Original Article

Antimicrobial Susceptibility Pattern of *Neisseria gonorrhoeae* Isolated from Floating Female Commercial Sex Workers in Dhaka, Bangladesh

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ABSTRACT

A prospective study was carried out from June to December 1998 among 270 female commercial sex workers (CSWs) in Dhaka. The purpose of the study was to determine the sensitivity of different strains of *Neisseria gonorrhoeae* to 5 antibiotics (penicillin, tetracycline, spectinomycin, ceftriaxone and ciprofloxacin). The results showed that 83 cases (30.7%) were positive for *N. gonorrhoeae*. Seventeen of the isolates (20.5%) were positive for penicillinase production (penicillinase producing *N. gonorrhoeae*, PPNG). Concerning susceptibility to penicillin, 68.7 percent of the isolates were resistant and 31.3 percent were moderately susceptible. Concerning tetracycline, 87.9 percent isolates were resistant and the remaining 21.1 percent were moderately susceptible. None of the isolates were fully susceptible to either penicillin or tetracycline. Concerning ciprofloxacin, a total of 36.2 percent of the isolates were found resistant, 13.3 percent intermediately susceptible and 50.6 percent susceptible. All of the isolates (100%) were susceptible to both ceftriaxone and spectinomycin. The significant rates of resistance of gonococci to ciprofloxacin is of great concern and makes the treatment of gonococcal infection difficult. (*J Infect Dis Antimicrob Agents 2002;19:93-9.*)

INTRODUCTION

Gonorrhoea remains as an important cause of morbidity in sexually active people. The consequences of the infection include infertility, ectopic pregnancy, and perinatal morbidity. Infant blindness is another through maternal infection which remains common throughout developing countries.[1] In addition, along with other sexually transmitted diseases, gonococcal infection has been implicated as a co-factor in HIV transmission.[2] Treatment of gonococcal infection has been complicated by the rapid emergence of resistance to commonly used antimicrobials. There have been

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found reservoirs of gonococcal infection in commercial sex workers (CSWs).[3,4] The screening of the CSWs for gonococcal infection and their effective treatment might play a vital role in control of the disease. Antimicrobial susceptibility test results of the isolated gonococci can detect the emergence of new forms of resistance, and can monitor drifts in susceptibility to the treatment of choice. In the present study, female non-brothel-based CSWs of Dhaka city in Bangladesh were screened for gonococcal infection and antimicrobial susceptibility tests were done for the isolates of *Neisseria gonorrhoeae* (*N. gonorrhoeae*) to five commonly used antimicrobial agents.

MATERIALS AND METHODS

Study population and study design. A total of 270 non-brothel-based female CSWs, attending a government vagrant home for rehabilitation, were studied for a period of seven months from June to December 1998 in Dhaka, Bangladesh. On each day of specimen collection, after taking verbal consents, the CSWs were registered with an identification number. A short history regarding their profession to find out any relation with the disease conditions (if any) and any complaints suggestive of gonococcal infection (such as per vaginal discharge, dysuria, abdominal pain) were recorded. The CSWs were then referred to the examination table for collection of specimens of endocervical swabs in presence of paramedic staff. One lady physician helped in sample collection.

Exclusion criteria. 1) Those who were menstruating, 2) Those who took antibiotics in the previous two weeks.

Isolation and identification of gonococci. The specimens of endocervical swabs were inoculated on Modified Thayer Martin (MTM) medium plate and incubated at 37°C with CO_2 and moisture for next 48 hours. Plates were examined and colonies of *N. gonorrhoeae* were identified using recommended methods as described previously.[5] The colonies were then subcultured for pure isolation and kept until the antimicrobial susceptibility testing had been completed.

Beta-lactamase test. Gonococcal isolates were tested for beta-lactamase (penicillinase) production by the paper acidometric method.[5]

Antimicrobial susceptibility testing. Susceptibility testing of gonococcal isolates was carried out with five antimicrobial agents, namely penicillin, tetracycline, ceftriaxone, ciprofloxacin and spectinomycin. Antimicrobial susceptibility testing was done by both disk diffusion and agar dilution methods. Both methods were performed with GC agar using 1 percent defined supplement (Isovitalex, Oxoid, UK), inoculum made comparing with 0.5 McFarland standard and incubated with CO_2 as described earlier.[6]

Disk diffusion method. Antimicrobial disks containing penicillin (10 U), tetracycline (30 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg) and spectinomycin (100 mcg) (all from Oxoid, UK) were used for each isolate. Diameters of the zone inhibition (inhibitory zone diameters, IZDs) for each antimicrobial agents were recorded for individual gonococcal isolate.

Agar dilution method. Minimum inhibitory concentrations (MICs) were measured by two-fold serial dilutions using the following range of concentrations: penicillin 0.06-32.0 mcg/ml, tetracycline 0.06-32.0 mcg/ml, ceftriaxone 0.008-4.0 mcg/ml, ciprofloxacin 0.008-16.0 mcg/ml, and spectinomycin 4.0-128.0 mcg/ml. The MICs of antimicrobial agents were recorded as the lowest concentrations at which growth of an isolate was inhibited. The MIC_{50}s and MIC_{90}s for each of the antimicrobial agent were

calculated at which 50 percent and 90 percent of the isolates were inhibited respectively.

Susceptibility interpretation. The results of susceptibility testing were interpreted by correlating inhibitory zone diameters (IZDs) with the MICs following the standard criteria (Table 1).[6,7] Isolates were then defined as susceptible, intermediately/ moderately susceptible and resistant for each of the antimicrobial agent tested.

Resistant phenotypes. The gonococcal isolates were categorized into seven resistant phenotypes (Table 2) as described by Gorwitz et al.[8]

RESULTS

The ages of the CSWs study group was from 12 to 45 years, with the mean age of 20 years. One hundred and seventy-two (63.7%) were 18-30 years, 79 (29.3%) were less than 18 years and only 19 (7.0%) were over 30 years. One hundred and twenty-one (44.8%) had been in the profession for less than 6 months, 70 (25.9%) for 1-2 years, 43 (15.9%) for 6-11 months and only 36 (13.3%) for more than 2 years.

N. gonorrhoeae were isolated in 83 cases (30.7%) of cases. The majority of the positive cases (51/83, 61.4%) were asymptomatic, and only 38.6 percent (32/83) had symptoms (discharge per

Table 1. Interpretative criteria of susceptibility of *N. gonorrhoeae* isolates.

Antimicrobial agents and disk contents	Inhibitory zone diameter (corresponding MIC in mcg/ml)		
	Susceptible	Moderate/intermediate susceptible	Resistant
Pencillin (10 U)	≥ 47 (≤ 0.06)	7-46 (0.12-1.0)	≤ 26 (≥ 2.0)
Tetracycline (30 mcg)	≥38 (≤ 0.25)	31-37 (0.5-1.0)	≤ 30 (≥ 2.0)
Ciprofloxacin (5 mcg)	≥ 36 (≤ 0.06)	30-35 (0.12-0.5)	≤ 29 (≥ 1.0)
Ceftriaxone (30 mcg)	≥ 35 (≤ 0.25)	NA	NA
Spectinomycin (100 mcg)	≥18 (≤ 32.0)	15-17 (64.0)	≤ 14 (≥ 128.0)

Note: NA = not available

Table 2. Resistant phenotypes of *N. gonorrhoeae*.

Sl no	Phenotypes	Criteria
01	Penicilinase producing *N. gonorrhoeae* (PPNG)	β-lactamase positive with tetracycline MIC < 16.0 mcg/ml
02	Plasmid-mediated tetracycline-resistant *N. gonorrhoeae* (TRNG)	β-lactamase negative with tetracycline MIC ≥ 16.0 mcg/ml
03	PPNG-TRNG	β-lactamase positive with tetracycline MIC ≥ 16.0 mcg/ml
04	Chromosomally mediated resistance of *N. gonorrhoeae* to penicillin only ($CMRNG^{PO}$)	Non-PPNG with penicillin MIC ≥ 2.0 mcg/ml and tetracycline cycline MIC < 2.0 mcg/ml
05	Chromosomally mediated resistance of *N. gonorrhoeae* to tetracycline only ($CMRNG^{TO}$)	Non-PPNG with penicillin MIC < 2.0 mcg/ml and tetracycline MIC 2.0-8.0 mcg/ml
06	Chromosomally mediated resistance of *N. gonorrhoeae* to both penicillin and tetracycline ($CMRNG^{PT}$)	Non-PPNG with penicillin MIC ≥ 2.0 mcg/ml and tetracycline MIC 2.0-8.0 mcg/ml
07	Moderately susceptible *N. gonorrhoeae* to either penicillin or tetracycline ($MSNG^{P/T}$)	Non-PPNG with penicillin MIC 0.12-1.0 mcg/ml and tetracycline MIC 0.5-1.0 mcg/ml

vagina, dysuria, abdominal pain) of gonococcal infection.

Seventeen isolates of *N. gonorrhoeae* (20.5%) were positive for beta-lactamase production, 14 of them were truly PPNG and the other 3 were PPNG-TRNG phenotypes (Table 3).

Fifty-seven isolates of *N. gonorrhoeae* (68.7%), were resistant to penicillin, and the rest 26 (31.3%) were moderately susceptible. Seventy-three isolates (87.9%) of the isolates were resistant to tetracycline and 10 isolates (12.1%) were moderately susceptible. None of the gonococcal isolate was susceptible to either penicillin or tetracycline. Thirty isolates (36.1%) were resistant to ciprofloxacin, 11 (13.3%) had intermediate susceptibility and (50.6%) were found susceptible to ciprofloxacin. All 83 isolates (100%) were susceptible to both ceftriaxone and spectinomycin (Table 4).

All of the 17 PPNG (14 PPNG + 3 PPNG-TRNG) strains were resistant to both penicillin and tetracycline, but 14 strains (82.3%) were susceptible to other three (ceftriaxone, ciprofloxacin and spectinomycin) of the remaining antimicrobials. At least 6 of the 15 TRNG strains (40%) were susceptible to remaining four antibiotics. Whereas, at least 13 of the 28 CMRNG ^{PT} strains (46.4%) were susceptible to three antibiotics. (Table 3).

The MIC parameters (MIC_{50}s, MIC_{90}s and the range of MICs) for each of the antibiotic is shown in the Table 5.

DISCUSSION

Antimicrobial resistance in *N. gonorrhoeae* is an important public health concern throughout the world. The CDC recommends the first-line treatment with fluoroquinolones along with few others.[9] But the highly adaptive gonococci are becoming resistant to this important drug. The first reported cases of fluoroquinolone resistance in gonococci were published in 1989 from North America by Yeung and Dillon.[10] Since then cases of fluoroquinolone-resistant *N. gonorrhoeae* have been then reported throughout the world i.e. USA[11], UK[12] and the Southeast Asia (Hong Kong and Sri Lanka).[13,14] The first ciprofloxacin-resistant *N. gonorrhoeae* in Bangladesh was reported by Bhuiyan et al in 1999[15] who found almost 12 percent of isolates were ciprofloxacin-resistant. Our study reports 30.6 percent ciprofloxacin-resistant isolates which has increased significantly from the previous

Table 3. Distribution of the isolates into seven resistant phenotypes of *N. gonorrhoeae* in relation to the individual antimicrobial agents tested.

Resistant phenotypes	No. (%) of isolates	No. (%) of isolates resistant to antimicrobial agents				
		PEN	TET	CIP	CRO	SPT
1. PPNG	14(16.9)	14(100)	14(100)	3(21.4)	0(0)	0(0)
2. TRNG	15(18.1)	9(60.0)	15(100)	6(40.0)	0(0)	0(0)
3. PPNG-TRNG	3(3.6)	3(100)	3(100)	0(0)	0(0)	0(0)
4. CMRNG^{PO}	3(3.6)	3(100)	0(0)	0(0)	0(0)	0(0)
5. CMRNG^{TO}	13(15.7)	0(0)	13(100)	4(30.7)	0(0)	0(0)
6. CMRNG^{PT}	28(33.7)	28(100)	28(100)	15(53.6)	0(0)	0(0)
7. MSNG$^{P/T}$	7(8.4)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	83(100)	57(68.7)	73(87.9)	30(36.1)	0(0)	0(0)

Note: PEN=penicillin, TET=tetracycline, CRO=ceftriaxone, CIP=ciprofloxacin, SPT=spectinomycin.

Table 4. Susceptibilities of *N. gonorrhoeae* isolates to the antimicrobial agents tested, classified into the 17 PPNG and 66 non-PPNG strains.

Antimicrobial agents	No. of isolates (%) in three susceptibility categories		
	Susceptible	Moderately/intermediately susceptible	Resistant
1. Penicillin			
Non-PPNG	0 (0)	26 (39.4)	40 (60.6)
PPNG	0 (0)	0 (0)	17 (100)
Total	0 (0)	26 (31.3)	57 (68.7)
2. Tetracycline			
Non-PPNG	0 (0)	10 (15.1)	56 (84.9)
PPNG	0 (0)	0 (0)	17 (100)
Total	0 (0)	10 (12.1)	73 (87.9)
3. Ciprofloxacin			
Non-PPNG	30 (45.5)	9 (13.6)	27 (40.9)
PPNG	12 (70.6)	2 (11.7)	3 (17.7)
Total	42 (50.6)	11 (13.3)	30 (36.1)
4. Ceftriaxone			
Non-PPNG	66 (100)	0 (0)	0 (0)
PPNG	17 (100)	0 (0)	0 (0)
Total	83 (100)	0 (0)	0 (0)
5. Spectinomycin			
Non-PPNG	66 (100)	0 (0)	0 (0)
PPNG	17 (100)	0 (0)	0 (0)
Total	83 (100)	0 (0)	0 (0)

Table 5. The MIC parameters of the isolates of *N. gonorrhoeae* for five antimicrobial agents tested.

Antimicrobial agents	MIC (mcg/ml)		
	90%	50%	Range
1. Penicillin	116.0	2.0	0.25-32.0
2. Tetracycline	216.0	2.0	0.5-64.0
3. Ceftriaxone	30.25	0.06	0.008-0.25
4. Ciprofloxacin	48.0	0.06	0.008-32.0
5. Spectinomycin	32.0	8.0	2.0-32.0

study in Bangladesh. This increasing level resistance to ciprofloxacin could indicate adaptive mutations of gonococci under selective pressure, possibly due to the indiscriminate use of the antibiotic.

All the isolates in this study were susceptible to ceftriaxone and spectinomycin, which are effective alternatives. However, these drugs should not be used as the primary choice, because gonococcal resistance to spectinomycin has been reported from the UK[16] and Korea[17] when using it primarily.

From our study and others, we conclude that penicillin and tetracycline are no longer useful for the treatment of gonococcal infection. Ciprofloxacin and ceftriaxone should remain as the primary treatment but susceptibility test should be undertaken. Spectinomycin should be reserved for the ciprofloxacin- and ceftriaxone-resistant isolates as well as for the cases of ciprofloxacin contraindication e.g. pregnancy. New drugs should be sought for gonococcal susceptibility being guided by the WHO multicentre Gonococcal Antimicrobial Susceptibility Project (GASP). Countries such as Bangladesh, should be included where antibiotics are used unscrupulously and without prescription. This project could help to monitor emerging resistant strains and aid in effective treatment to halt the further spread of the resistant organisms.

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