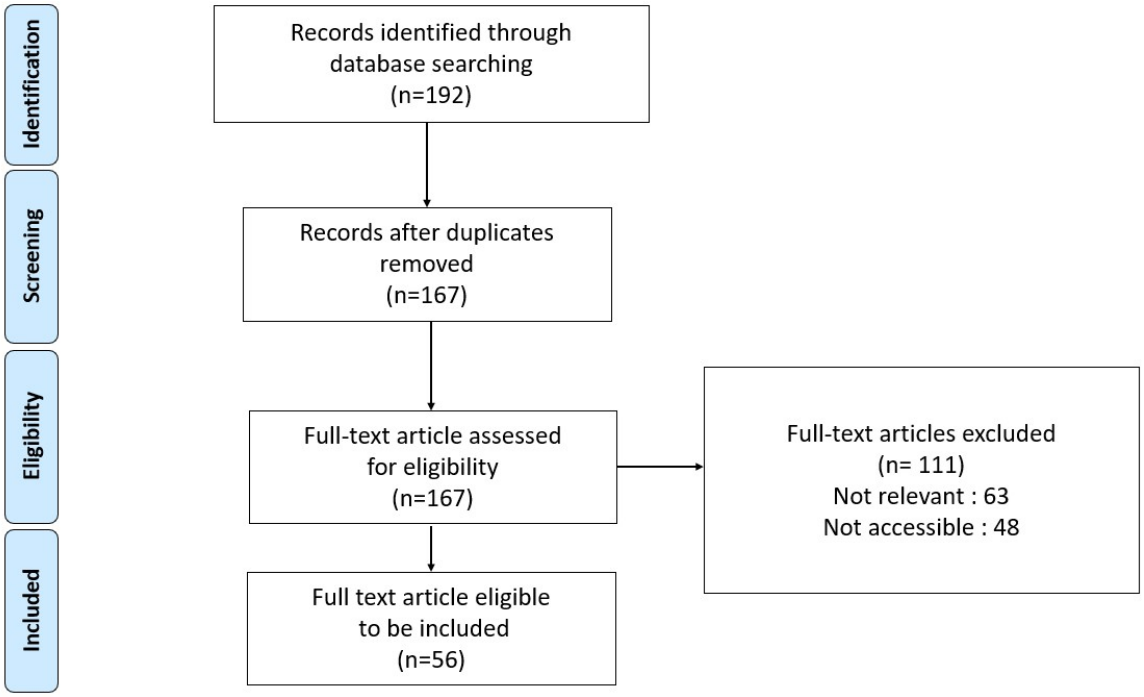

Figure 1. Schematic of literature study in journals using relevant keywords

Neisseria gonorrhoeae can live in a microaerophilic environment with low oxygen (2-10%) and high carbondioxide (5-10%) concentrations.[10-13] On direct microscopic examination of a specimen, a characteristic appearance of diplococci is found in polymorphonuclear leukocytes.[14]

Neisseria gonorrhoeae adheres to the mucous membranes of the genitourinary tract, eyes, rectum, and throat, producing an acute suppuration that can spread to the tissues.[15-17] Gonorrheal urethritis in men shows a clinical picture of yellowish pus and dysuria.[14,17] Complications can occur, including epididymitis, prostatitis, and periurethral abscess. Primary infections in women occur in endocervical and urethral columnar epithelial cells.[17] Gonorrheal infection in prepubertal children can occur in the vagina because of the immature mucosal epithelium. The atrophic vaginal mucosa of postmenopausal women can also be a site of *N.gonorrhoeae* infection. Clinical symptoms in women are generally a mucopurulent discharge from the vagina, dysuria and abdominal pain.[14,17,18]

Untreated infection can progress to chronic inflammation, including extension to tissues surrounding the site of infection or spread upward (ascending) into the fallopian tubes and cause salpingitis, fibrosis and tubal obliteration.[14] Infertility occurs in 20% of women with salpingitis.[7] Disseminated infection with septicemia and infection of the skin and joints occur in 1-3% of infected women.[14-16] Many symptoms are found, such as fever, pain on moving the joints, and skin lesions on the extremities, from hemorrhagic papules and pustules on the hands, feet, and legs to suppurative arthritis, usually in the knees (gonorrheal arthritis), ankles, and hands.[17] Endocarditis can also be caused by *N. gonorrhoeae* although it is rarely found.

Since 2016, the WHO has recommended a combination of a single dose of ceftriaxone 250 mg intramuscularly and a single dose of azithromycin 1 g orally OR cefixime 400 mg orally single dose and azithromycin 1 g orally single dose for empirical therapy of genital and anorectal gonorrhea. Single dose antibiotic therapy can be given (according to the sensitivity pattern) of a choice of ceftriaxone 250 mg intramuscular injection, OR cefixime 400 mg orally, OR spectinomycin 2 g intramuscular injection.[7]

Cefixime belongs to the class of β-lactam antibiotics and acts by inhibiting the peptidoglycan cross-link in the bacterial cell wall through binding to the β-lactam ring on penicillin binding protein 2 (PBP2).[2,19] Azithromycin belongs to the class of macrolide antibiotics that bind to the 50S ribosome and inhibit protein synthesis through elongation of the peptide chain.[20–25]

The mechanism of *N. gonorrhoeae* resistance to cefixime and azithromycin is influenced by mutations or transfer of *Neisseria* resistance genes (as shown in Table 1 and Figure 2), including *mtr*R mutations, single base mutations in the 23S rRNA gene, porB1B mutations and mosaic and non-mosaic alleles on PBP2 target proteins, efflux pump for antibiotics, and decreased antibiotic influx into cells.[1,3, 26, 29]

Cefixime resistance mechanism

Neisseria gonorrhoeae resistance to cefixime is mainly caused by mutations that modify PBP2 protein as an antibiotic target.[11,30–34] Changes in PBP2 target protein are most often caused by the presence of a mosaic gene consisting of 60-70 amino acid changes without Asp345a insertion in *N. gonorrhoeae* resistant to penicillin.[1,9,11,12,24] These mosaic genes are transferred through DNA transformation from commensal *Neisseria* in the oropharynx, because transfer of genetic material occurs during *N. gonorrhoeae* infection in the pharynx. These amino acid changes form varied patterns including patterns X, XXXIV, and XII, with the substitution of a single amino acid, for example: A501P and A501V as shown in Figure 3.[13,21,35-42]

Studies in the US and Canada reported that the *pen*A mosaic pattern XXXIV was most commonly found in clinical isolates of resistant *N. gonorrhoeae*. These results differ from those

Table 1. Resistance genes of *N. gonorrhoeae* to cefixime and azithromycin

NG Resistance Genes		Mechanism of resistance	Authors	Reference
Structural	Non mosaik *penA* XIII,		Bailey et al	1
	Mosaik *penA*, IX, X, XII, XIX, XXXIV		Berenger et al, Oree et al, Washington et al, Calado et al,Tanaka et al, Lourenço et al, Harris et al, Mahajan et al, Camara et al, Chen et al, Gianecini et al, Golparian et al, Jeverica et al, Yan et al, Xiu et al, Lewis et al, Wind et al, Lan et al, Thakur et al, Trembizki et al, Kueakulpattana et al,Thomas et al,Endimiani et al, Gose et al,Liang et al, Bonikowska1 et al, Ryan et al, Li et al, Pladevall et al, Sethi et al	2,7,10,11,12, 13,15,17,21,23,24,25,26,30,31,32,33,34,35,37,38,39,40,41,45,46,48,49,51,52,53,54
	23S rRNA A2059G	Modification of antibiotic targets	Berenger et al, Gernert et al, Reimche et al, Oree et al, Harris et al, Belkacem et al, Golparian et al, Jacobsson et al, Yan et al, Wind et al, Trembizki et al,Liang et al, Zhang et al	2,4,6,7,15,20,26,29,31,34,39,48, 55
	23S rRNA C2611T		Berenger et al, Gernert et al, Reimche et al, Oree et al, Harris et al, Belkacem et al, Golparian et al, Jacobsson et al, Yan et al, Wind et al, Trembizki et al,Liang et al, Zhang et al	2,4,6,7, 15,20, 26,29,31,34,39,48,55
	*por*B/*pen*B	Decreased antibiotic influx	Golparian et al, Jeverica et al, Yan et al, Lan et al, Thakur et al, Kueakulpattana et al,Endimiani et al, Olsen et al, Pladevall et al,	26,30,31,33,35,38,40,45,50,53
Non structural	*mtr*R repressor		Berenger et al, Gernert et al, Reimche et al, Lourenço et al, Belkacem et al, Gianecini et al, Golparian et al, Holderman et al, Jeverica et al, Yan et al, Lewis et al, Lan et al, Thakur et al, Kueakulpattana et al, Endimiani et al, Olsen et al, Ryan et al, Li et al, Pladevall et al, Sethi et al, Zhang et al	2,4,6, 13, 16,20,25, 26,27,30,31,33,34,35,38,40,45,50,51,52,53,54,55
	*mtr*R promotor	Overexpression of efflux pump	Bailey et al,Berenger et al, Gernert et al, Reimche et al, Calado et al, Lourenço et al, Harris et al, Mahajan et al, Belkacem et al, Camara et al, Gianecini et al, Golparian et al, Holderman et al, Jeverica et al, Yan et al, Lewis et al, Thakur et al, Kueakulpattana et al, Endimiani et al. Olsen et al, Li et al, Pladevall et al, Sethi et al, Zhang et al	1,2,4,6,11,13, 15, 17,20,21,25, 26,27,28,31,33,34,35,38,40,45,50,52,53,54, 55
	*erm*B, *erm*C	Enzymatic destruction	Berenger et al, Wind et al	2,34

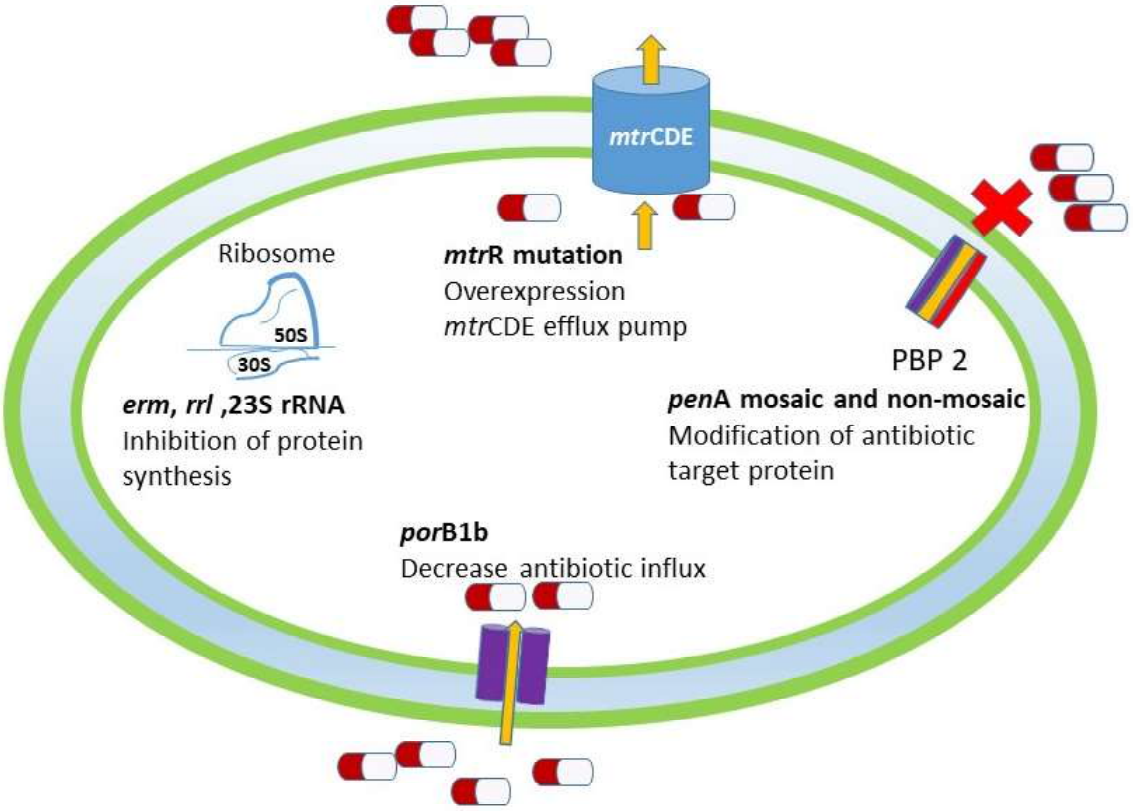

Figure 2. Mechanism of resistance of *N. gonorrhoeae* to cefixime and azithromycin

of a study conducted by Peng et al. [13] in China, reporting that pattern X predominated in *N. gonorrhoeae* resistance. A Korean study found that 16% of resistant *N. gonorrhoeae* isolates had *pen*A X patterns with high MIC values that will lead to treatment failure.[35] Non-mosaic *pen*A patterns, including patterns IV, V, XII, XVI, XVII, and XVIII, can also cause *N. gonorrhoeae* resistance such as those caused by *pen*A mosaic alleles including the PBP2 XIII allele which has five specific amino acid changes (A501V, F504L, A510V, A516G and P551S). This finding is supported by Lee et al.[35], in that non-mosaic PBP2 sequence pattern XIII is most commonly found in resistant *N. gonorrhoeae* isolates followed by patterns IV and V.

Several studies also reported that an increase in efflux of antibiotics caused *N.gonorrhoeae* resistance to β-lactams such as cefixime.[1,13] The presence of a missense mutation in the DNA binding domain in the codon region encoding the *mtr*R repressor causes mutations in the G45D helix-turn-helix domain.[35,36,46,48,53,60] This deletion causes frame shifts and premature stop codons to form an incomplete *mtr*R protein.[22,24,26,31] In addition to resistant *N. gonorrhoeae* strains with high MIC values, mutations are most often found in the form of a single base deletion at 13-bp inverted repeat sequences (IR) on the *mtr*R promoter.[32,51,53,60]

The outer membrane of *N. gonorrhoeae* acts as a permeability barrier to various components, including cefixime. The decreased influx of cefixime antibiotic that diffuses into the periplasmic space through a protein channel on the outer membrane, namely the porin protein, is one of the mechanisms of resistance of *N. gonorrhoeae*.[44,45,47,48,52,53] However, the PorB1a and PorB1b genes belonging to *N. gonorrhoeae* were reported to be more influential in the occurrence of *N. gonorrhoeae* resistance to penicillin and ceftriaxone antibiotics than in its resistance to cefixime.[10] A study in Korea reported that mutations in *por*B and *mtr*R contributed to increased MIC values in resistant *N. gonorrhoeae* isolates. The *pen*B resistance

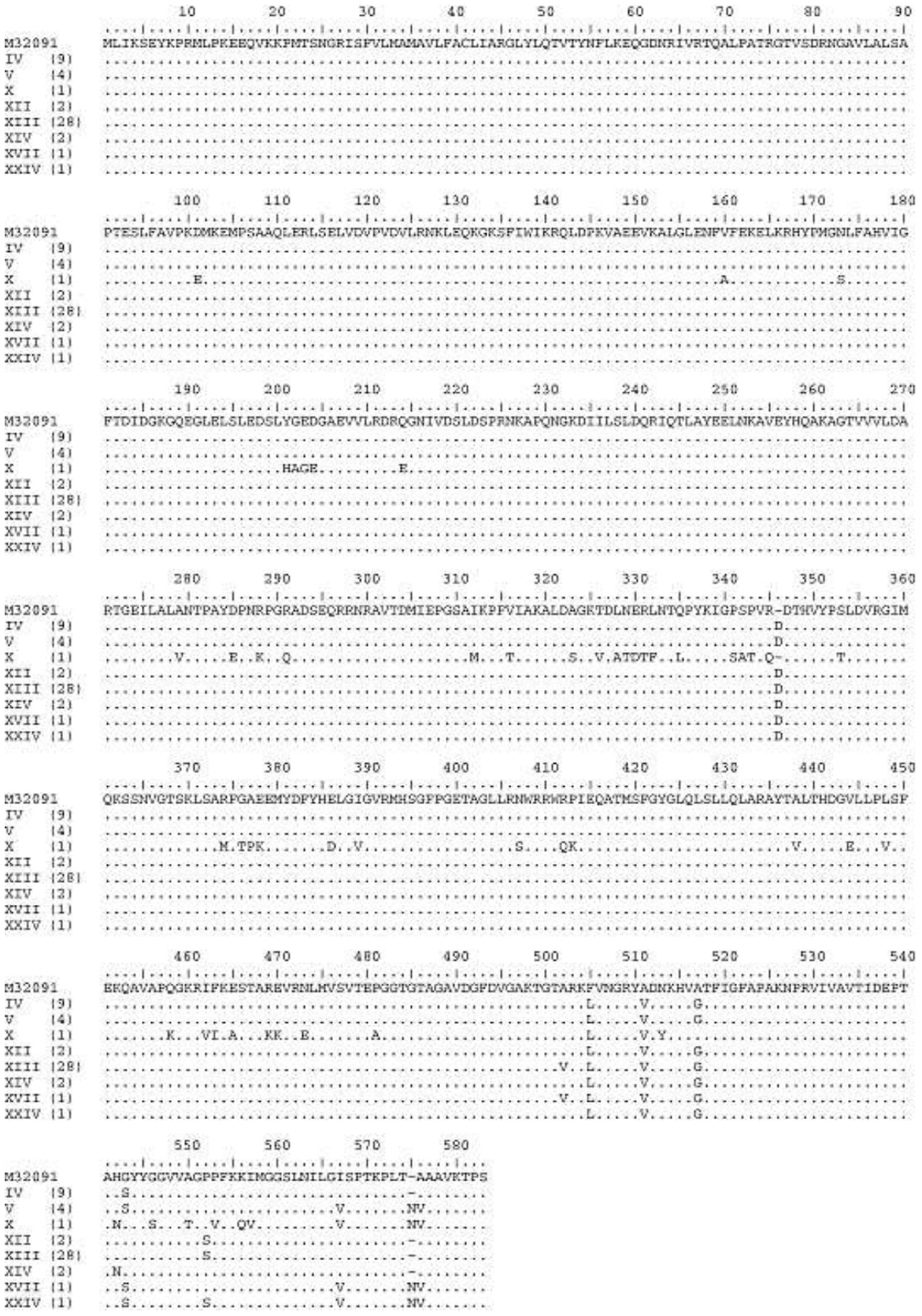

Figure 3. Amino acid alignment of penicillin binding protein 2 related to resistance of *N. gonorrhoeae* to cefixime.[35]

determinant causes amino acid changes at positions 120 and 121 (G120D/A121D), leading to a decrease in the entry of antibiotics. Interestingly, the effect of the *pen*B phenotype is only seen in the *N. gonorrhoeae* mutant strain that overexpresses the *mtr*CDE efflux pump.[54]

Azithromycin resistance mechanism

Many cases of macrolide resistance are associated with specific nucleotide changes in 23S rRNA in the 50S ribosomal subunit.[22,51,55,61] In general, bacterial resistance to macrolides can result from modification of ribosomal targets through modification of rRNA via the methylase enzyme of 23S rRNA or specific mutations in 23S rRNA itself and/or from the fact that the efflux pump system is overexpressed.[8,27,56] Modification of RNA via methylase enzymes is mediated by the *erm*B, *erm*C, and *erm*F genes from other bacteria, leading to modification of the antibiotic.[59,61]

The study conducted by Pham et al.[62] also reported that resistant *N. gonorrhoeae* strains showed single base mutations in the V domain of their 23S rRNA, namely mutations A2059G and C2611T.[30] Mutations at this position involved more than 1 of the 4 alleles of *Neisseria gonorrhoeae* (NG).[30,56,57] An enzymatic mechanism through rRNA methylase causing blocking of azithromycin binding to 23S rRNA at position 2058 as found in studies in Seattle and Uruguay was also reported as a mechanism of *N. gonorrhoeae* resistance to azithromycin.[30]

Another mechanism of azithromycin resistance involves mutations in *mtr*R leading to overexpression of the efflux pump. Mutations can occur in the *mtr*R promoter region in the form of adenine deletion or the mtrR A39T/G45D repressor as found in a study in Portugal. According to a study by Shigemura in Japan, deletion mutations in the *mtr*R promoter region can cause an increase in MIC values (>0.5 mcg/ml).[59] Mutations in the promoter region can also increase the binding of RNA polymerase or activator to the *mtr*C promoter due to decreased competition for binding to the same DNA region, thereby inducing higher resistance.[3]

CONCLUSION

Modification of target proteins is the main mechanism of *N. gonorrhoeae* resistance to cefixime. Meanwhile, *N. gonorrhoeae* resistance to azithromycin is more influenced by the overexpression of the efflux pump. By understanding these mechanisms, the WHO recommends giving cefixime and azithromycin for the treatment of gonorrhea which aims to reduce resistance rates.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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CONTRIBUTORS

LIU searched the journals. YR supervised the journals choice. LIU and YR analysed the result. LIU and YR wrote the draft of the report. All authors contributed to revisions and approved the final version.

REFERENCES

1. Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 2014;27:587–613. doi: 10.1128/CMR.00010-14

2. Sethi S, Golparian D, Bala M, et al. Antimicrobial susceptibility and genetic characteristics of *Neisseria gonorrhoeae* isolates from India, Pakistan and Bhutan in 2007-2011. BMC Infect Dis 2013;13:35. doi: 10.1186/1471-2334-13-35.
3. Unemo M, Shafer WM. Antibiotic resistance in *Neisseria gonorrhoeae*: origin, evolution, and lessons learned for the future. Ann N Y Acad Sci 2011;1230:E19-28. doi: 10.1111/j.1749-6632.2011.06215.x.
4. Bailey AL, Potter RF, Wallace MA, Johnson C, Dantas G, Burnham CAD. Genotypic and phenotypic characterization of antimicrobial resistance in *Neisseria gonorrhoeae*: a cross-sectional study of isolates recovered from routine urine cultures in a high-incidence setting. mSphere 2019;4:e00373-19. doi: 10.1128/mSphere.00373-19.
5. Berenger BM, Demczuk W, Gratrix J, Pabbaraju K, Smyczek P, Martin I. Genetic characterization and enhanced surveillance of ceftriaxone-resistant *Neisseria gonorrhoeae* strain, Alberta, Canada, 2018. Emerg Infect Dis 2019;25:1660-7. doi:10.3201/eid2509.190407
6. Gernert KM, Seby S, Schmerer MW, et al. Azithromycin susceptibility of *Neisseria gonorrhoeae* in the USA in 2017: a genomic analysis of surveillance data. Lancet Microbe 2020;1:e154-64. doi: 10.1016/S2666-5247(20)30059-8.
7. World Health Organization. WHO guidelines for the treatment of Neisseria gonorrhoeae. Geneva: World Health Organztion;2016.
8. Harris SR, Cole MJ, Spiteri G, et al. Public health surveillance of multidrug-resistant clones of *Neisseria gonorrhoeae* in Europe: a genomic survey. Lancet Infect Dis 2018;18:758–68. doi: 10.1016/S1473-3099(18)30225-1.
9. Ryan L, Golparian D, Fennelly N, et al. Antimicrobial resistance and molecular epidemiology using whole-genome sequencing of *Neisseria gonorrhoeae* in Ireland, 2014–2016: focus on extended-spectrum cephalosporins and azithromycin. Eur J Clin Microbiol Infect Dis 2018;37:1661–72. doi: 10.1007/s10096-018-3296-5.
10. Zheng Z, Liu L, Shen X, et al. Antimicrobial resistance and molecular characteristics among *Neisseria gonorrhoeae* clinical isolates in a Chinese tertiary hospital. Infect Drug Resist 2019; 12:3301-9. https://doi.org/10.2147/IDR.S221109.
11. Chen SC, Yin YP, Dai XQ, Unemo M, Chen XS. First nationwide study regarding ceftriaxone resistance and molecular epidemiology of *Neisseria gonorrhoeae* in China. J Antimicrob Chemother 2016;71:92-9. doi: 10.1093/jac/dkv321.
12. Demczuk W, Sidhu S, Unemo M, et al. *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance, a novel antimicrobial resistance multilocus typing scheme for tracking global dissemination of *N. gonorrhoeae* strains. J Clin Microbiol 2017;55:1454–68. doi: 10.1128/JCM.00100-17.
13. Peng JP, Yin YP, Chen SC, et al. A whole-genome sequencing analysis of *Neisseria gonorrhoeae* isolates in China: an observational Study. E Clinical Medicine 2019;7:47-54. doi: 10.1016/j.eclinm.2019.01.010.
14. Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology 7th ed. Philadelphia: Elsevier;2013.
15. Lee MH, Byun J, Jung M, et al. Disseminated gonococcal infection presenting as bacteremia and liver abscesses in a healthy adult. Infect Chemother 2015;47:60-3. doi: 10.3947/ic.2015.47.1.60.
16. Birrell JM, Gunathilake M, Singleton S, Williams S, Krause V. Characteristics and impact of disseminated gonococcal infection in the "Top End" of Australia. Am J Trop Med Hyg 2019; 101:753-60. doi: 10.4269/ajtmh.19-0288.
17. Brooks GF, Jawetz E, Melnick JL, et al. Jawetz, Melnick & Adelberg's medical microbiology. 26th ed. New York: McGraw-Hill Inc; 2013.
18. Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan Kementerian Kesehatan Republik Indonesia. Pedoman Nasional Penanganan Infeksi Menular Seksual. Jakarta: Kementerian Kesehatan Republik Indonesia;2016.
19. Shaskolskiy B, Dementieva E, Kandinov I, et al. Genetic diversity of *Neisseria gonorrhoeae* multi-antigen sequence types in Russia and Europe. Int J Infect Dis 2020;93:1–8. doi: 10.1016/j.ijid.2020.01.020.
20. Brunner A, Nemes-Nikodem E, Mihalik N, Marschalko M, Karpati S, Ostorhazi S. Incidence and antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from patients attending the national *Neisseria gonorrhoeae* reference laboratory of Hungary. BMC Infect Dis 2014;14:4-11. https://doi.org/10.1186/1471-2334-14-433
21. Tanaka M, Furuya R, Kobayashi I, Kanesaka I, Ohno A, Kanayama AK. Antimicrobial resistance and molecular characterisation of *Neisseria gonorrhoeae* isolates in Fukuoka, Japan, 1996–2016. J Glob Antimicrob Resist 2019;17:3–7. https://doi.org/10.1016/j.jgar.2018.11.011.
22. Belkacem A, Jacquier H, Goubard A, et al. Molecular epidemiology and mechanisms of resistance of azithromycin-resistant *Neisseria gonorrhoeae* isolated in France during 2013-14. J Antimicrob Chemother 2016;71:2471–8. doi: 10.1093/jac/dkw182.

23. Jacobsson S, Cole MJ, Spiteri G, Day M, Unemo M. Associations between antimicrobial susceptibility/resistance of *Neisseria gonorrhoeae* isolates in European Union/ European Economic Area and patients' gender, sexual orientation and anatomical site of infection, 2009–2016. BMC Infect Dis 2021;21:273. https://doi.org/10.1186/s12879-021-05931-0.
24. Lewis DA, Sriruttan C, Müller EE, et al. Phenotypic and genetic characterization of the first two cases of extended-spectrum-cephalosporin-resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. J Antimicrob Chemother 2013;68:1267–70. doi: 10.1093/jac/dkt034.
25. Sánchez-Busó L, Yeats CA, Taylor B, et al. A community-driven resource for genomic epidemiology and antimicrobial resistance prediction of *Neisseria gonorrhoeae* at Pathogenwatch. Genome Med 2021;13:1–22. doi: 10.1186/s13073-021-00858-2.
26. Kueakulpattana N, Wannigama DL, Luk-in S, et al. Multidrug-resistant *Neisseria gonorrhoeae* infection in heterosexual men with reduced susceptibility to ceftriaxone, first report in Thailand. Sci Rep 2021;11:1–16. doi :10.1038/s41598-021-00675-y.
27. Oree G, Naicker M, Maise H, Abbai NS. Comparison of endocervical swabs to cultured isolates for the detection of antimicrobial resistance determinants in *Neisseria gonorrhoeae*. J Med Lab Sci Technol South Africa 2022;3:40–6.
28. Qin X, Zhao Y, Chen W, et al. Changing antimicrobial susceptibility and molecular characterisation of *Neisseria gonorrhoeae* isolates in Guangdong, China: in a background of rapidly raising epidemic. Int J Antimicrob Agents 2019;54:757–65. https://doi.org/10.1016/j.ijantimicag.2019.08.015.
29. Holderman JL, Thomas JC, Schlanger K, et al. Sustained transmission of *Neisseria gonorrhoeae* with high-level resistance to azithromycin, in Indianapolis, Indiana, 2017-2018. Clin Infect Dis 2021;73:808–15. doi: 10.1093/cid/ciab132.
30. Kovari H, de Melo Oliveira MDG, Hauser P, et al. Decreased susceptibility of *Neisseria gonorrhoeae* isolates from Switzerland to cefixime and ceftriaxone: antimicrobial susceptibility data from 1990 and 2000 to 2012. BMC Infect Dis 2013;13:603. https://doi.org/10.1186/1471-2334-13-603
31. Karim S, Bouchikhi C, Banani A, et al. Molecular antimicrobial resistance of *Neisseria gonorrhoeae* in a Moroccan area. Infect Dis Obstet Gynecol 2018;7263849. doi: 10.1155/2018/7263849.
32. Day MJ, Jacobsson S, Spiteri G. et al. Significant increase in azithromycin "resistance" and susceptibility to ceftriaxone and cefixime in *Neisseria gonorrhoeae* isolates in 26 European countries, 2019. BMC Infect Dis 2022;22:524. https://doi.org/10.1186/s12879-022-07509-w.
33. Liang JY, Cao WL, Li XD, et al. Azithromycin-resistant *Neisseria gonorrhoeae* isolates in Guangzhou, China (2009-2013): Coevolution with decreased susceptibilities to ceftriaxone and genetic characteristics. BMC Infect Dis 2016;16:152. http://dx.doi.org/10.1186/s12879-016-1469-3.
34. Jiang FX, Lan Q, Le WJ, Su XH. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from Hefei (2014-2015): genetic characteristics of antimicrobial resistance. BMC Infect Dis 2017; 17:366. doi: 10.1186/s12879-017-2472-z.
35. Lee H, Unemo M, Kim HJ, Seo Y, Lee K, Chong Y. Emergence of decreased susceptibility and resistance to extended spectrum cephalosporins in *Neisseria gonorrhoeae* in Korea. J Antimicrob Chemother 2015;70:2536–42. doi: 10.1093/jac/dkv146. Calado J, Castro R, Lopes Â, Campos MJ, Rocha M, Pereira F. Antimicrobial resistance and molecular characteristics of *Neisseria gonorrhoeae* isolates from men who have sex with men. Int J Infect Dis 2019;79:116–22. https://doi.org/10.1016/j.ijid.2018.10.030.
36. Thomas JC, Seby S, Abrams AJ,et al. Evidence of recent genomic evolution in gonococcal strains with decreased susceptibility to cephalosporins or azithromycin in the United States, 2014-2016. J Infect Dis 2019;220:294-305. doi: 10.1093/infdis/jiz079.
37. Xiu L, Yuan Q, Li Y, Zhang C, Yang L, Peng J. Emergence of ceftriaxone-resistant *Neisseria gonorrhoeae* strains harbouring a novel mosaic penA gene in China. J Antimicrob Chemother 2020;75:907–10. doi: 10.1093/jac/dkz530.
38. Pham TL, Golparian D, Ringlander J, Le VH, Nguyen VT, Unemo M. Genomic analysis and antimicrobial resistance of *Neisseria gonorrhoeae* isolates from Vietnam in 2011 and 2015-16. J Antimicrob Chemother 2021;75:1432–8. doi 10.1093/jac/dkaa040.
39. Thomas JC, Joseph SJ, Cartee JC, et al. Phylogenomic analysis reveals persistence of gonococcal strains with reduced-susceptibility to extended-spectrum cephalosporins and mosaic penA-34. Nat Commun 2021;12. https://doi.org/10.1038/s41467-021-24072-1

40. Gose S, Nguyen D, Lowenberg D, Samuel M, Bauer H, Pandori M. *Neisseria gonorrhoeae* and extended-spectrum cephalosporins in California: Surveillance and molecular detection of mosaic *penA*. BMC Infect Dis 2013;13:570. doi: 10.1186/1471-2334-13-570.
41. Mlynarczyk-Bonikowska B, Serwin AB, Golparian D, et al. Antimicrobial susceptibility/resistance and genetic characteristics of *Neisseria gonorrhoeae* isolates from Poland, 2010-2012. BMC Infect Dis 2014;14:1–7.doi 10.1186/1471-2334-14-65
42. Gianecini RA, Golparian D, Zittermann S, et al. Genome-based epidemiology and antimicrobial resistance determinants of *Neisseria gonorrhoeae* isolates with decreased susceptibility and resistance to extended-spectrum cephalosporins in Argentina in 2011-16. J Antimicrob Chemother 2019;74:1551–9. doi https://doi.org/10.1111/apm.13060
43. Jeverica S, Golparian D, Matièiè M, Potoènik M, Mlakar B, Unemo M. Phenotypic and molecular characterization of *Neisseria gonorrhoeae* isolates from Slovenia, 2006-12: rise and fall of the multidrug-resistant NG-MAST genogroup 1407 clone? J Antimicrob Chemother 2014;69: 1517–25. doi: 10.1093/jac/dku026.
44. Yan J, Xue J, Chen Y, Chen S, et al. Increasing prevalence of *Neisseria gonorrhoeae* with decreased susceptibility to ceftriaxone and resistance to azithromycin in Hangzhou, China (2015-17). J Antimicrob Chemother 2019;74:29–37. doi: 10.1093/jac/dky412.
45. Thakur SD, Levett PN, Horsman GB, Dillon JA. Association of *Neisseria gonorrhoeae* genogroups and specific PBP2/MtrR/PorB mutation patterns with susceptibility to penicillin in a susceptible gonococcal population. J Antimicrob Chemother 2018;73:2682–6. doi: 10.1093/jac/dky233.
46. Endimiani A, Guilarte YN, Tinguely R, et al. Characterization of *Neisseria gonorrhoeae* isolates detected in Switzerland (1998-2012): Emergence of multidrug-resistant clones less susceptible to cephalosporins. BMC Infect Dis 2014;14. doi: 10.1186/1471-2334-14-106.
47. Serra-Pladevall J, Barberá MJ, Rodriguez S, et al. *Neisseria gonorrhoeae* antimicrobial susceptibility in Barcelona: *penA, ponA, mtrR,* and *porB* mutations and NG-MAST sequence types associated with decreased susceptibility to cephalosporins. Eur J Clin Microbiol Infect Dis 2016;35:1549–56. http://dx.doi.org/10.1007/s10096-016-2696-7.
48. Zhang C, Wang F, Zhu C, et al. Determining antimicrobial resistance profiles and identifying novel mutations of *Neisseria gonorrhoeae* genomes obtained by multiplexed MinION sequencing. Sci China Life Sci 2020;63:1063–70. doi: 10.1007/s11427-019-1558-8.
49. Reimche JL, Chivukula VL, Schmerer MW, et al. Genomic analysis of the predominant strains and antimicrobial resistance determinants within 1479 *Neisseria gonorrhoeae* isolates from the US Gonococcal Isolate Surveillance Project in 2018. Sex Transm Dis 2021;48:S78–87. doi: 10.1097/OLQ.0000000000001471.
50. da Costa-Lourenço APR, Abrams AJ, dos Santos KTB, et al. Phylogeny and antimicrobial resistance in *Neisseria gonorrhoeae* isolates from Rio de Janeiro, Brazil. Infect Genet Evol 2018;58:157–63. https://doi.org/10.1016/j.meegid.2017.12.003.
51. Golparian D, Bazzo ML, Golfetto L, et al. Genomic epidemiology of *Neisseria gonorrhoeae* elucidating the gonococcal antimicrobial resistance and lineages/sublineages across Brazil, 2015–16. J Antimicrob Chemother 2020;75:3163–72. doi: 10.1093/jac/dkaa318.
52. Thakur SD, Starnino S, Horsman GB, Levett PN, Dillon JR. Unique combined *penA/mtrR/porB* mutations and NG-MAST strain types associated with ceftriaxone and cefixime MIC increases in a "susceptible" *Neisseria gonorrhoeae* population. J Antimicrob Chemother 2014;69: 1510–6. doi: 10.1093/jac/dkt543.
53. Lee SG, Lee H, Jeong SH, Yong D, Chung GT, Lee YS, et al. Various penA mutations together with mtrR, porB and ponA mutations in Neisseria gonorrhoeae isolates with reduced susceptibility to cefixime or ceftriaxone. J Antimicrob Chemother 2010;65:669-75.
54. Wind CM, De Vries E, Schim Van Der Loeff MF, et al. Decreased azithromycin susceptibility of *Neisseria gonorrhoeae* isolates in patients recently treated with azithromycin. Clin Infect Dis 2017;65:37–45. doi: 10.1093/cid/cix249.
55. Pham CD, Nash E, Liu H, et al. Atypical mutation in *Neisseria gonorrhoeae* 23S rRNA associated with high-level azithromycin resistance. Antimicrob Agents Chemother 2021;65:1–5. doi: 10.1128/AAC.00885-20.
56. Johnson SR, Grad Y, Abrams AJ, Pettus K, Trees DL. Use of whole-genome sequencing data to analyze 23S rRNA-mediated azithromycin resistance. Int J Antimicrob Agents 2017;49:252-4. doi:10.1016/j.ijantimicag.2016.10.023.
57. Derbie A, Mekonnen D, Woldeamanuel Y, Abebe T. Azithromycin resistant gonococci: A literature review. Antimicrob Resist Infect Control 2020;9: 138. doi: 10.1186/s13756-020-00805-7.
58. Shigemura K, Osawa K, Miura M, et al. Azithromycin resistance and its mechanism in

Neisseria gonorrhoeae strains in Hyogo, Japan. Antimicrob Agents Chemother 2015;59:2695–9. doi: 10.1128/AAC.04320-14.

59. Handing JW, Ragland SA, Bharathan UV, Criss AK. The MtrCDE efflux pump contributes to survival of *Neisseria gonorrhoeae* from human neutrophils and their antimicrobial components. Front Microbiol 2018;9:2688. doi:10.3389/fmicb.2018.02688.
60. Chen SC, Yin YP, Dai XQ, Unemo M, Chen XS. Antimicrobial resistance, genetic resistance determinants for ceftriaxone and molecular epidemiology of *Neisseria gonorrhoeae* isolates in Nanjing, China. J Antimicrob Chemother 2014; 69:2959–65. doi: 10.1093/jac/dku245.
61. Pham CD, Nash E, Liu H, et al. Atypical mutation in Neisseria gonorrhoeae 23S rRNA associated with high-level azithromycin resistance. Antimicrob Agents Chemother 2021;65:e00885-20. doi: 10.1128/AAC.00885-20.