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Original Research Article

A study on antimicrobial sensitivity pattern in Neisseria gonorrhoeae in a tertiary care hospital

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ABSTRACT

Background: Sexually transmitted diseases are prevalent throughout the world. Sexually transmitted diseases (STD) play a major role in the transmission of HIV infection. The risk of acquiring HIV infection in non-ulcerative STD is 3 to 5 times more than that in persons without any sexually transmitted infections. One of the main non-ulcerative STD is gonorrhoea. The relative incidence of gonococcal infections is about 10 to 13% of total sexually transmitted infections in STD clinics. Aims and objectives was to identify gonococcal infection in patients attending the STD clinic and associated sexually transmitted infections and to study the antimicrobial susceptibility of *gonococcus* and to modify the disease intervention strategies.
Methods: A retrospective study was conducted in our institute of venereology, government general hospital and madras medical college, Chennai-03, Tamil Nadu, India. 43 patients with gram stained smear or culture positive for *gonococcus* who attended the institute from February 2013 to September 2014 were taken into the study. All the details were collected from the case records of the patients. The antibiotic sensitivity testing in N. gonorrhoeae had been done by Kirby-Bauer disc diffusion method. Screening for other sexually transmitted diseases had been done and were treated according to the institute guidelines.
Results: Specimens from 43 patients (40 male, 3 females) had been collected. 40 specimens were found to be culture positive. Antibiotic sensitivity tests were carried out on those 40 isolates of Neisseria gonorrhoeae obtained in pure culture. 70% of isolates were resistant to penicillin and 30% were less sensitive to it. 52.5% of the isolates were PPNG. 57.5% of isolates were resistant to ciprofloxacin and 42.5% were less sensitive to it. 7.5% were resistant to ceftriaxone, 12.5% were resistant to cefixime and 15% were resistant to spectinomycin. All the isolates were sensitive to Azithromycin. Three male patients had HIV (6.9%), three had syphilis and one had genital wart. One female patient had trichomoniasis.
Conclusions: The results of the study indicate that multidrug resistant Neisseria gonorrhoeae is prevalent in this region. Associated STDs must be investigated to prevent the transmission of HIV and further complications. The need for establishing a national surveillance programme for antibiotic resistance becomes clear with this study.

Keywords: Antimicrobial sensitivity, Gonococcal infections, Non-ulcerative STDs

INTRODUCTION

Sexually transmitted diseases are prevalent throughout the world and about 340 million cases are reported.[1] Prevalence of STD cases in India is about 30 million or 6% of population.[2] Globally around 36.7 million people are living with HIV/AIDS by 2015, and India constitutes 2.12 million of those PLHIV.[2,3] Sexually transmitted

diseases (STDs) play a major role in the transmission of HIV infection. Thus, prevention and control of STD is one of the major strategies for the control of HIV. The risk of acquiring HIV infection in ulcerative STD is 5 to 7 times and in non-ulcerative STD is 3 to 5 times more than that in persons without any sexually transmitted infections.[4] One of the main non-ulcerative STD is Gonorrhoea.

The relative incidence of gonococcal infections is about 10 to 13% of total sexually transmitted infections in STD clinics.[5,6] Common symptoms are urethritis in male and cervicitis is female. HIV positive men with urethritis had HIV concentrations in the semen more than those in seropositive men without urethritis. After treatment of urethritis the concentrations of HIV in semen decreased significantly.[7] Causative organism for gonorrhoea is Neisseria gonorrhoeae. Man is the only natural host. Penicillin has been the drug of choice for many years for treatment of gonorrhoea. In recent years, there has been an alarming increase in ciprofloxacin and penicillin-resistant N. gonorrhoeae in India.[8] Establishing appropriate treatment strategies is further complicated by the rapidly changing resistance patterns. Hence monitoring of drug resistance patterns at regular intervals, is essential. Other sexually transmitted infections like HIV, syphilis, trichomoniasis are common with gonorrhoea.[9] Presence of gonococcal infection aids in acquiring HIV infection. So proper diagnosis and treatment with sensitive antibiotics is necessary to prevent the complications of gonorrhoea and the spread of HIV.

Aims and objectives

To identify the gonococcal infection in patients attending the STD clinic and associated sexually transmitted infections. To study the antimicrobial susceptibility of *gonococcus* and to modify the disease intervention activities and therapy recommendations accordingly.

METHODS

A retrospective study was conducted in our institute of venereology, Rajiv Gandhi government general hospital and madras medical college, Chennai-03. 43 patients with gram stained smear or culture positive for *gonococcus* who attended the institute, from February 2013 to September 2014 were taken into the study. All the details were collected from the case records of the patients.

Details like routine history and clinical examination findings, appropriate specimens collected according to the duration of illness and site of gonococcal infection and data regarding the age, sex, risk factors associated with clinical illness and history regarding mode of transmission were collected. The specimen collected for the culture had been immediately inoculated on the selective medium (modified thayer martin medium with VCNT) and non-selective chocolate agar. The plates were examined after 24 hrs and if there were no growth the plates were further incubated and read after 48 hours (Figure 1).

Figure 1: Colonies of *Gonococcus* after 24 hours of incubation.

Colonies which showed gram negative diplococci in gram stain and oxidase positive were identified presumptively as N. gonorrhoeae and had been sub cultured on to chocolate agar for further tests like sugar degradation test, b-lactamase test and antibiotic sensitivity (Figure 2, 3).

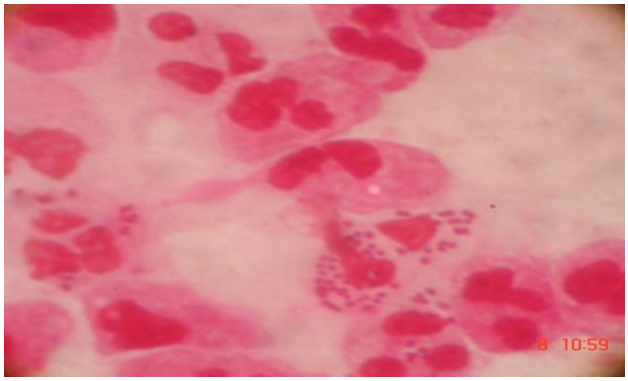

Figure 2: Gram stain of smear taken from urethral discharge showing gram negative intracellular diplococci.

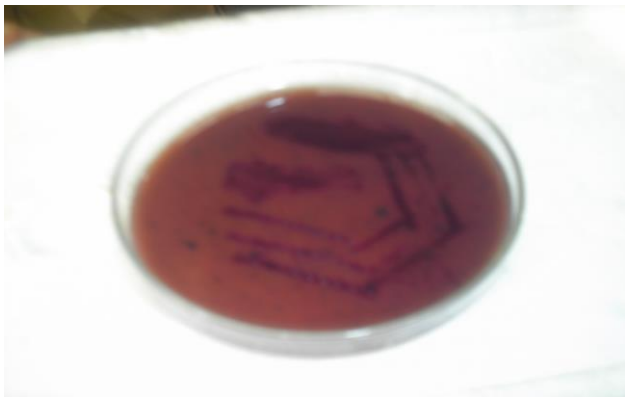

Figure 3: Oxidase test showing purple colour of the colonies.

The antibiotic sensitivity testing in N. gonorrhoeae had been done by Kirby-Bauer disc diffusion method. Sensitivity for Penicillin G (10 units), Ciprofloxacin (5mcg), Ceftriaxone (30mcg), Cefixime (5mcg), Spectinomycin (100mcg) and Azithromycin (15mcg) were tested (Figure 4).

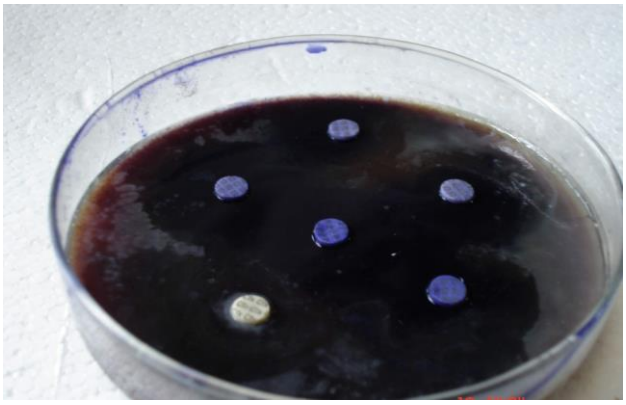

Figure 4: Antibiotic sensitivity test-Kirby Bauer disc diffusion method.

All patients were treated as per NACO guidelines with grey kit containing a single dose of Tab. Cefixime 400mg and Tab. Azithromycin 1 g. Follow up was done on 3, 7, 14 and 30 days.

RESULTS

Specimens from 43 patients (40 male, 3 females) had been collected. The number of patients diagnosed by smear and culture are given (Table 1).

Table 1: Smear and culture results.

	Positive		Negative	
	Male	**Female**	**Male**	**Female**
Smear	40	2	0	1
Culture	37	3	3	0

91% of patients were in the age group of 20-40 years. 45% of males and 100% of females were married. 90% of males and 66% of females were literate. 90% of males were employed. 85% of males had heterosexual orientation, 12.5% had bisexual orientation and 2.5% had homosexual orientation. 52.5% of males had last sexual exposure with CSWs and 32.5% of males with known contacts.

All male patients had urethral discharge. 42.5% of males had dysuria. 33% of females had no symptoms. Antibiotic sensitivity tests were carried out on 40 isolates of Neisseria gonorrhoeae obtained in pure culture. 70% of isolates were resistant to penicillin and 30% were less sensitive to it. 52.5% of the isolates were PPNG. 57.5% of isolates were resistant to ciprofloxacin and 42.5% were less sensitive to it. 7.5% were resistant to ceftriaxone, 12.5% were resistant to cefixime and 15% were resistant to spectinomycin. All the isolates were sensitive to azithromycin (Table 2).

Out of 43 patients 40 turned for follow up and all showed signs of cure. No treatment failures were noticed. Screening for other sexually transmitted diseases had been done and treated according to the institute guidelines.

Table 2: Anti-microbial sensitivity of Gonococcus.

Name of the drug	Sensitive		Moderately sensitive		Resistant	
	No	**%**	**No**	**%**	**No**	**%**
Penicillin	0	0	12	30	28	70
Ciprofloxacin	0	0	17	42.5	23	57.5
Ceftriaxone	37	92.5	-	-	3	7.5
Cefixime	35	87.5	-	-	5	12.5
Spectinomycin	34	85	0	0	6	15
Azithromycin	40	100	0	0	0	0

Associated STDs

Three male patients had HIV (6.9%). Three male patients had syphilis and one had genital wart. One female patient had trichomoniasis (Table 3).

TABLE 3: Associated STDs.

	Male (no. of patients)	Female (no. of patients)
Syphilis	3	0
HIV	3	0
Genital warts	1	0
Trichomoniasis	0	1

DISCUSSION

A total 43 patients with gonococcal infection were studied in detail. The analysis of age incidence in the present study showed 67.4% of patients were in the age group of 20-30 years which is the sexually active group where another STD are also common. The next predominant age group was 31-40 years. This analysis correlates with the study by Mehta Swami D et al.[10]

In present study 47.5% of the males were unmarried. Vlajinac BM et al showed that in both sexes gonococcal infection was commonest in populations who has never married and the present study had similar findings.[11]

According to our study 52.5% of males had contact with the high-risk group CSW who form the core group in the transmission of STD. Jaiswal AK et al reported that the main source of infection (74.5%) was female commercial sex workers.[12] In this study also CSW played a key role in the transmission of gonorrhoea. In present study 2.5% of males had homosexual orientation and 12.5% of males had bisexual orientation. Dafferty WF reported 8.4%

gonorrhoea in homosexuals which is higher than our present study.[13] Mcmillan A et al reported that unprotected receptive anal sex was most risky factor favouring transmission of gonorrhoea and HIV.[14] Hence nonreceptive contact tracing is an essential criterion to prevent transmission.

As per the present study 100% of males presented with urethral discharge and 33% of females were asymptomatic this correlates with Peter Leone who reported 90% of newly acquired infection were symptomatic and 50% of women with endocervical infection were asymptomatic.[15]

Symptomatic males report earlier for treatment, but females who are asymptomatic do not take treatment and come with later complications like pelvic inflammatory diseases and infertility.

According to our study Gram stained smear were positive in 100% of males and 66% of females. This correlates with the study by Sherrard J and Barlow D which showed urethral gonorrhoea was diagnosed by microscopy in 94.4% of gonorrhoea in men.[16] Gram staining of genital secretions remains the only widely accepted routine procedure for making an 'on the spot' diagnosis of gonococcal infection.

For interpretation of results of disk diffusion tests, three categories of results are proposed.[17]

- A susceptible result implies a < 5% likelihood of treatment failure
- An intermediate result indicates predictable failure rates of 5% - 15% if the patient is treated with the tested antibiotic in the standard dosage
- A resistant result is with clinical treatment failure rates of > 15%.

Treatment failure may result from variety of causes, and patients may experience failure of therapy even when infected with isolates that manifest in vitro susceptibility. Test result must be used as an adjunct to not in place of clinical evaluation.[17]

In this study 70% of the isolates were resistant to penicillin. In 1987, 74% of isolates were found resistant to penicillin from India.[18] In a recent study by Kumar S et al 17.2% of isolates were found resistant to penicillin in Ahmedabad in 2013.[8] 12.5% of isolates in this study were PPNG.

In a study conducted in the institute of venereology in 2001 the prevalence of PPNG was 35.8%. The prevalence of PPNG in different studies varies from 8-70% in South and South East-Asia. (8% in New Delhi, 15% in Dhaka, 17.8% in Thailand, 51.7% in Bombay, 52% in Indonesia and 70.7% in Philippines.

Ciprofloxacin resistance was found in 57.5% of isolates. In the study by Kumar S et al in Ahmedabad in 2013, 46.6% of the isolates were resistant to ciprofloxacin. This is comparable to our results. Not even a single isolate was sensitive to neither penicillin nor ciprofloxacin in our study. Penicillin is not recommended for treatment either by WHO or by CDC.

Continuing treatment with penicillin would contribute to treatment failures and prolonged period of infectivity and in turn spread of resistant strains in the community. Resistance to ciprofloxacin showed by all isolates was disturbing.

Resistance to ceftriaxone and cefixime were present in 7.5% and 12.5% of the isolates respectively. This is a matter of concern. The previous study from this Institute in 2001 showed 3.7% resistance to ceftriaxone. In 2003 5.9% of the isolates were found less sensitive to ceftriaxone in New Delhi.

Out of the 3 isolates taken from patients who showed resistance to ceftriaxone 2 were treated with Inj. ceftriaxone 250 mg im single dose. But these 2 patients responded well to treatment and there was no treatment failure (In vitro resistance). Resistance to spectinomycin which is least used was 15% in our study.

All the 40 isolates were sensitive to azithromycin in our study. This looks promising since NACO recommends grey kit in the treatment of gonorrhoea. It also has the added advantage of covering Chlamydial infection.

Since no control strains (reference strains) were used in the study, all results must be verified by disk-diffusion or preferable by agar dilution method which also incorporates reference strains. Quality control is necessary for comparing results from different laboratories done under different conditions.

Various studies conducted on antibiotic sensitivity pattern in gonococcal infections over the past decade around the world has been tabulated (Table 4).

Going by the results our study Azithromycin, Cefixime and Ceftriaxone appears to offer hope. Further studies using agar dilution method are to be done before treatment recommendations are to be made. Among the associated infections in this study, HIV infections were 6.9% of the total gonococcal cases.

Co infection with syphilis was present in 3 cases. All the three were latent syphilis. One patient had genital wart. Out of 3 female patients one (33%) had trichomonas vaginalis infection. Vazquez-F et al reported trichomonas vaginalis isolated in gonorrhoea was 40.4% which correlates with our present study.[22]

Table 4: Comparison of various studies on antibiotic sensitivity pattern in Neisseria gonorrhoea conducted over the years in various parts of the world.

	Cefixime	Ceftriaxone	Ciprofloxacin	Penicillin	Spectinomycin	Azithromycin	Tetracycline	Cefotaxime
Saleem K et al, Karachi.[19]	S-100%	S-100%	S-88.55% R-11.5%	S-59% R-41%	-	-	S-45% R-55%	-
Amito PF et al, Uganda.[20]		-	R-23.3%	R-23.4%	-	-	R-17.2%	R-1.8%
Hailemariam M et al, Ethiopia.[21]	S-100% R-0%	S-100% R-0%	S-55% I-27% R-18%	S-0% I-18% R-82%	S-82% I-18%	-	I-45% R-55%	-
Nanda S et al, Ahmedabad.[8]	R-0%	R-0%	R-46.4%	R-17.8%	-	-	R-23.2%	-
Present study, 2013-2014	S-87.5% I-0 R-12.5%	S-92.5% I-0 R-7.5%	S-0 I-42.5% R-57.5%	S-0 I-30% R-70%	S-85% I-0 R-15%	S-100% I-0 R-0	-	-

Surveillance for antibiotic resistant strains is essential for providing data for disease intervention. Treatment of patients diagnosed by gram-stained smear at primary care level should be done according to prevailing guidelines. At tertiary care levels, it is better to treat patients based on sensitivity reports at least for cases with treatment failures. Surveillance work should include both government and private sectors. Prompt analysis and review of the data should be done so that appropriate adjustments can be made in disease intervention activities and therapy recommendations. Appropriate funds should be allotted under STD control programmes for establishing laboratory facilities for surveillance. Training laboratory personnel and quality control procedures should be incorporated in surveillance programmes.

Gonococcal antimicrobial surveillance programme (GASP) was started by the WHO in 1990 with an aim to establish a network of laboratories for surveillance based on WHO regions. Adoption of uniform methodology by training programmes and quality control programmes are a prerequisite for involvement in GASP.

In 1987, the centers for disease control and prevention (CDC) in collaboration with other organizations implemented the gonococcal isolate surveillance project (GISP) to monitor antimicrobial resistance in the United States. WHO has proposed wisely and widely, points of action for over-coming antimicrobial resistance. These include adopting WHOs policies and strategies, educating health workers and the public on the use of medicines, measures to contain resistance in hospitals, increasing research for new drugs and increasing availability of essential drugs. WHO recommends that surveillance data be analysed and distributed to health care workers to assist them in prescribing drugs appropriately.

Educating the public and health care workers on the wiser use of antimicrobial drugs is imperative to halt the spread of resistance. Governments, professional societies and teaching institutions must keep health care priorities up-to-date by supplying necessary information on the selection of correct drugs, dosages and optimum treatment durations necessary for effective patient management. Educating consumers and the community on the judicious use of antimicrobials is also critical in tackling the problem of drug resistance. Patients need to recognize the value of antimicrobials, how to use them and how not to use them, the importance of taking them as required, and avoiding them when unnecessary.

WHO encourage hospitals to form drug and therapeutics committees aimed at establishing treatment guidelines. These encourage drug-use monitoring and infection control thereby preventing the transmission and spread of resistant organisms. Encouraging the research community to develop new compounds is essential as once-effective treatment become inefficient in the face of ever evolving resistant microbes. Research into dosage regimens calculated to minimize the likelihood of selecting for resistance is also important.

CONCLUSION

The results of the study indicate that multidrug resistant Neisseria gonorrhoeae is prevalent in this region. The value of Penicillin and Ciprofloxacin as drugs for the treatment of gonorrhea is doubtful as all the strains were either less sensitive or resistant to these drugs. Azithromycin looks promising with no drug resistance. Associated STDs must be investigated to prevent the transmission of HIV and further complications. The need for establishing a national surveillance programme for antibiotic resistance becomes clear with this study.

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