

REVIEW ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

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 *penA* genes encoding penicillin binding protein 2. In addition, mutations in the repressor and promoter genes of *mtrR* 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 *mtrR* 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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Date of first submission, July 24, 2022

Date of final revised submission,
February 9, 2023

Date of acceptance, February 21, 2023

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Cite this article as: Utami LI, Rosana Y. Resistance genes of *neisseria gonorrhoeae* to cefixime and azithromycin. Univ Med 2023;42:108-118. doi: 10.18051/UnivMed. 2023.v42:108-118.



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.

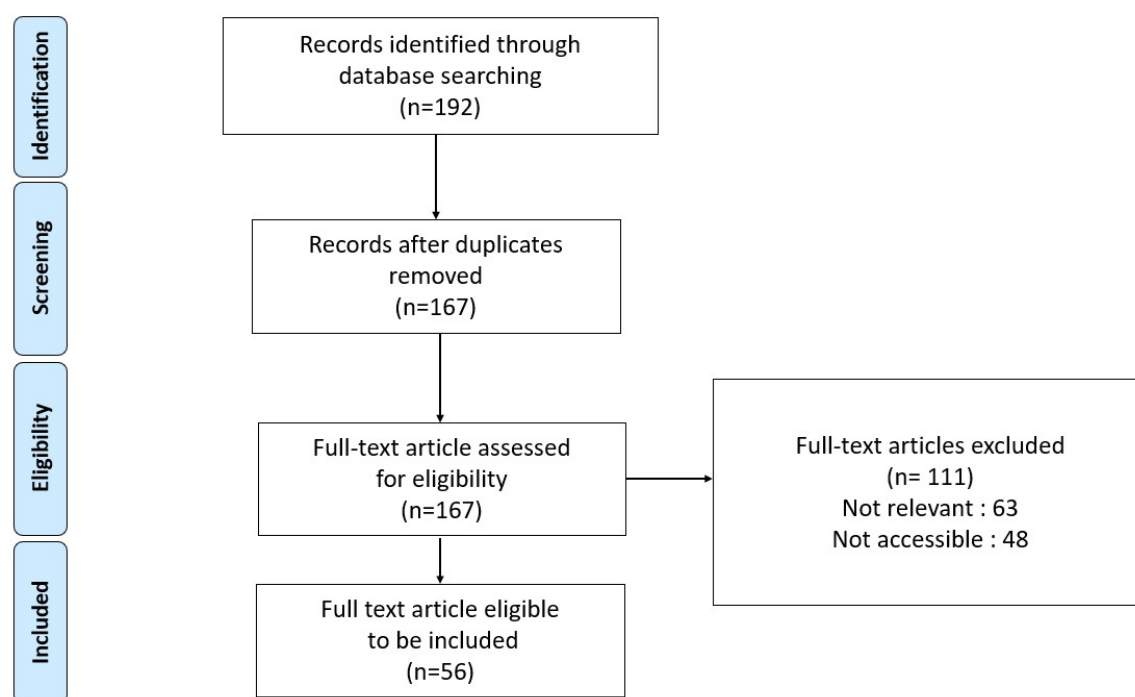


Figure 1. Schematic of literature study in journals using relevant keywords

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 *mtrR* 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 *penA* 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 mosaic <i>penA</i> XIII, Mosaic <i>penA</i> , IX, X, XII, XIX, XXXIV		Bailey et al	1
		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, Bonikowska I et al, Ryan et al, Li et al, Pladevall et al, Sethi et al	2,7,10,11,12, 13,15,17,21,23,24,25,26,30,31,3 2,33,34,35,37,38,39,40,41,45,46 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
<i>porB/penB</i>	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			
<i>mttR</i> 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, 26,27,30,31,33,34,35,38,40,45,5 0,51,52,53,54,55
<i>mttR</i> 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, 26,27,28,31,33,34,35,38,40,45,5 0,52,53,54, 55
<i>ermB</i> , <i>ermC</i>	Enzymatic destruction	Berenger et al, Wind et al	2,34

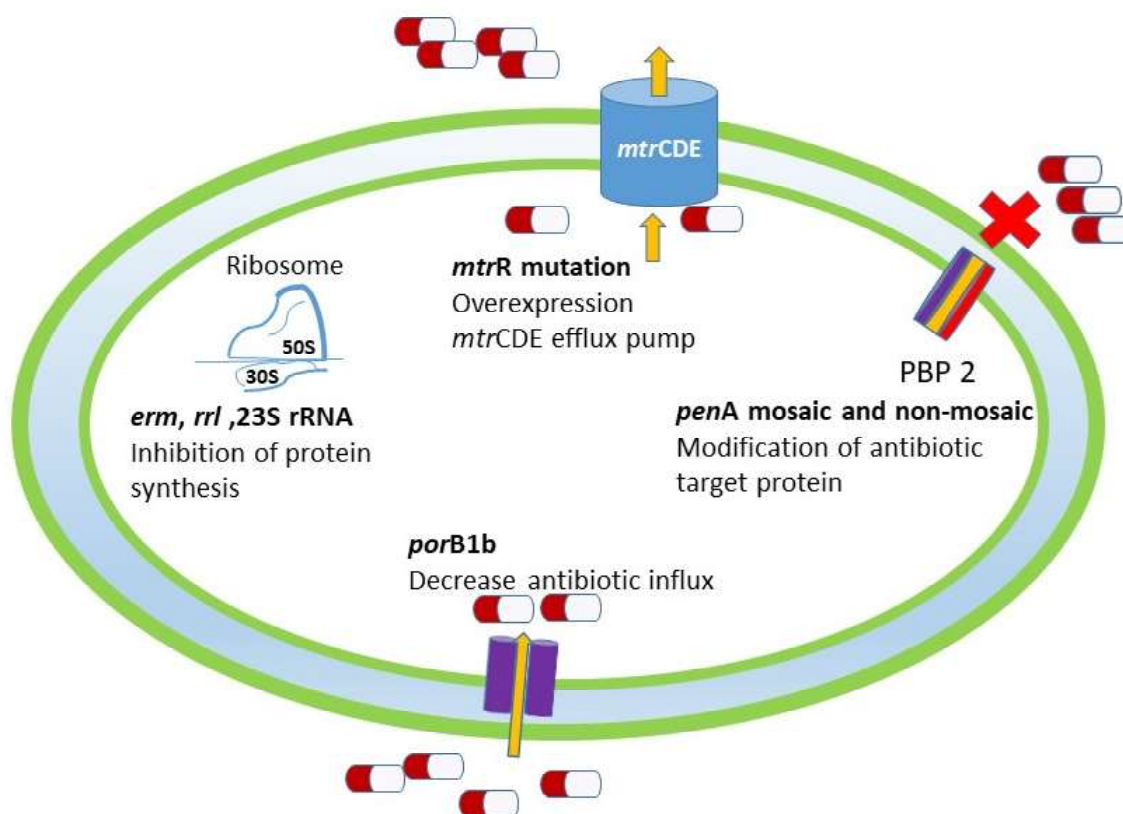


Figure 2. Mechanism of resistance of *N. gonorrhoeae* to cefixime and azithromycin

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 *penA* X patterns with high MIC values that will lead to treatment failure. ⁽³⁵⁾ Non-mosaic *penA* patterns, including patterns IV, V, XII, XVI, XVII, and XVIII, can also cause *N. gonorrhoeae* resistance such as those caused by *penA* 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 *mtrR* 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 *mtrR* 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 *mtrR* 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 *porB* and *mtrR* contributed to increased MIC values in resistant *N. gonorrhoeae* isolates. The *penB* resistance

	10	20	30	40	50	60	70	80	90
M32091	MLIKSEYKPRMLPKBEQVKKPMTSNGRISFVLNMAVLFACLIARGLYLQTVTYNPLKEQGDNRIVRTQALPATRGTVSDRNGAVIALSA								
IV (9)									
V (4)									
X (1)									
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	100	110	120	130	140	150	160	170	180
M32091	PTESLFAVPKIMKEMPSAAQLERLSELVDVPPVDVLRNKLQKQKSPFIWIKRQLDPKVAEEVKALGLENFVFEKELKRHYPMGNLFAHVIG								
IV (9)									
V (4)									
X (1)		E					A		S
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	190	200	210	220	230	240	250	260	270
M32091	FTDIDGKQEGLELSLEDGLYGEDGAEEVLRDRQGNIVDSLDSFKNKAPQNGKDIILSLDQRIQTLAYEELNKAVEYHQAKAGTVVVLDA								
IV (9)									
V (4)									
X (1)			HAGE		E				
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	280	290	300	310	320	330	340	350	360
M32091	RTGEILALANTPAYDPNRPGRADSEQRNRRAVTDMEIPGSAIKPFVIAKALDAGETDLNERLNTQPYKIGPSPVR-DTHVYPSLDVRGIM								
IV (9)								D	
V (4)								D	
X (1)		V	E	K	Q		M	T	S
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	370	380	390	400	410	420	430	440	450
M32091	QKSSNVGTSKLSARPGAEEMYDFYHELIGVVRMHSGFPGETAGLLRNWRWRPIEQATMSPGYGLQLSLQLARAYTALTHTDGVLLPLSP								
IV (9)									
V (4)									
X (1)			M	TPK		D	V		S
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	460	470	480	490	500	510	520	530	540
M32091	EQQAVAPQGRIFKESTAREVRNLMVSVTEPGGTGTAGAVDGFVVGAKTGTARKFVNGRYADNKHVATFIGFAPAKNFRVIVAVTIDEPT								
IV (9)						L	V	G	
V (4)									
X (1)		K	VI	A	KK	E	A		L
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									
	550	560	570	580					
M32091	AHGYYGGVVAGPPFKKINGGSLNIGISPTKPLT-AAAVKTPS								
IV (9)		S							
V (4)									
X (1)		S	S	T	V	QV			
XII (2)									
XIII (28)									
XIV (2)									
XVII (1)									
XXIV (1)									

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 *penB* phenotype is only seen in the *N. gonorrhoeae* mutant strain that overexpresses the *mtrCDE* 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 *ermB*, *ermC*, and *ermF* 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 *mtrR* leading to overexpression of the efflux pump. Mutations can occur in the *mtrR* 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 *mtrR* 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 *mtrC* 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.

FUNDING

No funding was received for conducting this study.

ACKNOWLEDGEMENTS

The authors would like to thank Magister Program Biomedical Science, Faculty of Medicine, Universitas Indonesia for giving the opportunity and facilitating the writing of this manuscript.

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.



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