



PREVALENCE OF *NEISSERIA GONORRHOEAE* AND OTHER STIS/RTIS WITH FOCUS ON GONOCOCCAL PHENOTYPIC ANTIMICROBIAL RESISTANCE

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ABSTRACT

Introduction: The aim of this study was to validate microbiologically the spectrum of STIs in a tertiary care centre in North India and to phenotypically detect the antimicrobial resistance in *Neisseria gonorrhoeae*.

Material And Methods: STIs were diagnosed clinically and confirmed by standard laboratory techniques. Antimicrobial resistance pattern of *N.gonorrhoeae* was determined by standard techniques.

Results: Among 201 cases, most common STIs detected were bacterial vaginosis (43.78%) followed by herpes genitalis (25.87%) and candidiasis (16.92%). *N.gonorrhoea* was identified in 10 (4.98%) patients. Of the 10 confirmed cases of gonorrhoea, antimicrobial sensitivity was performed in 7 patients. All the 7 patients tested were QRNG (Quinolone resistant *N.gonorrhoeae*), 5/7 were PPNG (Penicillinase producing *N.gonorrhoeae*), 1/7 was CMPRNG (Chromosomally mediated penicillin resistant *N.gonorrhoeae*), 3/7 were TRNG (Tetracycline resistant *N.gonorrhoeae*). MIC by E-test was performed in 5 strains and we observed that MIC₉₀ for ciprofloxacin was $\geq 4\mu\text{g/ml}$, penicillin was $\geq 6\mu\text{g/ml}$, and for tetracycline was $12\mu\text{g/ml}$ which clearly brackets them as resistant isolates.

Conclusion: Alarming increase in the resistance to commonly used antimicrobials for gonorrhoea in our study especially of fluoroquinolones is a clarion call for the urgent need for prudence in prescribing them.

KEYWORDS :

INTRODUCTION

Sexually transmitted infections (STIs) continue to present major health problems in the developing world, leading to considerable morbidity, mortality, and stigma¹. Their profile varies with changes in socioeconomic, cultural, geographic, and environmental factors prevalent in different parts of the country. However, due to lack of adequate laboratory infrastructure in the country, information regarding the profile of STIs relies essentially on syndromic diagnosis. Hence there is very limited data of laboratory-proven STIs^{2,3}. The aim of this study was to validate microbiologically the spectrum of STIs in a tertiary care centre in North India and to phenotypically detect the antimicrobial resistance in *Neisseria gonorrhoeae*.

MATERIAL AND METHODS

The present study was conducted from 2015 to 2017 in the Department of Microbiology, Jawaharlal Nehru Medical College in collaboration with Suraksha Clinics (Departments of Dermatology and Obstetrics and Gynaecology J.N. Medical College, Malkhan Singh Male and Female District Hospital, Aligarh) and Anti-Retroviral Centre, J.N. Medical College, Aligarh. The study group comprised of subjects attending the Suraksha clinics in the two hospitals with one or more of the complaints as enunciated by WHO in its syndromic approach for the diagnosis of STI⁴. STIs provisionally diagnosed by clinical features were confirmed by standard laboratory techniques, using appropriately collected specimens⁴.

All isolates of *N. gonorrhoeae* were tested for their susceptibility to clinically important antimicrobial agents. All the isolates were sent to (WHO GASP SEAR Regional Reference Laboratory) for confirmation. The following antimicrobial agents were tested. The antibiotics in the 'core' group are those currently recommended by the WHO⁵.

Antibiotics in the 'additional' group include those that are used

for the treatment of gonorrhoea in some parts of the world. The concentration of various antibiotics were as follows: penicillin (0.5 IU), ciprofloxacin (1ug), nalidixic acid (30 ug), ceftriaxone (0.5 ug), cefpodoxime (10 ug), spectinomycin (100 ug), tetracycline (10 ug), azithromycin (15 ug), Cefixime (10ug).

Techniques for antimicrobial susceptibility testing of *N. gonorrhoeae*

The disc diffusion technique was employed as the initial susceptibility test. All the isolates were sent to Apex Regional STD Teaching, Training & Research Centre (WHO GASP SEAR Regional Reference Laboratory) Safdarjung Hospital, New Delhi for confirmation of disc diffusion results and interpretation was done according to Bala M et.al, 2015⁶.

RESULTS

Out of 3120 patients screened, microbiologically the etiology was confirmed in 201 cases. Most common STIs detected in our study were bacterial vaginosis (88) followed by herpes genitalis (52) and candidiasis (34). *N. gonorrhoea* was identified in 10 male patients while Hepatitis C virus and Hepatitis B virus infections accounted for (5) and (1) cases respectively. Table 1 furnishes information on all the STIs identified in this study.

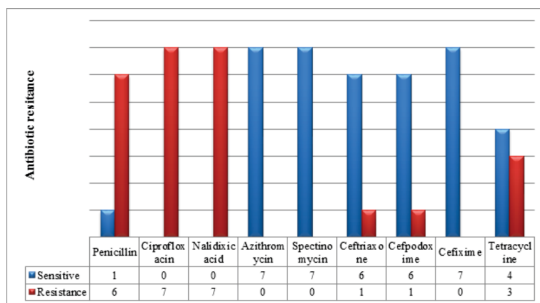
Table 1: Etiology of confirmed STI cases (n=201)

STI	Confirmed STI cases		
	Male	Female	Total No. of Isolates
Gonorrhoea	10	0	10
Chlamydiasis	0	3	3
Bacterial Vaginosis	0	88	88
Trichomoniasis	0	4	4
Candidiasis	0	34	34

Herpes genitalis	36	16	52
Syphilis	3	1	4
Hepatitis B	0	1	1
Hepatitis C	3	2	5
Total	52	149	201

Of the 10 confirmed cases of gonorrhoea, antimicrobial sensitivity was performed in 7 patients. All the patients were sensitive to azithromycin, spectinomycin and cefixime while in case of ceftriaxone and cefpodoxime, *Neisseria* was found resistant in 1 case only on the basis of disc diffusion technique. However, results of this isolate were not confirmed by E-test by the Apex STD Centre. One isolate was sensitive, 2 were less sensitive and 4 isolates were resistant to tetracycline. No isolate was sensitive to penicillin and 1 isolate was less sensitive to penicillin. 100% resistance was observed against ciprofloxacin and nalidixic acid as seen in Figure 1.

Figure 1: Antimicrobial sensitivity pattern for *N. gonorrhoeae*



MIC by E-test was performed in 5 strains and we observed that MIC 90 for ciprofloxacin was $\geq 4 \mu\text{g/ml}$, penicillin was $\geq 6 \mu\text{g/ml}$, and for tetracycline was $12 \mu\text{g/ml}$ which clearly brackets them as resistant isolates. MIC90 of ceftriaxone was $\leq 0.03 \mu\text{g/ml}$, spectinomycin was $\leq 16 \mu\text{g/ml}$, azithromycin was $\leq 0.5 \mu\text{g/ml}$ and cefpodoxime was $\leq 0.023 \mu\text{g/ml}$, which were in the sensitive range. In our study, all the 7 patients tested were QRNG (Quinolone resistant *N. gonorrhoeae*), 5/7 were PPNG (Penicillinase producing *N. gonorrhoeae*), 1/7 was CMPRNG (Chromosomally mediated penicillin resistant *N. gonorrhoeae*), 3/7 were TRNG (Tetracycline resistant *N. gonorrhoeae*).

DISCUSSION

Due to lack of adequate laboratory infrastructure in the country, information regarding the profile of STIs relies essentially on syndromic diagnosis. Hence there is very limited data of laboratory-proven STIs^{7,8}. In our study, GUD Syndrome (80.9%) was the most common presenting complaint followed by genital discharge syndrome (13.43%) while patients presenting with systemic infections with genital mucosal involvement comprised of only 5.67% patients. In the context of HIV transmission genital ulcer disease not only facilitates the transmission but also enhances susceptibility to HIV infection by sexual contact^{9,10} while non-ulcerative STIs like gonorrhoeae and chlamydia increase shedding of the HIV virus in the genital tract by recruiting HIV-infected inflammatory cells as part of normal host response^{9,11}.

In this study, 419 patients presented with genital discharge syndrome. Amongst these, 139 (33.17%) were confirmed as cases of STIs. Both Choudhry S et al (2010)¹² and Ray et al (2006)¹³ also observed that genital discharge syndrome was the most common STI. In this group patients presented with urethritis, cervicitis, burning micturition and vaginal discharge. In our study genital discharge (69.15%) was the most common syndrome, with bacterial vaginosis predominating (63.3%), followed by candidiasis (24.5%), gonorrhoeae (7.19%) and trichomoniasis (2.88%). Surprisingly chlamydia (2.16%) was the least prevalent

STI. According to a study conducted under AIDS Prevention and Control Project, jointly undertaken by Voluntary Health Services (VHS) and US Agency for International Development (USAID), in a community of urban and rural adult population of Tamil Nadu, a high HIV prevalence state, it was revealed that the genital discharge was the most common syndrome (41.5%) in women, with trichomoniasis accounting for 5.1%, *Chlamydia trachomatis* 3.9% and gonorrhoea 3.7%. In another study 41.5% of the women had bacterial vaginosis, 18.6% candidiasis, 4.3% trichomoniasis and 28.7% chlamydia trachomatis¹⁴.

Patients with urethritis mainly comprised of male patients, with complaints of painful urethral discharge and burning micturition. The most common etiology in them was gonorrhoea (7.19%) in our centre. Ray et al (2006)¹³ also found in their study that in this group, the most common etiology was gonorrhoea (15.24%). Another study by Bhargava et al (2017)¹⁵ observed 65.8% (586/891) of men with UD syndrome were diagnosed with gonococcal infection by microscopy or/and culture. According to The NMJI, Review Article (2004) in India the prevalence of gonorrhoea among STI clinic attendees in different regions varied from 3% to 19%. A steady decline in prevalence was observed in Chandigarh, Delhi and Patiala, while a marginal increase was reported from Rohtak and Ahmedabad¹³.

In our study it was observed that all the patients were sensitive to azithromycin, spectinomycin and cefixime. Resistance was observed against ceftriaxone and cefpodoxime in 14.2% cases. 4 patients were sensitive and 3 patients were resistant to tetracycline. As expected a large number (85.8%) of cases were resistant to penicillin. Alarmingly 100% resistance was observed against nalidixic acid and ciprofloxacin. Other Indian studies have also reported increase in the spectrum and level of antibiotic resistance of *N. gonorrhoeae* isolates in recent years as compared to that seen previously^{17,18}. Rishud A (2005)¹⁹ in a study in Pune observed that during 1996-2002 resistant isolates showed an increasing trend over the years for all the antibiotics. Penicillin resistant isolates increased from 11 per cent in the year 1996 to 33 per cent in 2002. In our study it escalated to 85.8%. Ciprofloxacin resistant isolates increased from 74 to 100 per cent, which mirrors our resistance profile against fluoroquinolones. Tetracycline resistance increased from 28 to 80 per cent during the same period. However in our study it remained at 42.8%. Isolates showing lower susceptibility to ceftriaxone appeared from 1999 onwards with one in 1999 and two each in the years 2000, 2001 and 2002, respectively¹⁸. In our study too one case exhibited resistance to ceftriaxone and cefpodoxime. However, we could not preserve this isolate and results of this isolate were not confirmed by MIC testing by the Apex STD Centre.

Antimicrobial susceptibility of *N. gonorrhoeae* isolated in Pune during the past decade was characterized by high rates of resistance to penicillin and ciprofloxacin²⁰. Cefixime with azithromycin is the first-line treatment recommended under syndromic management of STIs according to the recent NACO guidelines for treatment of gonorrhoea. In our study, we too found that all the strains tested were found 100% sensitive to azithromycin and cefixime, indicating that kit 1 (comprising azithromycin and cefixime) as approved by NACO for syndromic management of discharge patients was appropriate. In our study, all the 7 patients tested were QRNG (Quinolone resistant *N. gonorrhoeae*), 5/7 were PPNG (Penicillinase producing *N. gonorrhoeae*), 1/7 was CMPRNG (Chromosomally mediated penicillin resistant *N. gonorrhoeae*), 4/7 were TRNG (Tetracycline resistant *N. gonorrhoeae*).

In our study, MIC determination was performed in 5 strains and we observed that MIC90 for ciprofloxacin was $\geq 4 \mu\text{g/ml}$

and penicillin was $\geq 6 \mu\text{g/ml}$, which clearly brackets them as resistant isolates. MIC₉₀ of ceftriaxone was $\leq 0.03 \mu\text{g/ml}$, spectinomycin was $\leq 16 \mu\text{g/ml}$, azithromycin was $\leq 0.5 \mu\text{g/ml}$ and cefpodoxime was $\leq 0.023 \mu\text{g/ml}$, which were in the sensitive range. MIC₉₀ for tetracycline was $12 \mu\text{g/ml}$, all being classified as resistant. In a study by Kulkarni et al, 2012²⁰ 98.4%, 57.8% and 48.4% were found resistant to ciprofloxacin, tetracycline, and penicillin, respectively. Minimum inhibitory concentration (90%) for ciprofloxacin, tetracycline, penicillin, ceftriaxone, and spectinomycin was 32, 4, 12, 0.008, and 8 $\mu\text{g/ml}$, respectively. 15.6% strains were found to be penicillinase-producing *N. gonorrhoeae* (PPNG) in their study. Minimum inhibitory concentrations (MICs) of ciprofloxacin and penicillin against the isolates studied in the years 1996 to 1999 and 2000 to 2001 showed increase in resistant isolates from 21.8 to 67.3 per cent, and from 10.9 to 35.3 per cent, respectively^{17,18}.

CONCLUSION

The alarming increase in the resistance to commonly used antimicrobials for gonorrhoea in our study especially to fluoroquinolones is a clarion call for the urgent need for prudence in prescribing them. Observing the rampant resistance exhibited by *N. gonorrhoeae*, it is clear that the day is not far when it will acquire a superbug status and become intractable for treatment by the available antibiotics.

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