

**Evaluation of *in vitro* antimicrobial susceptibility of *Neisseria gonorrhoeae* strains isolated from symptomatic men and women in Yangon**

\*Wah Wah Aung & \*\*Kyi Kyi Thinn

\*Bacteriology Research Division  
Department of Medical Research (Lower Myanmar)  
\*\*Department of Microbiology, University of Medicine (1)

The antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* varies from one country to another and may also change with time. Periodic monitoring of the susceptibility of *N. gonorrhoeae* strains is important in order to adopt empirical treatment strategies. Antibiotic susceptibility of 40 *N. gonorrhoeae* strains isolated from urethral swab and endocervical swab specimens of symptomatic men and women was evaluated by disc diffusion method and determination of minimum inhibitory concentration (MIC) using E test method at Bacteriology Research Division, Department of Medical Research (Lower Myanmar) from August 2006 to July 2007. The susceptibility results of both methods showed satisfactory agreement for ciprofloxacin ( $\kappa=0.646$ ), ceftriazone ( $\kappa=0.806$ ) and penicillin ( $\kappa=0.536$ ). The tested *N. gonorrhoeae* isolates were susceptible to azithromycin (87.5%), cefixime (80%), ceftriaxone (>70%) and ciprofloxacin ( $\geq 55\%$ ) which are currently recommended drugs for gonococcal infections. High level ciprofloxacin resistance ( $MIC \geq 4 \mu g/ml$ ) was found in 30% of tested isolates. The present study highlighted that majority of the tested *N. gonorrhoeae* isolates were susceptible to azithromycin, cefixime, ceftriaxone and the occurrence of high level ciprofloxacin resistant strains in the study population. Both disc diffusion and E test susceptibility testing methods gave reproducible results. Thus cost-effective and more feasible disc diffusion method could remain as the method of choice for routine diagnostic laboratories.

## INTRODUCTION

Gonorrhoea, a classical sexually-transmitted disease (STD) caused by the bacterium *Neisseria gonorrhoeae*, still remains one of the common STDs in developing countries. Approximately about 62 million new cases of gonorrhoea occurred worldwide in 1999 [1]. The onset of gonococcal infection is abrupt and the main symptoms are dysuria, frequency of micturition, urethral or cervical discharge. It infects only humans and causes lower genital tract infections, pelvic inflammatory diseases and related sequelae (infertility and ectopic pregnancy) in women, urethritis and epididymitis in men and proctitis, pharyngitis, conjunctivitis and disseminated infections in both sexes. It can

also be transmitted from an infected mother to a baby during child birth and causes ophthalmia neonatorum which can lead to perforation of the globe and blindness in the newborn [2].

The third generation cephalosporins, fluoroquinolones, spectinomycin and azithromycin are included for the treatment recommendation of gonorrhoea by the World Health Organization. The global health problem of gonorrhoea is concerned with the development of antimicrobial resistance in *N. gonorrhoeae* and Southeast Asia is the most likely origin of drug resistant *N. gonorrhoea*. The rate and pattern of antimicrobial resistance in *N. gonorrhoeae* vary with geographic regions and change over time. Therefore periodic monitoring of the

susceptibility of *N. gonorrhoeae* strains is important in order to adopt empirical treatment strategies [3].

A study carried out on 161 *N. gonorrhoeae* strains obtained from female sex workers in North Sumatra, Indonesia in 1996 showed that all strains exhibited plasmid mediated resistance to penicillin and/or tetracycline but all were susceptible to ceftriaxone, ciprofloxacin, kanamycin and spectinomycin [4]. Lawung *et al.*, found that multiple resistant determinants coexisted in a single isolate and double resistance to penicillin and quinolone were significantly increased among the isolates from patients attending Bangkok Hospital [5].

There are only limited studies on gonococcal antibiotic sensitivity pattern in Myanmar. Khin Mya Lwin *et al.* reported that 6.7% of gonorrhoea cases were detected among 71 pregnant women, 26 STD patients and 5 commercial sex workers and the gonococcal isolates were found to be less sensitive to penicillin, but susceptible to ciprofloxacin and ceftriaxone [6]. Gonococcal antimicrobial susceptibility of 14 isolates of *N. gonorrhoeae* isolated from married women in a community based study showed that 92.9% were resistant to penicillin, 85.7% were resistant to septrin, and 71.4% were resistant to amikacin and kanamycin [7].

Antimicrobial susceptibility testing of gonococcal isolates can be carried out by disc diffusion and determination of minimum inhibitory concentration (MIC) by agar dilution tests or E tests. Disc diffusion is a convenient method used by intermediate level laboratory in screening of individual isolates for antibiotic resistance. In disc diffusion test, discs containing known amount of an antimicrobial agents are placed on the surface of an agar plate seeded with tested *N. gonorrhoeae* strain. The antimicrobial agent diffuses into the medium, causing a zone of inhibition around the disc corresponding to the susceptibility of the strain to the agent [8].

Agar dilution technique is the reference method for determination of gonococcal MIC. The procedure is laborious and recommended for use only in central laboratories to provide epidemiological information. E tests provide an effective and simple alternative to reference agar dilution method for direct quantification of *N. gonorrhoeae* susceptibility. This method uses a plastic carrier strip containing a known gradient of an antimicrobial agent and calibrated to give results as MICs of the agent. E test strips are applied to the surface of a previously seeded agar plate and the MIC is recorded by reading the value at the point of intersection between the edge of the zone of growth inhibition and the strip [9].

Thus this study was carried out to determine the antimicrobial susceptibility pattern of currently recommended antibiotics for the treatment of gonococcal infection in Myanmar and to evaluate the antimicrobial susceptibility results of disc diffusion method compared with E test, so that a simple, reliable and cost-effective method could be recommended for antimicrobial susceptibility testing of gonorrhoea.

## MATERIAL AND METHODS

Urethral swab specimens from 48 men presenting with urethral discharge and/or burning micturition and/or frequency of micturition, attending Central STD Clinic, Yangon and endocervical swab specimens from 124 women presenting with vaginal discharge and/or dysuria and/or pelvic inflammatory diseases, attending Central STD Clinic and out-patient clinics of Central Women Hospital and Thingangyan Sanpya Hospital were collected during August 2006 to July 2007. *Neisseria gonorrhoeae* strains were isolated by culture using modified Thayer- Martin media and chocolate agar. The isolates were confirmed by gram staining, oxidase test, catalase test, superoxol test, carbohydrate degradation test and Phadebact GC monoclonal antibody coagglutination test (Boule, Sweden).

Antibiotic susceptibility tests were carried out on 40 culture confirmed *N. gonorrhoeae* isolates using Gonococcal (GC) agar base with 1% IsoVitaleX supplement by disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) [8]. Antibiotic discs (Oxoid, Hampshire, England) containing penicillin G (10 IU), tetracycline (30 µg), chloramphenicol (30 µg), sulphamethoxazole/ trimetho-prim (25 µg), gentamycin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cefixime (5 µg) and kanamycin (30 µg) were tested. The isolates were defined as susceptible, intermediate and resistant according to CLSI.

The susceptibility of penicillin, ciprofloxacin, ceftriaxone and azithromycin were detected by determination of MIC using E test method on 40 culture confirmed *N. gonorrhoeae* isolates. A fresh overnight culture of *N. gonorrhoeae* was suspended in Mueller Hinton broth until a turbidity equivalent to 0.5 McFarland standard was obtained (corresponding to 10<sup>4</sup> colony forming units per ml). The cell suspension was inoculated onto GC agar plate by gently spreading with a sterile cotton-tipped swab.

After drying of the plates for 3-4 minutes, the E test strips were placed in the centre of plate, and then incubated at 35°C in 3-5% CO<sub>2</sub> for 24 hours. The MIC values were determined by recording the point of intersection of the growth elliptic margin zone with the MIC scale value on the E test strip. Based on MIC values described by the manufacturer (AB Biodisk, Sweden), the isolates were interpreted as susceptible, intermediate and resistant [9]. Both disc diffusion and E test were performed simultaneously with the same inoculums. Reference *N.gonorrhoeae* ATCC 49226 was used as quality control for disc diffusion and E test. Kappa statistics was applied to determine the agreement between the two methods.

This study was approved by Research and Ethical Committee, University of Medicine (1).

## RESULTS

*N. gonorrhoeae* strains were isolated from 12/48 (25%) men and 28/124 (22.6%) women comprising 40/172 (23.3%) of total study population.

### *Antibiotic susceptibility by disc diffusion method*

The antibiotic susceptibility pattern revealed by using disc diffusion method is shown in Table 1. Although most of the isolates were sensitive to cefixime (80%), ceftriazone (75%) and ciprofloxacin (60.0%), they were resistant to older antibiotics like penicillin (80.0%), tetracycline (82.5%), chloramphenicol (82.5%) and septrin (77.5%).

Table 1. Antibiotic susceptibility pattern of *Neisseria gonorrhoeae* strains tested by disc diffusion method (n=40)

Tested antibiotics	Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)
Penicillin	4 (10)	4 (10)	32 (80)
Tetracycline	3 (7.5)	4 (10)	33 (82.5)
Gentamycin	8 (20)	12 (30)	20 (50)
Trimethoprim/ Sulphamethoxazole	4 (10)	5 (12.5)	31 (77.5)
Chloramphenicol	3 (7.5)	4 (10)	33 (82.5)
Ciprofloxacin	24 (60)	4 (10)	12 (30)
Ceftriaxone	30 (75)	-	10 (25)
Cefixime	32 (80)	2 (5)	6 (15)
Amikacin	20 (50)	4 (10)	16 (40)
Kanamycin	16 (40)	4 (10)	20 (50)

Zone inhibition diameter (mm) for interpretative criteria recommended by CLSI: penicillin (S ≥ 47, I= 27-46, R≤26), tetracycline (S≥38,I=31-37,R ≤ 30), gentamycin ( S≥15, I=13-14, R≤12 ), trimethoprim/ sulphamethoxazole ( S≥16, I=11-15, R≤16 ), chloramphenicol ( S≥18, I=13-16,R≤12), ciprofloxacin ( S≥41, I=28-40-46, R≤27), ceftriazone ( S≥35 ), cefixime ( S≥31), amikacin ( S≥17, I=15-16,R≤14 ), kanamycin ( S≥18, I=14-17, R≤13 )

R= resistant, I= intermediate, S= susceptible

### *Antibiotic susceptibility determined by E test*

The antibiotic susceptibility of penicillin, ciprofloxacin, ceftriaxone and azithromycin tested by determination of MIC using E test method is shown in Table 2. The suscep-

tibility revealed as: azithromycin (87.5%), ceftriaxone (72.5%) and ciprofloxacin (55.0%). Penicillin showed high resistant rate of 80.0%.

Table 2. Antibiotic susceptibility pattern of *N. gonorrhoeae* determined by E test

Tested anti-biotics (n=40)	Susceptible no.(%)	Intermediate no. (%)	Resistant no. (%)
Azithromycin	35 (87.5)	-	5 (12.5)
Ciprofloxacin	22 (55.0)	8 (20.0)	10 (25)
Ceftriaxone	29 (72.5)	-	11 (27.5)
Penicillin	3 (7.5)	5 (12.5)	32 (80.0)

AB Biodisk's Interpretative criteria according to MIC ( $\mu\text{g/ml}$ ): azithromycin ( $R \geq 2, I=1, S \leq 0.5$ ), ciprofloxacin ( $R \geq 1, I=0.12-0.5, S \leq 0.06$ ), ceftriaxone ( $S \leq 0.25$ ), penicillin ( $R \geq 2, I=0.12-1, S \leq 0.06$ )  
R= resistant, I= intermediate, S= susceptible

#### Comparison of antibiotic susceptibility by disc diffusion and E test method

Antibiotic susceptibility to ciprofloxacin, ceftriaxone and penicillin by disc diffusion and E test methods are compared and shown in Table 3. Statistically moderate level of agreement for ciprofloxacin ( $\kappa=0.646$ ) and penicillin ( $\kappa=0.536$ ) and high level of agreement for ceftriaxone ( $\kappa=0.806$ ) were found.

Table 3. Comparison of antibiotic susceptibility to ciprofloxacin, ceftriaxone and penicillin by disc diffusion and E test method

Antibiotics tested	E test susceptibility	Disc Diffusion Test Susceptibility			Total
		Susceptible no.	Intermediate no.	Resistant no.	
Ciprofloxacin	S	20	1	1	22
	I	3	2	1	6
	R	1	1	10	12
	Total	24	4	14	40
Ceftriaxone	S	28	-	1	29
	I	-	-	-	-
	R	2	-	9	11
	Total	30	-	10	40
Penicillin	S	1	1	1	3
	I	2	2	1	5
	R	1	1	30	32
	Total	4	4	32	40

R= resistant, I= intermediate, S= susceptible

#### MIC values determined by E test

Anti gonococcal activities of azithromycin, ciprofloxacin, ceftriaxone and penicillin

tested by E test expressed in range of minimum inhibitory concentrations is shown in Table 4. High level ciprofloxacin resistance ( $\text{MIC} \geq 4\mu\text{g/ml}$ ) was seen in 10/40 (25%) of tested isolates.

Table 4. Range of MIC values ( $\mu\text{g/ml}$ ) of penicillin, ciprofloxacin, ceftriaxone and azithromycin by E test

Antibiotic (MIC range on E test strip)	Sensitivity pattern	No. of strains	MIC values of tested antibiotic
Azithromycin (0.016-256)	Susceptible	35	0.25-0.5
	Resistant	5	2-6
Ciprofloxacin (0.002-32)	Susceptible	22	0.032- 0.064
	Intermediate	6	0.125- 0.5
Ceftriaxone (0.002-32)	Susceptible	29	0.094 – 0.25
	Resistant	11	0.75-1.5
Penicillin (0.002-32)	Susceptible	3	0.064
	Intermediate	5	0.12-0.1
	Resistant	32	2-32

## DISCUSSION

In the present study 40 *N. gonorrhoeae* strains were isolated from a total population of 172 symptomatic patients. Of these strains, 24/82 (29.3%) strains were isolated from STD clinic attendees (48 men and 34 women) and 16/90 (17.8%) were isolated from women attending gynaecological OPDs. The gonorrhoea patients were within the age of 24-37 years which is the reproductive age group. The majority (75%) were dependents, labourer and commercial sex workers. Only 23.5% of study population had the high school and university education. The similar finding was also seen in a study by Jolly *et al.* (1995) which stated core transmitters of gonorrhoea were young age, low socioeconomic status and high risk group for STDs eg. commercial sex workers [10].

The most common presentations were dysuria and urethral/vaginal discharge. Inguinal lymphadenopathy was seen in only 5% of patients and the systemic manifestation like fever, arthralgia and rash were seen in only one patient. Only 5% gave

history of previous STDs and 6% of patients had history of prior antibiotic treatment.

In the present study, there was satisfactory level of agreement for ciprofloxacin, ceftriaxone and penicillin between disc diffusion and E tests. This finding was compactable with the result reported by Bala *et al.*, [11]. Although E test MIC testing has advantage of producing more defined estimation of the level of resistance, it is expensive (approximately USD 15 for one test), disc diffusion which is simple, less expensive (approximately USD 8 for 50 tests) and feasible could remain the method of choice for routine testing.

The World Health Organization (WHO) recommended that antibiotic susceptibility testing should be performed against agents that are being used as primary therapies against uncomplicated gonorrhoea and agents that are used as alternative therapies. The worldwide prevalence of gonorrhoea and the emergence of antibiotic resistant *N. gonorrhoeae* reinforce the need for surveillance of its susceptibility to antibiotics commonly used for treatment. The antibiotics included in the currently recommended WHO regimen are ciprofloxacin, ceftriazone, cefixime, azithromycin and spectinomycin. Kanamycin and trimethoprim/sulphamethoxazole are the drugs for the alternative regimen.

A large portion of gonococcal isolates worldwide are now resistant to penicillin, tetracycline and other older antimicrobial agents [3]. The present study also exhibited the high percentage resistance to penicillin, tetracycline, chloramphenicol, trimethoprim/ cotrimoxazole, gentamycin, kanamycin and amikacin. The majority of *N. gonorrhoeae* isolates were susceptible to azithromycin (87.5%), cefixime (80%), ceftriaxone (>70%) and ciprofloxacin ( $\geq 55\%$ ). Recently, there were reports on the occurrence of high level resistance to ciprofloxacin ( $\text{MIC} \geq 4 \mu\text{g/ml}$ ) in Argentina, Israel and Taiwan [12, 13, 14] and our study also found out 30 % of high level

ciprofloxacin resistant cases among the tested isolates.

In Khin Mya Lwin's study during 1998, 6.7% of the gonococcal isolates from STD patients in Hlaingtharya township in Yangon were found to be less sensitive to penicillin, but susceptible to ciprofloxacin and ceftriazone [6]. Thus decreased susceptibility of gonococcal isolates to ceftriaxone and ciprofloxacin were noted in the present study. Fluoroquinolone-resistant gonococci have appeared in several Asian countries since early 1990 [15]. Although quinolones such as ciprofloxacin are recommended as the first line of therapy for gonorrhoea, the emergence of significant resistance to ciprofloxacin and presence of high level resistant strains will limit the usefulness of this drug. According to the *in vitro* susceptibility results, azithromycin, cefixime and ceftriazone were found to be effective against the majority of tested *N. gonorrhoeae* strains. However, we could not report the susceptibility of spectinomycin as its susceptibility result on the reference quality control strain (*N. gonorrhoeae* ATCC 49226) showed inconclusive results.

Our study revealed the susceptibility pattern of currently recommended antibiotics used for the treatment of gonococcal infection and these results could provide useful information to National AIDS/STD Control Program in Myanmar. The present study also highlighted that majority of the gonococcal isolates were susceptible to azithromycin, cefixime, ceftriaxone and the occurrence of high level ciprofloxacin resistant strains in the study population. Both disc diffusion and E test susceptibility testing methods gave reproducible results, disc diffusion method could remain as the first choice as it is cost-effective and more feasible in routine diagnostic laboratories in developing countries.

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## REFERENCES

1. World Health Organization (2001). Global prevalence and incidence of selected curable STDs overviews and estimates. [http://www.who.int/docstore/hiv/GRSTI/who\\_hiv\\_aids\\_2col\\_02pdf](http://www.who.int/docstore/hiv/GRSTI/who_hiv_aids_2col_02pdf) . Accessed on 12-4-06.
2. Brooks GF, Butel JS & Morse SA. The Neisseriae. In: *Jawetz, Melnick and Adelberg's Medical Microbiology* 23<sup>rd</sup> Edition . Lange Medical Brook / Mc Graw - Hill Medical Publishing Division, 2004; 295-304.
3. World Health organization Guidelines (2001). Guidelines for the management of STIs. [http://www.who.int/mipfiles/2226/who\\_hiv\\_aids\\_2001.01.pdf](http://www.who.int/mipfiles/2226/who_hiv_aids_2001.01.pdf). Accessed on 12-4-06.
4. Su X, Hutapea N, Tapsall JW & Lind I. Plasmid-mediated resistance of *Neisseria gonorrhoeae* strains isolated from female sex workers in North Sumatra, Indonesia, 1996. *Sexually Transmitted Disease* 2003; 30(2): 178-182.
5. Lawung R, Buatiang A, Jittawoutipoka T, Rittiroongrag S & Prachayasittikul V. Increasing trend of multiple resistance and genomic mobility of *Neisseria gonorrhoeae* to penicillin and quinolone. *Experimental and Clinical Sciences Journal* 2005; 4: 130-140.
6. Khin Mya Lwin, San San Min, Sann Myint, Mya Thidar & Soe Nyein . Prevalence of syphilis, gonorrhoea and chlamydial infections in women attending maternal and child health care centre in Hlaing Tharyar Township. *Myanmar Medical Journal* 1998; 42 (3, 4): 38-42.
7. Mar Mar Nyein, Ba Thike K, Khin Thet Wai, Khin May Oo, Wah Wah Aung, *et al.* Non-ulcerative STDs among married women in selected urban and semi urban area. *Myanmar Health Sciences Research Journal* 2003; 15(1-3): 5-9.
8. Van Dyck E, Meheus AZ & Piots P. Gonorrhoea. In: *Laboratory diagnosis of sexually transmitted diseases*, WHO publication, 1999; 1-21.
9. Biedenbach DA & Jones RN. Comparative assessment of E test for testing susceptibilities of *Neisseria gonorrhoeae* to penicillin, tetracycline, ceftrizone, cefotaxime, and ciprofloxacin: investigation using 510(k) Review Criteria, recommended by the Food and Drug Administration. *Journal of Clinical Microbiology* 1996; 34(12): 3214-3217.
10. Jolly AM, Orr P, Harmond G & Young TK. Risk factors for infection in women undergoing testing for *C. trachomatis* and *N. gonorrhoeae* in Manisota, Canada. *Journal of the American Venereal Disease Association* 1995; 22(5): 289-295.
11. Bala M, Ray K & Gupta SM. Comparison of disc diffusion results with mean inhibitory concentration (MIC) values for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. *Indian Journal of Medical Research*; July 2005 122:48-51.
12. Fiorito S, Galarza P, Pagano I & Oviedo C. Emergence of high level ciprofloxacin resistant *Neisseria gonorrhoeae* strains in Buenos Aries, Argentina. *Sexually Transmitted Infections* 2001; 77: 77.
13. Dan M, Poch F & Sheinberg B. High prevalence of high level ciprofloxacin resistance in *Neisseria gonorrhoeae* in Tel Aviv, Israel: Correlation with response to therapy. *Antimicrobial agents and chemotherapy* 2002; 46: 1671-1673.
14. Hsueh PR, Tseng S-P, Teng LJ & Ho SW. High prevalence of ciprofloxacin resistant *Neisseria gonorrhoeae* in northern Taiwan. *Clinical Infectious Diseases* 2005; 40: 188-192.
15. Ieven M, Van Looveren M, Sudidgoadi S, Rosana, Y, Goossens W, Lammens C, Neheus A & Goosens H. Antimicrobial susceptibilities of *Neisseria gonorrhoeae* strains isolated in Java, Indonesia, *Sexually Transmitted Diseases* 2003; 30(1): 25-29.