PREVALENCE OF ANTIMICROBIAL RESISTANCE OF NEISSERIA GONORRHOEAE ISOLATED IN FIVE HEALTH CENTRES IN VIETNAM

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This was the first study in Vietnam which showed the prevalence of antimicrobial resistance of Neisseria gonorrhoeae in 5 health centres across the country. This study aimed to investigate the prevalence of N. gonorrhoeae in patients with urethral and/or vaginal discharge symptoms who attended one of five health centres in Vietnam, and to investigate the prevalence of antimicrobial resistance of isolated N. gonorrhoeae. In sample collection, pus or discharge was collected from 5,082 patients with urethral and/or vaginal discharge symptoms. Gram-staining, culture, determination and antimicrobial susceptibility methods were used to detect N. gonorrhoeae and its resistance to antibiotics. In this study, the prevalence of N. gonorrhoeae was 18.16%; N. gonorrhoeae showed its resistance to ciprofloxacin ((96.64%), tetracycline (86.78%) penicillin (57.64%), and its non-susceptibility to azithromycin (39.00%), cefixime (1.95%), ceftriaxone (1.52%) and cefotaxime (0.52%). No isolate showed resistance to spectinomycin. In conclusion, spectinomycin, ceftriaxone, and cefixime should be recommended for N. gonorrhoeae treatment while ciprofloxacin, tetracycline and penicillin should not.

Keyword: gonorrhoeae, Neisseria gonorrhoeae, antimicrobial resistance.

I. INTRODUCTION

According to the World Health Organization (WHO), annually there were an estimated 500 million new cases of four sexually transmitted diseases (STDs) including gonorrhoeae, syphilis, chlamydia and trichomoniasis.

Corresponding author: Le Van Hung, Hanoi Medical University Email: levanhung@hmu.edu.vn Received: 10/04/2020 Accepted: 19/05/2020 Of which, 106 million new cases were gonorrhoeae.¹ In Vietnam in 2018, with WHO's support, antimicrobial susceptibility testing for *Neisseria gonorrhoeae* was performed in 5 health centres across the nation, including the National hospital of dermatology and venereology (NHDV).

The NHDV receives STD reports from provincial health centres every year, with about 9.6% of STD cases reported as gonorrhoeae.² In addition, using antibiotics without prescription

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or overuse of antibiotics in community and health centres causes increased antimicrobial resistance of N. gonorrhoeae, which creates difficulties for diagnosis and treatment.3,4 Without correct diagnoses and timelv treatment, gonorrhoeae may further spread in the community and induce many different complications in the ano - genital tract, such as infertility.5 The antimicrobial resistance of N. gonorrhoeae has received much attention from many researchers in Vietnam and also in the world. Thus, we performed this study to investigate the prevalence of N. gonorrhoeae infections in patients with urethral and/or vaginal discharge syndrome who attended 5 health centres above, and to determine the antimicrobial resistant levels of isolated N. gonorrhoeae.

II. METHODS

1. Subjects

This cross - sectional study included 5,082 male and female patients with urethral and/ or vaginal discharge syndrome who attended one of the following health centres in 2019: NHDV, Hai Phong centre of dermatology and venereology, Quang Ninh centres for disease control and prevention, Dong Nai hospital of dermatology and venereology and Ho Chi Minh City hospital of dermatology and venereology. Patients who used antibiotics within 7 days and/ or declined to participate were excluded from this study.

2. Method

Sample collection

For men, samples were collected from the urethra by inserting a pre - wet cotton tip about 2cm long into the urethral meatus and rubbing against the inner walls. In women, samples were collected from the urethra, cervix, 2 sides

of Bartholin's gland, and 2 sides of Skene's Gland by using a pre - wet cotton tips. Then, the tips were used for making smear for gram staining, and culturing in Thayer - Martin agar.

Neisseria gonorrhoeae detection

As for gram staining, samples were considered positive for *N. gonorrhoeae* if the bacteria in smears showed negative gram results, coffee - bean - like shape, in and outside of neutrophils in microscope field.

Remel BactiCard Neisseria test kit (Thermo Scientific) was used for *N. gonorrhoeae* determination.

Antimicrobial susceptibility testing

Antibiotic discs (Oxoid) were used to determine the antimicrobial susceptibility of *N. gonorrhoeae*. The zone of inhibition was measured by using mm ladder and interpreted according to the guideline of Clinical and Laboratory Standards Institute (CLSI) in 2018,⁶ except for the measurement of Azithromycin which was interpreted according to CDC's guideline.⁷

Minimum inhibitory concentration (MIC) test was performed by using E - test (Oxoid). In our study, MIC was only performed for *N. gonorrhoeae* isolates with the zone of inhibition being close to sensitive or resistant level.

2. Statistical analysis

Statistical analyses were performed by using SPSS Version 24.0 for Windows. The chi - square test or Fisher's exact probability test was used, and P - values of < 0.05 were considered statistically significant.

3. Medical ethics

The study protocol was reviewed and approved by the Council of Master's thesis outline in Hanoi Medical University, Vietnam (No. 2503/QĐ - ĐHYHN on July 2nd, 2019).

III. RESULTS

1. The prevalence of N. gonorrhoeae infections in patients with urethral and/or vaginal discharge syndrome

 Table 1. The prevalence of *N. gonorrhoeae* infections in patients with urethral and/or vaginal discharge syndrome (n = 5082)

Test results	Gram staining (%)	Culture (%)	p value
Positive	905 (17.81)	923 (18.16)	
Negative	4177 (82.19)	4159 (81.84)	0.64
Total	5082	5082	

Of 5,082 patients with urethral and/or vaginal discharge syndrome, 923 (18.16%) were positive for *N. gonorrhoeae*. As compared with gram staining method, the detection rate of *N. gonorrhoeae* using culture method was higher (18.16% vs 17.81%) thought not significantly different (p = 0.64) (Table 1).

Table 2. Prevalence of N	gonorrhoeae in five health c	entres usina culture me	thod (n = 923)
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Name of Health Centre	Positive	%	p value
National hospital of dermatology and venereology (n = 755)	162	21.46	
Haiphong centre of dermatology and venereology ($n = 452$)	71	15.71	
Quang Ninh centres for disease control and prevention (n = 131)	17	12.98	
Dong Nai hospital of dermatology and venereology (n = 861)	135	15.68	< 0.01
Ho Chi Minh City hospital of dermatology and venereology (n = 2883)	538	18.66	
Total	923	18.16	

The prevalence of *N. gonorrhoeae* infection was significantly different among 5 health centres (p < 0.01). The prevalence of *N. gonorrhoeae* infection was highest in NHDV (21.46%) (Table 2).

2. Antimicrobial resistant level of isolated N. gonorrhoeae

Table 3. The resistant levels against antibiotics from main group (n = 923)

Antibiotico	Abbreviation	Susc	Susceptible		Intermediate		Resistant	
Anubioucs		n	%	n	%	n	%	
Penicillin	PG	0	0	391	42.36	532	57.64	
Spectinomycin	SPT	923	100	0	0	0	0	
Ciprofloxacin	CIP	8	0.87	23	2.49	892	96.64	
Ceftriaxone	CRO	909	98.48	Not susceptible: 14 (1.52%)				
Tetracycline	TE	2	0.22	120	13.00	801	86.78	
Tetracycline	TE	TRNG: 277(30.01%)						

TRNG: tetracycline resistant Neisseria gonorrhoeae is the term used to describe isolates with the

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inhibition zone of tetracycline being less than or equal to 19 mm.

N. gonorrhoeae showed its high resistance to ciprofloxacin and tetracycline (96.64% and 86.78%, respectively). Almost all *N. gonorrhoeae* isolates were susceptible to spectinomycin and ceftriaxone (100% and 98.48%, respectively) (Table 3).

Antibiotics	Abbreviation	Susc	Susceptible		Intermediate		Resistant	
		n	%	n	%	n	%	
Azithromycin*	AZM	No	t resistant	: 563 (61.0	0%)	360	39.00	
Cefotaxime**	CTX	383	99.48	Not	susceptik	ole: 2 (0.	52%)	
Cefixime	CFM	905	98.05	Not s	susceptib	le: 18 (1	.95%)	

Table 4. The resistant levels against antibiotics from additional groups

*The zone of inhibition of azithromycin was interpreted according to CDC guideline.

**Only 385 *N. gonorrhoeae* isolates were performed the antimicrobial susceptibility testing for cefotaxime

Of *N. gonorrhoeae* isolates, 99.49% and 98.05% of the isolates were susceptible to cefotaxime and cefixime, respectively, while 39.00% of the isolates were resistant to azithromycin (Table 4).

No	Antibiotico	Number of	MIC (μg/ml)			
NO.	Antibiotics	isolates	Susceptible	Intermediate	Resistant	
1	Donioillin	22	≤ 0.06	0.12 - 1	≥ 2	
I	Penicillin	33		3	30	
2 Spe	Spectinomycin	16	≤ 32	64	≥ 128	
	Specunomycin		16			
3	Ciprofloxacin	45	≤ 0.06	0.12 - 0.5	≥ 1	
				3	42	
4	Ceftriaxone	xone 26	≤ 0.25	-	-	
			12	Not suscep	tible: 14	
5	Tetracycline	35	≤ 0.25	0.5 - 1	≥ 2	
				3	32	

Table 5. MIC value of several antibiotics to N. gonorrhoeae

As for MICs, the results showed that 30/33 isolates were resistant to penicillin (MIC $\ge 2 \ \mu g/ml$), 42/45 isolates to ciprofloxacin (MIC $\ge 1 \ \mu g/ml$), 32/35 isolates to tetracycline (MIC $\ge 2 \ \mu g/ml$), and 14/26 isolates were not susceptible to ceftriaxone (MIC $\ge 0,25 \ \mu g/ml$). No isolate showed resistance to spectinomycin (MIC $\le 32 \ \mu g/ml$) (Table 5).

IV. DISCUSSION

This was the first study in Vietnam, which showed the large data of prevalence of antimicrobial resistance of *N. gonorrhoeae* in 5 health centres across the country. Of 5,082 patients with urethral and/or vaginal discharge syndrome attending one of the five health centres, 923 (18.16%) were positive for *N. gonorrhoeae* infection. This rate was higher as compared with previous studies in Vietnam.^{2,8}

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It was because we performed bacterial culture, determination and antimicrobial susceptibility testing for all suspected gonorrhoeae patients with WHO's support for labour and consumables.

Nguyen SH² reported that of 20,260 patients who attended NHDV for STDs from 2006 to 2010, 1946 (9.6%) were positive for *N. gonorrhoeae*. In fact, the real number of gonorrhoeae patients in the community may be higher because many patients decide to be examined at private clinics or even prefer self - treatment without a prescription due to perceived stigma.¹⁰⁻¹² In the other hands, the detection of N. gonorrhoeae infections also depends on several factors, such as the experience of technicians and quality of laboratory.¹³⁻¹⁵

N. gonorrhoeae seems to have resistance to several antibiotics which were reco mmended for gonorrhoeae treatment, such as penicillin, tetracycline, fluoroquinolone, erythromycin and azithromycin. Some N. gonorrhoeae strains even showed resistance in vitro to extended spectrum cephalosporin antibiotics, which are reco mmended as the first - line antibiotic for gonorrhoeae treatment in most countries.¹⁶

As for ciprofloxacin, *N. gonorrhoeae* showed high resistance to ciprofloxacin (96.64%) in our study. In Le HV's report, the resistant rate of *N. gonorrhoeae* to ciprofloxacin was 96.33%8. This rate was 85.2% in China, 79.5% in Hong Kong and 40% in Japan, 4.89% in England 4.89%, 7.8% in Scotland 7.8% and 0.9% in Papua New Guinea.^{17,18} The resistance of *N. gonorrhoeae* to ciprofloxacin has increased rapidly due to the usage of quinolones not only for gonorrhoeae treatment but also for other infections, such as urinary tract infections or injuries. Thus, ciprofloxacin should not be reco mmended for gonorrhoeae treatment as previous studies' report.^{2,8}

As for tetracycline, our study showed

that the resistance rate of N. gonorrhoeaeto this antibiotic was 86.78% with 30.01% of tetracycline resistant Neisseria gonorrhoeae (TRNG - the term used to describe isolates with the inhibition zone of tetracycline being less than or equal to 19 mm). The study conducted at the NHDV in 2014 showed that the resistance rate of N. gonorrhoeae to tetracycline was 83.4%.8 In 2011, the rate of tetracycline - resistant N. gonorrhoeae was 82% as Olsen B12 reported in the same hospital. Thus, this resistance rate in Vietnam has shown an increasing trend. Though tetracycline is an old antibiotic, this antibiotic is often applied in multiple doses for N. gonorrhoeae and C. trachomatis treatment in urethritis and cervicitis, leading to increased antimicrobial resistance of N. gonorrhoeae to this drug.

As for penicillin, the first discovered antibiotic, our study showed 57.64% of *N. gonorrhoeae* isolates were resistant and 42.36% of isolates showed reduced susceptibility while no isolate was susceptible to this antibiotic. In 2014, Le HV et al⁸ reported the rate of penicillin - resistant *N. gonorrhoeae* was 47.7%. According to the WHO's report, in Asia Pacific countries, this rate was more than 90% in Korea and the Philippines; was 80% in China and 60% in Singapore.¹⁷ Recently, WHO reco mmended that penicillin was not used for gonorrhoeae treatment among countries with penicillin high resistant rate of *N. gonorrhoeae*.

As for ceftriaxone, our study showed that most *N. gonorrhoeae* isolates were susceptible to this antibiotic (98.48%). However, we found the reduced susceptibility of *N. gonorrhoeae* to this antibiotic in 14 (1.52%) isolates which were isolated only in Ho Chi Minh City hospital of dermatology and venereology. This finding was consistent with a previous study which showed the reduced susceptibility of *N. gonorrhoeae* to

ceftriaxone was 5.0%.¹² Our result was different compared to previous studies which showed that all N. gonorrhoeae isolates were susceptible to ceftriaxone.^{8,17} However, our study suggested that the resistant rate of N. gonorrhoeae may vary according to the geographical area or population.

As for spectinomycin, all N. gonorrhoeae isolates were susceptible to this drug, consisted of previous studies in Vietnam. Thus, spectinomycin has been reco mmended for gonorrhoeae treatment. However, some studies reported the resistance of N. gonorrhoeae to spectinomycin (0.47%).

Our study showed that N. gonorrhoeae was susceptible to cefotaxime and cefixime, with susceptibility rates of 99.48 % and 98.05%, respectively. As for azithromycin, our study showed that 39.00% of isolates were resistant to this drug according to interpretation of CDC guideline.⁷ According to CLSI guideline for antimicrobial resistance of N. gonorrhoeae,⁶ MIC should perform for azithromycin, but not disc diffusion. However, we did not perform MIC for azithromycin due to a large number of isolates in our study. Further studies may need to confirm the resistance of N. gonorrhoeae to azithromycin.

MIC results showed 30/33 isolates were resistant to penicillin (MIC $\ge 2 \ \mu g/ml$), 42/45 isolates to ciprofloxacin (MIC $\ge 1 \ \mu g/ml$), 32/35 isolates to tetracycline (MIC $\ge 2 \ \mu g/ml$), 14/26 strains to ceftriaxone (MIC $> 0.25 \ \mu g/ml$). No isolate showed the resistance to spectinomycin (All MIC $\le 32 \ \mu g/ml$).

V. CONCLUSION

The prevalence of N. gonorrhoeae infection in patients with urethral and/or vaginal discharge syndrome who attended one of five health centres in 2019 in Vietnam was 18.16%. As for the antimicrobial resistance of N. gonorrhoeae, the rate of resistance was highest for ciprofloxacin (96.64%), followed by tetracycline (86.78%) and penicillin (57.64%). Thus, these antibiotics should not recommend for gonorrhoeae treatment. 100% of isolates were susceptible to spectinomycin and most isolates were susceptible ceftriaxone, cefotaxime and cefixime, which should reco mmend for gonorrhoeae treatment.

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REFERENCES

1. Newman L, Rowley J, Vander Hoorn S, et al. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS One.* 2015;10(12):e0143304.

2. Nguyen SH. Study the situation, characteristics of gonorrhoeae at the National hospital of Dermatology and Venereology. *Journal of Vietnam Medical Research*. 2012;1:32 - 35.

3. Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Ther Adv Drug Saf.* 2014;5(6):229 - 241.

4. Cristillo AD, Bristow CC, Torrone E, et al. Antimicrobial Resistance in Neisseria gonorrhoeae: Proceedings of the STAR Sexually Transmitted Infection - Clinical Trial Group Progra mmatic Meeting. *Sex Transm Dis.* 2019;46(3):e18 - e25.

5. Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. *Am J Obstet Gynecol.* 2017;216(1):1 - 9.

6. Clinical and Laboratory Standards Institude. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Wayne, PA: *Clinical and Laboratory Standards Institude*; 2018.

7. Centers of Disease Control and Prevention. Neisseria gonorrhoeae reference strains for antimicrobial susceptibility testing. https://www.cdc.gov/std/gonorrhea/lab/diskdiff. htm#InterpretiveCriteria. Updated May 2nd, 2018. Accessed Mar 1st, 2020.

8. Le HV, Tran TK, Nguyen SH. Survey on antibiotic resistance of Neisseria gonorrhoeae at the National hospital of Dermatology and Venereology in 2014. *Journal of Vietnam Dermatology*. 2016;21:28 - 36.

9. Nguyen S. Study the situation, characteristics of gonorrhoeae at the National hospital of Dermatology and Venereology. *Journal of Vietnam Medical Research.* 2012;1:32 - 35.

10. Unemo M, Seifert HS, Hook EW, 3rd, Hawkes S, Ndowa F, Dillon JR. Gonorrhoea. *Nat Rev Dis Primers.* 2019;5(1):79.

11. Fortenberry JD, McFarlane M, Bleakley A, et al. Relationships of stigma and shame to gonorrhea and HIV screening. *Am J Public Health.* 2002;92(3):378 - 381.

12. Olsen B, Pham TL, Golparian D, Johansson E, Tran HK, Unemo M. Antimicrobial susceptibility and genetic characteristics of Neisseria gonorrhoeae isolates from Vietnam, 2011. *BMC Infect Dis.* 2013;13:40.

13. Lunz ME, Castleberry BM, James K, Stahl J. The impact of the quality of laboratory staff on the accuracy of laboratory results. *JAMA*. 1987;258(3):361 - 363.

14. Church DL, Don - Joe C, Unger B. Effects of restructuring on the performance of microbiology laboratories in Alberta. *Arch Pathol Lab Med.* 2000;124(3):357 - 361.

15. Weston EJ, Wi T, Papp J. Strengthening Global Surveillance for Antimicrobial Drug - Resistant Neisseria gonorrhoeae through the Enhanced Gonococcal Antimicrobial Surveillance Program. *Emerg Infect Dis.* 2017;23(13).

16. Unemo M, Nicholas RA. Emergence of multidrug - resistant, extensively drug - resistant and untreatable gonorrhea. *Future Microbiol.* 2012;7(12):1401 - 1422.

17. World Health Organization. Surveillance of antibiotic resistance in Neisseria gonorrhoeae in the WHO Western Pacific Region, 2000. *Co mmun Dis Intell Q Rep.* 2001;25(4):274 - 276.

18. Creighton S, Tenant - Flowers M, Taylor CB, Miller R, Low N. Co - infection with gonorrhoeae and chlamydia: how much is there and what does it mean? *Int J STD AIDS*. 2003;14(2):109 - 113.

19. Le H, Tran T, Nguyen S. Survey on antibiotic resistance of Neisseria gonorrhoeae at the National hospital of Dermatology and Venereology in 2014. *Journal of Vietnam Dermatology*. 2016;21:28 - 36.

20. Clinical and Laboratory Standards Institude C. Performance Standards for *Antimicrobial Susceptibility Testing.* 28th ed2018.