

Prevalence and antibiotic susceptibility of *neisseria gonorrhoeae* in HIV positive and hiv negative cases at a tertiary care hospital

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Abstract

Introduction: Gonorrhoea is one of the most common sexually transmitted infections (STIs) in developing countries, and the emergence of resistance to antimicrobial agents in *Neisseria gonorrhoeae* is a major hindrance in the control of gonorrhoea. Over the past decade, *Neisseria gonorrhoeae* has developed resistance against commonly used antibiotics.

Method: This was a prospective study done for a period of 3 years, both in men and women with the age group of (15 – 50 years) with any signs and symptoms for STIs were included. Discharge specimens like urethral, vaginal and endocervical were collected under strict aseptic conditions and smears were prepared for Gram staining and culture was done on selective and non-selective agar media. Antibiotic susceptibility was done according to Clinical and Laboratory Standards Institute guidelines.

Results: Antibiotic resistance was higher in gonorrhoeal patients co-infected with HIV. The overall prevalence of gonorrhoea and its co-infection with HIV was 9% and 23.6% respectively. Highest resistance was seen with Penicillin, Tetracycline, Nalidixic acid, Ciprofloxacin, Cefotaxime and Cefoxitin in both positive and negative cases of HIV.

Conclusion: Periodic monitoring of antimicrobial sensitivity profile will help in detecting change in susceptibility pattern and the emergence of drug-resistant strains.

Key words: *Neisseria gonorrhoeae*, Sexually Transmitted Infections (STI), Human Immune Deficiency Virus (HIV), Antibiotics, Resistance

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Introduction

Sexually Transmitted Infections (STIs) present a major public health problem in both developed and developing countries. Gonorrhoea is caused by a bacterium *Neisseria gonorrhoeae* (*N. gonorrhoeae*), which is the second commonest bacterial STI in the world^[1,2]. Gonorrhoea primarily infects the urogenital tract leading to intense local inflammation and a wide range of clinical manifestations^[3]. Infection caused by *Neisseria gonorrhoeae* has a disproportionate impact on the health of the patient. It can be symptomatic or asymptomatic, and in some cases it may lead to Disseminated Gonococcal Infections (DGI)^[4]. Studies conducted in India have reported prevalence of gonorrhoea between 0 to 22%^[5,6].

The large majority of infection in women is asymptomatic. Asymptomatic infections are less commonly seen in men, but still represent an important reservoir for transmission. In India the prevalence of HIV infection is alarmingly higher among female sex workers and men attending clinics for Sexually

Transmitted Disease. STIs facilitate HIV transmission by penetrating protective mucosal barriers and drafting susceptible immune cells to the site of infection. Ulcerative and non-ulcerative STI create gateways for the entry of HIV to access susceptible cells. The association between ulcerative STI and HIV transmission is well established. STI causes genital bleeding, which increases the risk of exposure to HIV during sexual activity. Gonorrhoea coinfecting with HIV can transmit HIV more easily and the detection of *Neisseria gonorrhoeae* especially in HIV infected patients is of paramount importance^[7].

Over the past decade, *Neisseria gonorrhoeae* has developed resistance against commonly used antibiotics such as penicillin, tetracycline and quinolones in different countries^[8-10]. Data on antimicrobial susceptibility of *N. gonorrhoeae* will help in selection of an appropriate antimicrobial agent especially in the treatment of gonorrhoeal patients co-infected with HIV. In our study an increase in the resistance of commonly used antimicrobials in HIV positive and HIV negative co-infected cases indicates the need for alertness in their use for the treatment, as well as the importance of laboratory based surveillance for guidance to modify the STD management guidelines of the country from time to time. The aim of this study is to determine the prevalence and drug susceptibility pattern of *Neisseria gonorrhoeae* and its correlation in HIV positive and HIV negative case.

Material and Methods

A prospective study was conducted in the department of Gynecology OPD, General Medicine, Dermatology and STD clinic of Khaja Bandanawaz Institute of Medical Sciences in south India from January 2013 to December 2015 to find correlation between *Neisseria gonorrhoeae* and HIV and its antibiogram. Both the genders of 15 – 50 years age group with any one of the sign and symptoms of STIs were included.

The research study was approved by the Institutional Ethics Committee and the written questionnaire contained information on name, Age, sexual partners, residence, married life, no of children's, occupation, Clinical history/complaint of white discharge colour, odor, appearance-thick viscous, mucoid, scanty and with blood and conditions like lower abdominal pain, burning micturition, itching, dysperunia, pharyngitis, rectal infection and HIV positive or HIV negative, were obtained from participants. The diagnostic test results were given to the patients and proper treatment was also delivered according to NACO guidelines.

Specimen processing

Discharge specimens like urethral vaginal, endocervical were collected from patients suspected of having gonorrhoea. Specimens was collected under strict aseptic conditions and smears were prepared for Gram staining and were cultured on enriched and selective media. Two samples were taken, one for culture and second for direct microscopy.

A sterile, Dacron swab was used for collection of discharge or sterile bacteriological loop was passed 3-4 cm into the urethra. Gram-stained smears of urethral exudate were examined under 1000 x magnification in oil immersion for the presence of polymorphonuclear cells and Gram-negative diplococci. The results of the smears were considered positive if typical Gram-negative diplococci were seen, whether located intracellularly or extracellularly.

Other swab was inoculated immediately on Chocolate agar and Modified Thayer Martin (MTM) medium plate and the plates were incubated at 36°C enriched with CO₂ for 24-48 hours. CO₂ was provided by candle extinction jar. Colonies of *N. gonorrhoeae* was identified by colony morphology, gram stain and biochemical tests^[11]. *Neisseria gonorrhoeae* was differentiated from other *Neisseria* species based on the fermentation of glucose with the production of acid only and not from other sugars like sucrose, lactose, maltose and mannitol. Oxidase test and Superoxal test were also found positive in all culture positive isolates.

Serum samples were collected from all the gonococcal cases and detection of HIV was done with

NACO approved kits like TRIDOT test (Diagnostic Enterprises) and confirmed by MICROLISA 4th generation kit (J.Mitra & Co.Pvt.Ltd).

Antibiotic sensitivity testing The inoculum was prepared from 24 hours of pure culture from chocolate agar medium and a homogenized suspension was prepared in 5 ml of sterile saline solution and turbidity was adjusted to an equivalent of 0.5 McFarland opacity standard test tubes. Same suspension was used for the sensitivity testing within 15 min.

Antibiotic susceptibility was done using Modified Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotics were used. Penicillin (10 IU), Tetracycline (30µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Cefixime (5µg), Cefoxitin (30µg), Cefotaxim (10µg), Nalidixic acid (30µg). The antibiotics were placed on the surface of an agar plate containing a nonselective medium that was inoculated with a suspension of a strain of *N. gonorrhoeae* to give a confluent lawn culture of growth. *N. gonorrhoeae* ATCC 49226 was used as a control strain. The antibiotics were placed on the media and incubated at 36°C for 24 hours, the drug diffuses into the medium, causing a zone of inhibition of growth of the strain around the disk corresponding to the susceptibility of the strain to the agent.

Results

A total of 611 cases were evaluated for gonorrhoea attending the STD and Non- STD clinic. Out of 611 cases, 55 were positive for gonorrhoea. Among the 55 cases, 42 were HIV negative patients and 13 were HIV positive patients. The age and gender distribution of the cases is shown in Table 1.

Table 1: Age and gender wise distribution of the cases

Age group	Male n (%)	Female n (%)	Total
15-20	5 (9.1)	6 (10.9)	11
21-24	6 (10.9)	10 (18.2)	16
26-30	4 (7.2)	13 (23.6)	17
31-35	1 (1.8)	3 (5.5)	4
36-40	1 (1.8)	3 (5.5)	4
41-45	0 (0)	1 (1.8)	1
46-50	1 (1.8)	1 (1.8)	2
Total	18 (32.7)	37 (67.3)	55

Maximum cases were in Females and in the age group of 26-30 years. Overall gonorrheal infection among males were 32.7% and in females 67.3%

The sex distribution of gonorrhoea cases in relation to HIV status is shown in Table 2.

Table 2: Sex distribution of gonorrhoea cases in relation to HIV status

Gender	HIV positive		HIVnegative		Total
	Number	%	Number	%	
Male	7	12.7	11	20	18
Female	6	10.9	31	56.3	37
Total	13	23.6	42	76.4	55

Maximum cases of gonorrhoea (76.4 %) were isolated from HIV negative patients. The female predominance (56.3%) was higher in HIV negative patients when compared to HIV positive cases.

Pattern of Gonorrhoea in HIV positive and HIV negative cases are shown in Table 3.

Table 3: Pattern of Gonorrhoea in HIV positive and HIV negative cases

Specimen	HIV Positive n=13	%	HIV Negative n =42	%	Total n =55	%
Urethral discharge	2	15.3	4	9.5	6	10.9
Vaginal discharge	3	23.0	9	21.4	12	21.8
Cervical discharge	2	15.3	5	11.9	7	12.7
Urine	1	7.7	2	4.7	3	5.4
Oropharyngeal	0	0	1	2.3	1	1.8
Conjunctiva	0	0	1	2.3	1	1.8
Sterile body fluids	1	7.7	1	2.3	2	3.6
Molluscum contagiosum	1	7.7	2	4.7	3	5.4
Balanoposthitis	0	0	2	4.7	2	3.6
Genital ulcer disease	1	7.7	3	7.1	4	7.2
Non-genital ulcer disease	1	7.7	5	11.9	6	10.9
Lower abdominal pain syndrome	1	7.7	7	16.6	8	14.5

Maximum cases of gonorrhoea in HIV and Non HIV male patients were obtained from urethral discharge, whereas in females maximum samples were obtained from vaginal discharge.

The pattern of cases isolated from STD and the non STD clinic is shown in Table 4.

Table 4: Pattern of cases isolated from STD and non STD clinic

Sex	STD Clinic		Non STD Clinic	
	Number	%	Number	%
Male	13	23.6	5	9.1
Female	21	38.2	16	29.1
Total	34	61.8	21	38.2

STD = Sexually Transmitted Diseases

Maximum cases of *Neisseria gonorrhoeae* among male and female (61.8 %) were obtained from STD clinic were as (38.2 %) patients were found from NonSTD clinic.

Percentage of Antibiotic resistance pattern of *Neisseria gonorrhoeae* in relation to HIV cases is shown in Fig. 1.

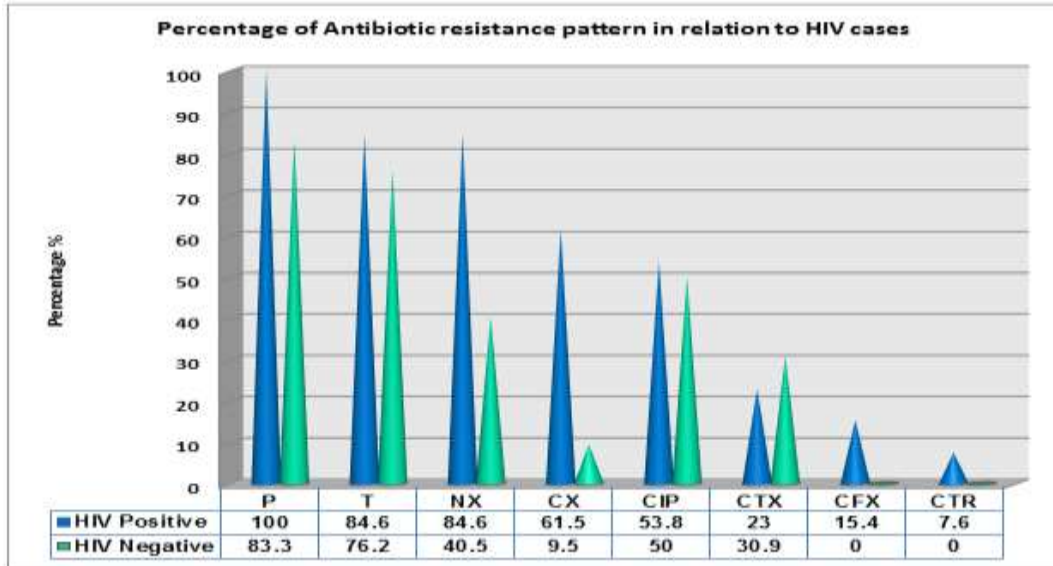


Fig 1: Percentage of Antibiotic resistance pattern of *Neisseria gonorrhoeae* in relation to HIV cases

P-Penicillin, T-Tetracycline, NX-Nalidixic acid, CX-Cefoxitin, CIP-Ciprofloxacin, CTX-Cefotaxime, CFX-Cefixime, CTR-Ceftriaxone

The resistance of *Neisseria gonorrhoeae* was higher in HIV positive cases for Penicillin, Tetracycline, Nalidixic acid and Cefoxitin and least resistance were seen with Ceftriaxone and Cefixime. Were as gonorrhoea in HIV negative cases was highly resistant to Penicillin and Tetracycline and no resistance was observed with Ceftriaxone and Cefixime.

Discussion

The overall prevalence of gonorrhoea was 9%. Among the 55 positive cases of *Neisseria gonorrhoeae*, 42 (76.7%) were HIV negative cases and co-infection with HIV positive cases was found in 23.6%. Studies conducted in India have reported prevalence of gonorrhoea between 0 to 22%^[5,6]. Prevalence of gonorrhoea in HIV patients is reported between 0 to 92%^[13-15]. Maximum cases were in Females and in the age group of 26-30 years (Table 1). Other studies by Srifeungfung S et al. (2009) & Nusrat F et al. (2014) have reported 20-30 year age group as most commonly affected, but with a male preponderance. The factors found to be significantly associated with the prevalence of STI gonorrhoea were, low Socio economic status, unmarried, multiple sexual partners, living in urban area, profession involving long stay away from home and past history of STI.

Neisseria gonorrhoeae was isolated from various specimens. The maximum isolation was from discharge samples from HIV positive and HIV negative patients. Gonorrhoea was isolated from 3 Molluscum contagiosum and 3 urine specimens The most common symptoms seen in males was urethral discharge whereas in females it was vaginal discharge (Table 3). Gender

wise the maximum cases were obtained from the STD clinic (Table 4). It may be because most of the female patients were referred from department Gynaecology to the STD clinic after they did not respond to the treatment.

The antibiotic resistance pattern of *Neisseria gonorrhoeae* is shown in Fig. 1. *Neisseria gonorrhoeae* were resistant to most commonly used antibiotics and the resistance was high in HIV positive cases. Resistance to Penicillin and Tetracycline was 100% and 84% respectively in HIV positive cases. While the resistance to above two antibiotics was slightly less (83.3% and 76.1%) in HIV negative cases. Other studies have also found increased resistance to Penicillin and Tetracycline but in varying between 12% to 70%^[19-21]. The global spread of high level resistance to Penicillin and Tetracycline among *N. gonorrhoeae* isolates in the last two decades has almost made these antibiotics obsolete in gonorrhoeal treatment as described by Lewis DA, (2010). The reasons for this outbreak of *Neisseria gonorrhoeae* strains with reduced sensitivity to Penicillin and Tetracycline could be due to coinfection with HIV and increasing misuse of the antibiotics as the pragmatic first-line treatment for *Neisseria gonorrhoeae* for the last several years, and re-infection due to the presence of a large reservoir of asymptomatic carriers that unknowingly transmit the disease to their sexual contacts and also reduced CD4 count and increasing viral load.

Resistance to Ciprofloxacin was 50% and 53.8% in HIV negative and positive cases respectively. This is an alarming sign as quinolones group of antibiotics (ciprofloxacin and nalidixic acid) are one of the most commonly used antibiotics for gonorrhoea. Other

studies reported 7% to 65% resistance to quinolones in countries like Bhutan, Thailand and Spain^[21,23].

Among the Cephalosporin group, highest resistance was seen with Cefotaxime and Cefoxitin in both HIV positive and HIV negative co-infected patients. HIV negative patients showed zero percent resistance to Ceftriaxone and Cefixime, but HIV positive patients showed 7% and 15% resistance to Ceftriaxone and Cefixime respectively. *N. gonorrhoeae* isolates with decreased susceptibility to third generation cephalosporin have been reported in the South East Asia Region including India as shown by Bala M et al. (2013). Treatment failure to Cefixime has been reported from other countries like Japan, France, Canada, Austria and Norway^[19,24,25]. Recently Ceftriaxone resistant isolates have been identified in Japan, France and Spain^[24,26,27].

Apart from the above antibiotic, some studies have used Azithromycin and Spectinomycin for antibiogram of *N. gonorrhoeae* and it has been reported that <5% of gonococcal isolates were resistant to Azithromycin^[23]. This forms the basis of dual therapy of gonococcal infections (cephalosporin + azithromycin, to improve treatment efficacy and potentially slow the emergence and spread of resistance to cephalosporins. But Spectinomycin-resistant strains have recently been reported from India and Bhutan^[23,28]. There are several mechanisms responsible for resistance of antibiotics in gonococcus, major being production of enzymes, the acquisition of plasmids, and genetic mutations^[29].

Due to the ability of gonococcus to develop resistance to antimicrobials, the treatment of gonococcus has evolved through time^[30]. Periodic monitoring of antimicrobial sensitivity profile will help in detecting change in susceptibility pattern and emergence of drug-resistant strains, helping physicians in choosing an appropriate antibiotic at the same time it helps the institution in developing an effective hospital antibiotic policy for the control of gonococcal infections.

Conclusion

Despite low rates of gonorrhoeal infection, it is important to focus on high risk population (15-30 years) because of the great physical and emotional costs of the disease. In the present study high rate of resistance to panel of antibiotics was seen which suggests a careful selection of antibiotic in the treatment of gonorrhoea in HIV patients. Better results are expected by the use of Ceftriaxone and Cefixime as a first line of drugs. Periodic monitoring of antimicrobial sensitivity profile will help in detecting changes in susceptibility pattern and the emergence of drug-resistant strains, and will help the physician to choose an appropriate antibiotic, and it also helps the institution in developing an effective hospital antibiotic policy.

Conflict of interest: None

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