

Changing trends of antimicrobial susceptibility patterns of *Neisseria gonorrhoeae* in India and the emergence of ceftriaxone less susceptible *N. gonorrhoeae* strains

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Objectives: To monitor the trend of antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from 2002 to 2006 in New Delhi, India under the Gonococcal Antimicrobial Susceptibility Programme and to document the emergence of any new antimicrobial resistance.

Methods: Antimicrobial susceptibility of 382 *N. gonorrhoeae* isolates from clinical cases in males and females to penicillin, tetracycline, ciprofloxacin, spectinomycin and ceftriaxone was determined by disc diffusion technique, using WHO reference strains as controls and WHO interpretative criteria. MICs were determined using Etests.

Results: A significant increasing trend of penicillin and ciprofloxacin resistance up to 2003 and 2004, respectively, and subsequent decrease in resistant strains with a concomitant increase in less susceptible strains, was observed. Tetracycline-resistant *N. gonorrhoeae* increased significantly from 6.7% in 2002 to 22.9% in 2005. Only one isolate was resistant to spectinomycin and nine isolates were less susceptible to ceftriaxone, during this 5 year period. A substantial proportion (23.3%) of strains were multiresistant.

Conclusions: Emergence of ceftriaxone less susceptible *N. gonorrhoeae* isolates is a cause for concern, although treatment failure was not observed. An active, continuous and comprehensive programme for monitoring and surveillance of antimicrobial resistance needs to be established in many laboratories, and a search for new effective agents needs to be initiated to respond to the emergence of resistant isolates.

Keywords: gonococci, antimicrobial surveillance, GASP, multiresistant strains

Introduction

Gonorrhoea is one of the most common sexually transmitted diseases (STDs) in developing countries and is a global health problem. The prevalence of gonorrhoea in males aged between 15 and 49 years was estimated to be ~2% in Sub-Saharan Africa, 1% in South and Southeast Asia and 0.6% in South and Central America, whereas the prevalence is almost one-tenth in the industrialized countries.¹ Over the last decade, *Neisseria gonorrhoeae* strains have developed a high level of resistance against several antimicrobial agents such as penicillin, tetracycline and quinolones in different countries including India,^{2–10} posing an increasing problem in the management of gonorrhoea. Reduced susceptibility to third-generation cephalosporins used

at present as first-line therapy, although rare, has been reported from some countries,^{10–14} but not from India and many other countries.^{2–8} Therefore, continuous surveillance of antimicrobial resistance of *N. gonorrhoeae* is essential to monitor its emergence and spread and to provide a rational basis for formulating national treatment guidelines. Moreover, surveillance can detect empirical levels of 5% resistance suggested by the WHO as a trigger for treatment change in terms of disease control.¹⁵

The WHO, therefore, established a surveillance programme in different regions of the world known as the Gonococcal Antimicrobial Surveillance Programme (GASP) in 1990.¹⁶ GASP is important in assisting health providers in making recommendations regarding effective antibiotics for treatment.

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Antimicrobial susceptibility pattern of *N. gonorrhoeae*

The regular monitoring of antimicrobial susceptibility has been carried out at this centre from 1995 to date.^{8,9,17} Since December 1999, the work was sponsored by WHO-Southeast Asia Region (SEAR) for 3 years under GASP.

In this article, we report the trend of antimicrobial resistance of *N. gonorrhoeae* isolated from 2002 to 2006 and the emergence of ceftriaxone less susceptible strains, which first appeared in 2001.⁹

Materials and methods

Study population

A total of 563 male patients clinically presenting with acute urethritis and 4153 female patients presenting with cervical/vaginal discharge to a male and female STD clinic of the Regional STD Teaching, Training and Research Centre in New Delhi from January 2002 to December 2006 were included in the study.

Isolation and identification of *N. gonorrhoeae*

N. gonorrhoeae was grown and identified by standard procedures.¹⁸

β -Lactamase testing

N. gonorrhoeae isolates were tested for β -lactamase production by the chromogenic cephalosporin method using nitrocefin freeze-dried powder (Oxoid).¹⁸

Antibiotic susceptibility testing

Antibiotic susceptibility testing of 382 *N. gonorrhoeae* isolates was performed by the calibrated dichotomous sensitivity test as per standard methodology.¹⁸ The strains were defined as susceptible, less susceptible and resistant.^{18,19} The MICs of penicillin, tetracycline, ciprofloxacin, spectinomycin and ceftriaxone were determined by the Etest method (AB Biodisk, Solna, Sweden). Multiresistant isolates were defined as: quinolone-resistant *N. gonorrhoeae* (QRNG) + penicillinase-producing *N. gonorrhoeae* (PPNG); QRNG + tetracycline-resistant *N. gonorrhoeae* (TRNG) and QRNG + PPNG + TRNG.

Control strains

WHO reference strains A–E, G, L, O and Q were used as controls for disc diffusion and MIC testing.

External quality assurance scheme

This centre participated in external quality assurance scheme (EQAS) of Gonococcal Antimicrobial Susceptibility Testing from 2001 to 2006 conducted by the *Neisseria* Reference Laboratory, WHO Collaborating Centre for STD and HIV, Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia. Under this EQAS, six lyophilized QA strains were received every year from the reference laboratory. The results of antimicrobial susceptibility testing of these strains were communicated and feedback was received.

Statistical analysis

The differences in percentages were statistically compared, tested for significance by using the χ^2 test and *P* values were determined.

Results

A total of 386 (68.6%) specimens from male patients and 26 (0.6%) from female patients were smear positive for *N. gonorrhoeae*. *N. gonorrhoeae* was isolated in 76.8% of male patients and in 0.8% of female patients.

Antibiotic susceptibility testing by disc diffusion technique revealed that 181 (47.4%) isolates were resistant to penicillin, out of which 81 (21.2%) were β -lactamase-positive. Out of 301 (78.8%) β -lactamase-negative isolates, 164 (42.9%) had reduced susceptibility to penicillin. Penicillin resistance increased significantly ($\chi^2 = 4.12$, $P < 0.05$) from 53.8% in 2002 to 68.4% in 2003 and thereafter there was a highly significant ($\chi^2 = 32.31$, $P < 0.001$) decrease to 18.2% in 2006 with a concomitant significant ($\chi^2 = 28.95$, $P < 0.001$) increase to 70.9% in 2006 in penicillin less susceptible strains. An increasing trend of TRNG was observed ($\chi^2 = 9.89$, $P < 0.01$) from 6.7% in 2002 to 22.9% in 2005. Ciprofloxacin resistance increased significantly ($\chi^2 = 10.61$, $P < 0.01$) from 80.7% in 2002 to 97.2% in 2004 and subsequently decreased to 83.6% in 2006 ($\chi^2 = 7.14$, $P < 0.01$). All of the isolates were susceptible to spectinomycin

Table 1. Trend of resistance of *N. gonorrhoeae* to different antimicrobials on the basis of disc diffusion results (2002–06)

Year	No. tested	n (%)					
		penicillin		tetracycline	ciprofloxacin		ceftriaxone
		LS	R	TRNG	LS	R	LS
2002	119	42 (35.3)	64 (53.8)	8 (6.7)	19 (16.0)	96 (80.7)	2 (1.7)
2003	76	18 (23.7)	52 (68.4)	9 (11.8)	6 (7.9)	66 (86.8)	1 (1.3)
2004	71	29 (40.8)	35 (49.3)	11 (15.5)	2 (2.8)	69 (97.2)	1 (1.4)
2005	61	36 (59.0)	20 (32.8)	14 (22.9)	7 (11.5)	54 (88.5)	2 (3.3)
2006	55	39 (70.9)	10 (18.2)	10 (18.2)	8 (14.5)	46 (83.6)	3 (5.5)
Total	382	164 (42.9)	181 (47.4)	52 (13.6)	42 (11.0)	331 (86.6)	9 (2.4)

LS, less susceptible; R, resistant.

Table 2. Susceptibility pattern of ceftriaxone less susceptible strains to other antimicrobials

Year	CRO		CIP		PEN		TET		SPE		β-Lactamase	Treatment given	Response to treatment
	AR (mm)	MIC (mg/L)	AR (mm)	MIC (mg/L)	AR (mm)	MIC (mg/L)	AR (mm)	MIC (mg/L)	AR (mm)	MIC (mg/L)			
2002	7	0.064	5	0.38	3	2	8	0.75	9	3	negative	inj. CRO 250 mg	responded
2002	7	0.064	1	ND	3	1.5	9	0.5	7	4	negative	inj. CRO 250 mg	responded
2003	6	0.094	0	3	8	0.38	12	0.25	12	1	negative	inj. CRO 250 mg	responded
2004	6	0.064	0	2	0	0.50	5	2	9	6	negative	inj. CRO 250 mg	responded
2005	4	0.094	3	1.0	4	0.38	7	0.38	7	3	negative	tab. CFM 400 mg	responded
2005	6	0.064	0	3	5	0.19	6	0.75	7	4	negative	inj. CRO 250 mg	responded
2006	6	0.064	0	12	0	2	9	0.75	10	4	positive	tab. CFM 400 mg	responded
2006	7	0.064	0	2	4	0.25	10	0.25	10	2	negative	tab. CFM 400 mg	responded
2006	6	0.064	0	3	1	2	0	16	9	16	negative	tab. CFM 400 mg	responded

CRO, ceftriaxone; CIP, ciprofloxacin; PEN, penicillin; TET, tetracycline; SPE, spectinomycin; CFM, cefixime; AR, annular radius; ND, not done.

except one which was found resistant in 2002.¹⁷ Ceftriaxone less susceptible strains varied from 1.3% to 1.7% between 2002 and 2004, but an insignificant rise ($\chi^2 = 1.92$) to 5.5% was observed in 2006 (Table 1).

Multiresistant strains were observed in 89 (23.3%) cases, out of which 50 (13.1%), 27 (7.1%) and 12 (3.1%) were QRNG + PPNG, QRNG + TRNG and QRNG + PPNG + TRNG, respectively.

EQAS results every year showed almost 100% agreement with the reference laboratory expected results, except that in 2002 one strain for ceftriaxone and in 2004 one strain for penicillin showed some disagreement of results. On repeat testing, 100% agreement was observed.

Table 2 shows the susceptibility pattern of ceftriaxone less susceptible strains to other antimicrobials. Out of these nine strains, eight strains were either resistant to quinolones or quinolones and penicillin/tetracycline. Two strains were multiresistant, i.e. one strain was QRNG + PPNG and one was QRNG + TRNG. However, all these strains were susceptible to spectinomycin.

Discussion

During the period of study, the number of isolates decreased every year, from 119 in 2002 to 55 isolates in 2006. It may be either due to actual decreasing incidence of gonorrhoea over the years or due to the fact that gradually fewer patients were reporting to the clinic because of easy availability of antimicrobials as a part of syndromic management of STDs in peripheral and private health set-ups. The isolation rate was quite low in females; maybe because most of the female patients were referred from Gynaecology clinic to STD clinic after they did not respond to syndromic management of genital discharges. Besides, most of the female patients were not having purulent/mucopurulent cervical discharge and thus were not cases of gonorrhoea. Culture positivity was higher than smear positivity in both males and females, indicating the higher sensitivity of culture.

Effective surveillance of antimicrobial resistance is expected to monitor trends in established types of resistance and promptly identify new types of resistance. Data from this centre, of the

first 7 years,^{8,9} have documented the pattern of antimicrobial resistance in *N. gonorrhoeae*, highlighting the alarming increase in ciprofloxacin and penicillin resistance from 1996 to 2001. These formed the basis for the national guidelines for the treatment of gonorrhoea, i.e. the use of ceftriaxone as first-line therapy.

The continued high prevalence of penicillin resistance up to 2003 followed by the decrease up to 2006 may reflect the loss of selective pressure from the disuse of penicillin as treatment for gonorrhoea. In the present study, 21.2% of isolates were found to be PPNG and the results compare well with a recent study from India.⁵ In contrast, Bhalla *et al.*^{6,7} in Delhi reported 8% and 11.1% of isolates to be PPNG in 1998 and 2002, respectively. In SEAR countries like Bangladesh,¹⁴ 16.1% of strains in 1997 and 8% in 1998 were reported to be PPNG; in Indonesia,²⁰ 63.1% strains were PPNG; and in Sri Lanka,²¹ PPNG rates had declined considerably. In WHO Western Pacific Region (WPR), the prevalence of PPNG varied from 1% to 90%.¹³

The increasing trend of TRNG observed in this study may reflect ongoing selective pressure produced by the use of tetracyclines to treat other infections and its use as adjunct therapy in the syndromic management of STDs. Tetracycline resistance was observed to be 51% in another city from India without any mention of TRNG.⁵ In contrast, Bhalla *et al.*^{6,7} reported 28% and 2.8% of isolates as TRNG in 1998 and 2002, respectively. In WHO SEAR, our results are comparable with the data from Bangladesh,¹⁴ where 14% and 17.4% isolates were TRNG in 1998 and 1999, respectively, but in contrast to a study from Indonesia²⁰ reporting 34.4% strains as TRNG. In the WHO WPR, reported TRNG ranged from 1% to 97%.¹³

The use of the quinolone group of antibiotics for the treatment of gonorrhoea has been discontinued in India for quite some time because of reported high levels of resistance. However, there were considerable differences in rates of quinolone resistance in different studies in India.^{5-7,22} QRNG is also widely distributed in other WHO SEAR and WPR countries.^{13,14,20-23}

It is fortunate that, except for one strain,¹⁷ spectinomycin resistance has not been reported from this centre as it is an alternative drug of choice for cases having hypersensitivity to cephalosporins. Spectinomycin is not easily available in India

and this may explain the retention of efficacy of this antibiotic. Resistance to spectinomycin was not observed in other Asian countries like Bangladesh¹⁴ and Indonesia.²⁰ However, another study from Indonesia²⁴ reported 18.1% strains as resistant. A small number of spectinomycin-resistant strains were found in China and Vietnam.¹³ Only very occasional strains resistant to this injectable antibiotic were observed in WPR surveys.¹³

The frequency of multiresistant isolates found (23.3%) in the present study is quite high in comparison with that found in Greece (16.5%),² Bangladesh (6.1%)¹⁴ and Europe (15.6%).²⁵

Ceftriaxone, a third-generation cephalosporin, has been used as a first-line drug for the treatment of gonorrhoea for the last 10 years in this centre. In most countries, including India,^{26,27} a 250 mg intramuscular dose is used in contrast to the 125 mg regimen recommended in the USA. This recommendation for a higher dose treatment would appear to be appropriate, in view of the increase in MICs of ceftriaxone observed for *N. gonorrhoeae* in some countries.^{10–14} Ceftriaxone less susceptible strains were detected for the first time in 2001 from this centre.⁹ Reduced susceptibility towards ceftriaxone was also reported by some laboratories in India and neighbouring SEAR countries.²² However, two strains isolated from one of the focal point laboratories in India, showing reduced susceptibility towards ceftriaxone, could not be confirmed at the Regional Reference Laboratory (RRL), New Delhi. Ceftriaxone less susceptible strains (unconfirmed by MIC) from other focal point laboratories were not received at the RRL for confirmation.²² In the present study, the number of isolates remained low at ~1% of all isolates tested up to 2004 and an insignificant increase thereafter was observed up to 2006. These strains almost always exhibited resistance to quinolones or quinolones and penicillin. These findings are consistent with recent data from Australia and Japan, suggesting increasing prevalence of these multiresistant strains in those regions.^{28–31} All the nine cases having strains less susceptible to ceftriaxone responded to treatment with ceftriaxone or cefixime. Treatment failures are documented with oral third-generation cephalosporins such as cefixime and cefdinir but not as yet with ceftriaxone.^{28,30}

Emergence of ceftriaxone less susceptible/resistant strains, as a consequence of excess utilization of oral third-generation cephalosporins for other infectious conditions, may pose a serious problem in the management of gonorrhoea in countries like India and emphasizes the importance of maintaining a robust GASP all over the country to monitor trends in antimicrobial susceptibility, so as to enable the public health managers to modify the national treatment guidelines from time to time. At the same time, a search for new effective agents should be initiated in important centres in collaboration with national authorities.

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Transparency declarations

None to declare.

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