Gene Expression Of Some Antibiotic Resistance Mechanisms In Neisseria Gonorrhoeae Isolated From Iraqi Patients

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KEYWORDS
Neisseria gonorrhoeae, gene expression, AMR, Real-time qPCR

ABSTRACT
Background: Treatment of N. gonorrhoeae infection is restricted by the rising commonness of multidrug-resistance strains. The point of this study was to evaluate antibiotic resistance and mRNA expression of penA, pilQ, norM, farA, and mtrE of N. gonorrhea. Method: one hindered samples collected from patients infected with N. gonorrhoeae. The bacteria were isolated and diagnosed using culture media and biochemical tests. Using the MIC method, the sensitivity of bacteria to penicillin, ciprofloxacin, ceftriaxone, spectinomycin, tetracycline, and azithromycin was determined. After RNA extraction and cDNA production, gene expression of penA, pilQ, norM, farA, and mtrE was assessed using real time - qPCR. Results: we found all isolates sensitive to ceftriaxone and azithromycin. Clear increase in penA, pilQ, farA, and mtrE gene expression of penicillin-resistant isolates (1.571, 3.135, 0.010 and 34.24 respectively), while norM gene expression decreased in those isolates. High gene expression for pilQ, farA, and mtrE among isolates resistant to ciprofloxacin (0.0041, 0.00072, 0.011 respectively). Tetracycline-resistant isolates appeared to have higher gene expression for penA, norM, farA, and mtrE (0.0033, 8.669, 37.85, 15.84 respectively). Spectinomycin-resistant isolates have high gene expression for penA, pilQ, norM, farA, and mtrE (0.222, 0.021, 0.036, 0.0002, 19.63 respectively) . Moreover, gene expression of penA, norM, and farA increased in males (6.938, 1.095, 7.946 respectively) while the rate of gene expression for pilQ and mtrE increased in females (0.161 and 7.832 respectively). Conclusion: gene expression of penA, pilQ, norM, farA, and mtrE increased in most antibiotics resistance isolates especially of bacterial infection that isolated from males.

1. Introduction

Expanding antimicrobial resistance (AMR) to Neisseria gonorrhoeae (N. gonorrhoeae) is presently a general wellbeing need as it compromises the ongoing World Health Organization (WHO) suggested double treatment (ceftriaxone and azithromycin) [1-3]. Molecular tools to reduce drug efficacy have been described everywhere mainly due to mutational modifications of the drug target, plasmids and efflux pump [4]. Urethral infections in males cause urethritis (aggravation of the urethra), the side effects of which include purulent release and dysuria [5].The sequelae of untreated gonorrhea incorporates intense urethritis, cervicitis, pelvic incendiary diseases (PID), barrenness, early termination, ectopic pregnancy, maternal passing, and neonatal visual deficiency [6]. The AMR systems through which N. gonorrhoeae has created opposition has not been completely evaluated. These incorporate antimicrobial inactivation, adjustment of target destinations, expanded send out by means of efflux pump, and diminished take-up through porins [7]

One of the most significant is the occurrence and development of antibiotic resistance systems of N. gonorrhoeae to beta-lactam anti-infection agents. Chromosomal protection from penicillins and oxyimino-cephalosporins and plasmid protection from penicillins are discussed [8]. Chromosomal resistance is connected with the finding of mutations in the PBP2 protein, containing mosaic variants and nonmosaic amino acid substitutions in the transpeptidase domain, and their link with mutations in the mtrR gene and its promoter regions (the MtrCDE membrane pump repressor) and in many other genes, which together detected reduced sensitivity or resistance to ceftriaxone and cefixime [9]. Plasmid resistance to penicillins produce from the present of beta-lactamases. There are different types of beta-lactamases as well as penicillinase plasmids. Furthermore to resistance to beta-lactam antibiotics, the manuscript covers the occurrence and mechanisms of resistance to macrolides (azithromycin), ciprofloxacin and some other antibiotics[10]. The aim of this study was to correlate mRNA expression levels of N. gonorrhoeae antibiotic target genes and efflux pump genes to antibiotic resistance in our population using real-time qPCR. A secondary goal was to determine whether patient gender affects the extent of bacteria's resistance to antibiotics.

2. Methodology

Samples collection: The current study is a cross – sectional study that included collecting 100 swab from patients infected with *N. gonorrhoeae*. Samples were collected from the General Maternity and Children's Teaching Hospital, Al-Shifa Private Hospital, and outpatient clinics during the period from 1/2/2021 to 9/12/2022. The required tests for the samples were conducted in the laboratories of the aforementioned hospitals.

Isolation and identification of *N.gonorrhea*: *N. gonorrhoeae* isolated from vagina and urethra were resuscitated on nonselective Thayer Martin media (supplemented with 1% Vitox, excluding antimicrobial enhancements) for 18–24 h in a 37°C, 5% CO incubator. ID was affirmed utilizing brilliant field microscopy (*N. gonorrhoeae* is a gram-negative diplococcus), Bactident® Oxidase quick test (Merck, Germany) (*N. gonorrhoeae* is oxidase positive), and Phadebact® Monoclonal GC test (Pharmacia, Sweden) (a coagglutination procedure utilized for the conclusive ID of *N. gonorrhoeae*).

Antibiotics sensitivity test (AST): AST was performed, utilizing Etest® (bioMérieux, Marcy l'Etoile, France), for all segregates, utilizing GC agar base medium (utilized for the disengagement and development of *N. gonorrhoeae*) enhanced with 1% Vitox (Oxoid). The base inhibitory fixation not entirely set in stone as the most reduced centralization of the medication to repress the development of the creature outwardly. The medications and focus ranges were as per the following; penicillin (0.0161-2561 µg/mL), ciprofloxacin (0.0020-320 µg/mL), ceftriaxone (0.002-32 µg/mL), spectinomycin (0.0640-10240 µg/mL), tetracycline (0.016-256 µg/mL), and azithromycin (0.0161-2561 µg/mL). Defenselessness was deciphered according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [11, 12].

RNA Extraction and cDNA production: RNA was separated from the N. gonorrhoeae segregates utilizing TRIzol™ reagent (Invitrogen) with the PureLink™ RNA Scaled down Unit (ThermoFisher Logical) and PureLink™ DNase (ThermoFisher Logical) according to the maker's directions. The all out RNA fixation was evaluated utilizing a nanodrop spectrophotometer, and tests were utilized provided that the optical thickness at 260nm (OD261/281) was ~2.0. RNA honesty was affirmed utilizing a sanitizer gel technique. One microgram of absolute RNA from each example was turned around translated utilizing the iScript™ Switch Record Supermix for Quantification polymerase chain reaction method (real time -qPCR) (BIO-RAD) according to the producer's guidance and response convention. The all out cDNA fixation was evaluated, and tests were utilized provided that the optical thickness at 260 nm (OD260/280) was >1.8 [13,14].

RNA Quantification by Real-Time PCR: performed for the preliminary successions recorded in Table 1 cDNA was weakened utilizing a 1 : 10 proportion for RT- PCR examination. Each PCR combination (5 µl absolute volume) comprised of the individual groundworks (0.5 pmol/µl for farB and mtrD; 0.7 pmol/µl for 16S rRNA, penA and macA; 0.3 pmol/µl for any remaining preliminaries), 2.5 µl PowerUp™ SYBR™ Green Expert Blend (ThermoFisher Logical, USA), 1 µg cDNA and sans nuclease water. Responses were run in copy on the Quant Studio 5 (ThermoFisher, CA, USA) (1 cycle at 95°C, 2 min.), trailed by 40 cycles comprising of denaturation at 95°C (15 sec.), tempering at 60°C (15 s), expansion at 72°C (1 min). Followed by a liquefy bend stage (95°C, 15°s) incline rate 1.6°C/s, 60°C (1 min) incline rate 1.6°C/s, and 95°C (15 s) incline rate 0.15°C/s. Intensification particularity was affirmed utilizing softening bend examination and gel electrophoresis [14].

Table (1): Primers sequences of studied genes

Gene (locus)	Primer sequence (5′-3′)	Molecular weight	Characteristics

		(bp)	
***penA* (NGO1542)**	penAF_ACCGAAAGACATCGTCGCCT penAR_CGTCGGCACAAGCAAACTGT	172	Penicillin-binding protein
pilQ (NGO0094)	pilQF_ACGAGGCTTTGGATTGCGAG pilQR_TTATGCTTTTTGCCGCGACCG	234	Type IV pilus secretin PilQ.
norM (NGO0395)	norMF_ATCGAAACGGTAGGCGAGCA norMR_AACCGGCAGACTTCACCCAA	140	Multidrug efflux protein
farA (NGO1683)	farAF_GCGGATTGCCCGAGGATTTC farAR_GCTGAACCGCGAAGATGTGG	183	Multidrug resistance protein
mtrE (NGO1363)	mtrEF_AGACGGCATTTGTTTGCCCG mtrER_ATTTGCTCGATGCGGAACGC	165	Multidrug transporter

Ethical Consideration

1. General Maternity and Children's Teaching Hospital, Al-Shifa Private Hospital were permitted to do this work.

2. Written consent was taken from all participants.

Statistics: Statistical analysis was conducted using the Statistical Package for the Social Sciences, version 19, with Microsoft Excel 2012, where variables were considered statistically different when the probability value was less than five percent ($P< 0.05$).

3. Results and discussion

The current study is a cross sectional study that included collecting 100 samples from gonorrhea patients whose ages ranged from 17 to 57 years, with an average age of 34.5 ± 8.15years. The infection rate in males (51%) was higher than in females (49%), as in Table 2. After isolating and diagnosing *N. gonorrhea* and conducting an antibiotic sensitivity test, we found all the isolates sensitive to Ceftriaxone and Azithromycin (Table 3). Moreover, We found a high percentage of the isolates (88%) sensitive to Spectinomycin. Penicillin became not successful for treatment *N. gonorrhea* infection when 98% of isolates appeared resist to it. In figure (1) Antibiotics resistance increased in males compared with females however *P* value more than 0.05 when compared resistance between males and females for each antibiotic.

Table (2): Age range and gender distribution of patients

Age characteristics	
Age range	17 to 57 years
Mean	34.5 year
Standard deviation	± 8.15
Standard Error	0.82
Females (%)	49 (49%)
Males (%)	51 (51%)
Total number	100

Table (3): Results of antibiotics sensitivity of studied antibiotics

Antibiotics	**Resistance (%)**	**Sensitive (%)**	**P value**
Penicillin	98(98%)	2 (2%)	< 0.0001
Ciprofloxacin	79 (79%)	21 (21%)	0.0007
Tetracycline	82 (82%)	18 (18%)	0.0002
Ceftriaxone	0 (0%)	100 (100%)	< 0.0001
Spectinomycin	12 (12%)	88 (88%)	0.0002
Azithromycin	0 (0%)	100 (100%)	< 0.0001

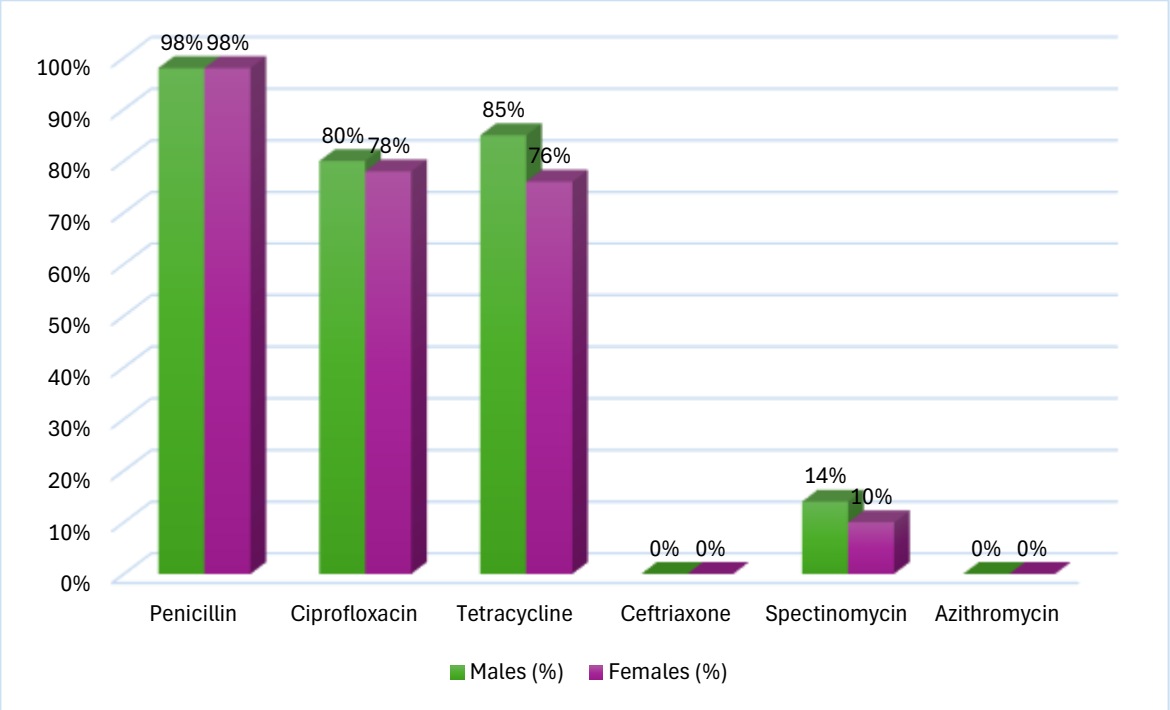

Figure (1): Distribution percentage of antibiotics resistance according to patients gender (P value>0.05 when compared resistance between males and females for each antibiotic)

We evaluation role of some antibiotics resistance mechanisms by determine gene expression of their genetic basis. Evaluation of gene expression for *penA*, *pilQ*, *norM*, *farA*, and *mtrE* showed an increase in most of these genes in antibiotic-resistant isolates. In Table 4 found a clear increase in *penA*, *pilQ*, *farA*, and *mtrE* gene expression in penicillin-resistant isolates (1.571, 3.135, 0.010 and 34.24 respectively), while *norM* gene expression decreased in those isolates (0.019). On the other hand, we found high gene expression for *pilQ*, *farA*, and *mtrE* among isolates resistant to ciprofloxacin (0.0041, 0.00072, 0.011 respectively) compared to the susceptible isolates had a lower rate of gene expression for those markers, while their gene expression for *norM* increased (0.003) but not significantly (P>0.05) (except for *mtrE*, P = 0.041), as in Table 5. Tetracycline-resistant isolates appeared to have higher gene expression for *penA*, *norM*, *farA*, and *mtrE* (0.0033, 8.669, 37.85, 15.84 respectively) compared to susceptible isolates (0.003, 2.633, 19.22, 13.68 respectively) as in Table 6. Spectinomycin-resistant isolates have high gene expression for *penA*, *pilQ*, *norM*, *farA*, and *mtrE* (0.222, 0.021, 0.036, 0.0002, 19.63 respectively) comparison with the susceptible isolates (0.163, 0.017, 0.035, 0.00013, 6.77 respectively) as in Table 7. When evaluating the expression of the isolates isolated from females compared to males in Table 8 we found gene expression of *penA*, *norM*, and *farA* increased in males (6.938, 1.095, 7.946 respectively) while the rate of gene expression for *pilQ* and *mtrE* increased in females (0.161 and 7.832 respectively).

Table (4): Evaluation resistance genes expression according o penicillin sensitivity test

Genes	Mean of gene expression		P value
	Sensitive	Resistance	
penA	0.0122	1.571	0.0012
pilQ	0.101	3.135	0.0007
norM	0.022	0.019	0.712
farA	0.007	0.010	0.0413
mtrE	13.08	34.24	0.012

Table (5): Evaluation resistance genes expression according to ciprofloxacin sensitivity test

Genes	**Mean of gene expression**	**P value**

	Sensitive	Resistance	
penA	0.001	0.001	1
pilQ	0.0036	0.0041	0.502
norM	0.003	0.0027	0.495
farA	0.0007	0.00072	0.619
mtrE	0.006	0.011	0.041

Table (6): Evaluation resistance genes expression according o tetracycline sensitivity test

Genes	Mean of gene expression		**P value**
	Sensitive	**Resistance**	
penA	**0.003**	**0.0033**	**0.379**
pilQ	**0.0026**	**0.0022**	**0.311**
norM	**2.633**	**8.669**	**0.037**
farA	**19.22**	**37.85**	**0.026**
mtrE	**13.68**	**15.84**	**0.116**

Table (7):Evaluation resistance genes expression according to spectinomycin sensitivity test

Genes	**Mean of gene expression**		**P value**
	Sensitive	Resistance	
penA	0.163	0.222	0.045
pilQ	0.017	0.021	0.084
norM	0.035	0.036	0.415
farA	0.00013	0.0002	0.107
mtrE	6.77	19.63	0.036

Table (8): Evaluation genes expression of *penA*, *pilQ*, *norM*, *farA*, and *mtrE* according to patient gender

Genes	**Mean of gene expression**		**P value**
	Females	Males	
penA	2.444	6.938	0.072
pilQ	0.731	0.161	0.031
norM	0.326	1.095	0.017
farA	6.614	7.946	0.116
mtrE	18.06	7.832	0.043

Discussion

Mutations can interrupt cellular processes and often hold the key to understanding gene function. The antibiotic target genes in *N. gonorrhoeae* need to more research because increase its infection and resistance to wide range of antibiotics [4, 12]. Alterations in antibiotic target genes are associated with increased MICs and resistance. we found significant difference in gene expression between resist and sensitive isolates. In contact to our findings, Mitchev *et al.*, analysis showed that expression levels of antibiotic target genes are significantly higher in susceptible isolates compared to nonsusceptible isolates [14]. High gene expression of *penA*, *pilQ*, *farA*, and *mtrE* significantly associated with penicillin resistance while *mtrE* significantly associated with ciprofloxacin resistance. *norM* and *farA* significantly associated with tetracycline resistance. Moreover, *penA* and *mtrE* significantly associated with resistance to spectinomycin. this results confirmed *penA* gene response for beta lactam antibiotics resistance through beta lactam binding protein, *PilQ* secretin of type IV pili plays an important role in antibiotic influx, *FarA* efflux pump (export host-derived antimicrobials, including cationic antimicrobial peptides and long-chain fatty acids) and mtrE represent multidrug transporter [15,16]. Pervious study showed A combination of mutations and other factors contribute to increased MICs: however, in pervious cohort, the majority of resistance to penicillin and tetracycline is attributed *tonorM*, *farA blaTEM* and *tetM*, respectively [15]. Other researchers showed that resistance to ciprofloxacin is due to mutations in gyrA and parC, and mutations in the norM promoter results in overexpression of NorM efflux pump, which increases ciprofloxacin MICs [17,18]. In previous studies, researchers found that *N. gonorrhoeae* contain *penA*

are resistant not only to penicillin, but also to most of the beta-lactam group, such as ceftriaxone and cefixime. However, in our study, resistance to penicillin appeared and did not show a tendency towards ceftriaxone, and the reason for this may be due to the presence of other specialized resistance genes for each one of beta-lactam antibiotics [19].

In constant with our results, many pervious studies found high sensitivity of *N. gonorrhoeae* to azithromycin that may be due to Azithromycin binds to a four-nucleotide fragment of rRNA (in the peptidyltransferase region) within the V domain of the 23S rRNA in the 50S subunit of the ribosome, which results in the inhibition of protein synthesis [20,21]. A study by Mlynarczyk-Bonikowska and his coworkers found that resistance of bacteria to the beta-lactam group is more likely in Asian countries where there is a high rate of beta-lactamase production by *N. gonorrhoeae*. In addition, of concern is the increase in the number of azithromycin-resistant *N. gonorrhoeae* strains observed in Europe in the period 2016-2019. In light of these risks, it is appropriate to search for new drugs effective against *N. gonorrhoeae* [22].

It was previously reported that transcriptional responses of *N. gonorrhoeae* to infection differed in genital samples from men and women, and AMR gene expression was increased in men, with higher expression of MtrCDE efflux pump-related genes, suggesting that the expression of AMR genes is driven by special environments. By gender [15]. While overall gene expression signatures may be sex-specific, we found that in the previous cohort of South African patients, there were no significant differences in the expression of resistance-associated targets between isolates from men and women. Based on this outcome, we found that while therapeutic strategies could be based on gender, when using a diagnostic assay, there was no need to streamline the gene target profile based on gender, and that the same targets can be used for AMR detection for specimens from males and females in our setting [24,43].

4. Conclusion and future scope

We found ceftriaxone and azithromycin are effective in treating N. gonorrhoeae infection. We also found a clear association with gene expression penA, pilQ, norM, farA, and mtrE with resistance to the antibiotics penicillin, ciprofloxacin, ceftriaxone, spectinomycin, tetracycline, and azithromycin especially penicillin, which is no longer effective in treating this infection.

Conflicts of Interest

The authors declare no conflict of interest

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