



A PROSPECTIVE STUDY ON ANTIMICROBIAL RESISTANCE IN NEISSERIA GONORRHEAE: A RE-EMERGING PATHOGEN

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ABSTRACT

Gonorrhea is one of the oldest known human illnesses. Despite the highly effective antimicrobial therapy available, it remains as a common sexually transmitted disease. Complicating this situation, new multi-drug resistant strains are emerging, making it a re-emerging public health threat. Presently, cephalosporins are the drug of choice and cephalosporin resistant strains are getting reported increasingly worldwide. This was a hospital-based prospective study conducted over 2 years to study the drug resistance profile of *Neisseria gonorrhoeae* strains prevalent in our population. Eighteen clinical samples were obtained and *Neisseria gonorrhoeae* was isolated from 15 samples. All isolates were obtained from male patients (Mean-age:25.89(±3.16)years). Direct smear examination was positive in 17 cases (94.44%). Antimicrobial susceptibility was studied and two isolates showing decreased susceptibility to ceftriaxone were found. Unrestricted, inappropriate selection and overuse of antibiotics, suboptimal quality of antibiotics and inherent genetic mutations within the organism have contributed to the development of this pattern of resistance in *N. gonorrhoeae*. The resistance will render it untreatable, increasing the reproductive morbidities. Furthermore, it facilitates the transmission of HIV by increasing the risk five-fold. Only an effective surveillance of the resistance profile and appropriate management regulations can curb the rise of this re-emerging threat

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INTRODUCTION

Gonorrhea is one of the oldest known human illnesses that is transmitted almost exclusively by sexual contact or perinatally. This bacterial infection caused by *Neisseria gonorrhoeae* primarily affects the mucous membranes of the urethra and cervix and, less frequently, those of the rectum, oropharynx, and conjunctivae. (1)

Gonorrhoea is a major public health challenge today, due to the high incidence of infections accompanied by a dwindling of treatment options. Gonorrhoea represents 21% of the 498 million new cases of curable sexually transmitted infections (STIs) that occur globally every year and the actual incidence is much more than this reported incidence. (2)

The prevention and control of gonorrhoea is an important public health intervention because of the magnitude of the problem and the long-term sequelae of untreated gonococcal infections. Complications include persistent urethritis, cervicitis,

proctitis and disseminated infections that could lead to pelvic inflammatory disease, infertility, first-trimester abortion, ectopic pregnancy and maternal death. Health consequences to neonates include severe infections that may lead to blindness. In addition, gonococcal urethritis, like many other STIs, significantly increases the risk of acquiring and transmitting HIV infection.

Despite the highly effective antimicrobial therapy available, it remains as a common sexually transmitted disease, owing to the influence of the social, behavioral, and demographic factors on the epidemiology of this disease. (1) Complicating this situation, new multi-drug resistant *Neisseria gonorrhoeae* strains are emerging, making it a re-emerging public health threat. Initially, gonococci were almost uniformly susceptible to the sulfonamides, penicillin, tetracyclines, macrolides and fluoroquinolones, but none of these is now suitable for routine therapy for uncomplicated gonorrhea. (3–7) Unrestricted access to antimicrobials, inappropriate selection and overuse of

antibiotics, and suboptimal quality of antibiotics, as well as inherent genetic mutations within the organism contribute to the development of resistance. (8)

Presently, third generation cephalosporins are the drug of choice and a major cause for concern is that decreasing susceptibility to this "last-line" third generation cephalosporins has begun to manifest as clinical treatment failures. Reports of clinical treatment failures with these agents have been verified and reported from diverse countries. (2) Antimicrobial resistance (AMR) surveillance is essential to optimize treatment recommendations and detect emerging antimicrobial resistance. World Health Organization is coordinating Gonococcal Antimicrobial Susceptibility Programme (GASP), consisting of six regional networks/reference centers and India has the reference centre for South-East Asian Region. More than 70% of the participating countries have reported *Neisseria gonorrhoeae* strains with decreased susceptibility to third generation (Extended spectrum) cephalosporins. Based on the data gathered by this programme in 2011, in India, 96-100% of the isolates were resistant to quinolones, 4-10% of the isolates were resistant to ceftriaxone and 4% were resistant to azithromycin (8)

Here, this study was undertaken with objectives of determining the incidence of gonorrhoea in the patients attending our hospital and analysis of their antimicrobial susceptibility pattern.

MATERIALS AND METHODS

This was a hospital based prospective study conducted in our department over 2 years (2014-2015) to study the antimicrobial resistance (AMR) profile of *Neisseria gonorrhoeae* strains prevalent in our population. All the urethral discharge samples obtained from patients suspected of gonorrhoea were processed according to standard laboratory guidelines. Though, the study was intended to include both male (urethral discharge) and female (endocervical discharge) patients, all the samples received were only from male patients during the study period.

Initially, microscopic examination of direct gram-stained smears was done and presence of gram-negative intracellular diplococci and pus cells were noted. The samples were immediately inoculated in Modified Thayer Martin (MTM) medium along with sheep blood agar and Chocolate agar as recommended for isolation of *Neisseria gonorrhoeae*. The plates were incubated at 37°C with 5% CO₂ for 48 hours. After incubation, the colonies suggestive of *Neisseria gonorrhoeae* were presumptively identified by gram stain, oxidase test and superoxol test. Confirmation of identity was based on carbohydrate utilization tests. The acid production from glucose, maltose, lactose and sucrose were tested. (9)

Antimicrobial susceptibility testing was performed using disc-diffusion method described by CLSI standards (M100-S25). (10) Drug resistance profile was studied using following antimicrobial disks: Penicillin (10U), Tetracycline (30 µg), Ciprofloxacin (5 µg), Ceftriaxone (30µg) and Cefixime (5 µg). The minimum inhibitory concentration (MIC) of penicillin and ceftriaxone for the test isolates were studied using E-test method.

Incidence of Penicillin-resistant *N. gonorrhoeae* (PRNG), Quinolone-resistant *N. gonorrhoeae* (QRNG) and tetracycline resistant *N. gonorrhoeae* (TRNG) strains were noted

RESULTS

A total of 18 samples (urethral discharge/pus) were obtained during the study period (all 18 from men). The mean age of the patients was 25.89 (±3.16). In the direct microscopic examination of smears (gram stain), pus cells with intracellular gram-negative diplococci suggestive of *Neisseria gonorrhoeae* were seen in 17 samples (94.44%). A total of 15 samples (83.33%) showed growth of *N. gonorrhoeae*. The identity of these isolates were confirmed using the tests mentioned above. All the isolates included were consecutive, patient-specific and non-duplicate.

The following pattern of drug resistance was detected: All the fifteen isolates (100%) showed non-susceptibility to penicillin. Eight isolates (53.33%) were resistant and seven isolates (46.67%) showed intermediate susceptibility to penicillin. The minimum inhibitory concentration studied using E-test correlated with the disk diffusion method. The eight isolates which were resistant to penicillin in disk diffusion method had mean MIC of 2.75µg/ml (±0.89) and the seven isolates which showed intermediate susceptibility had mean MIC of 1.25µg/ml (±0.32).

All the 15 (100%) isolates were not susceptible to tetracycline. Resistant pattern was seen with 10 (66.67%) and rest of the isolates (33.33%) showed intermediate susceptibility. Among all the isolates, only 2 (13.33%) were sensitive to ciprofloxacin. Three isolates (20%) showed resistance and 10 isolates (66.67%) showed intermediate susceptibility.

All the fifteen isolates (100%) were susceptible to ceftriaxone and cefixime in disk diffusion method (zone size 35mm and 31mm respectively). However, in E-test method two isolates showed an increased MIC to ceftriaxone. The mean MIC of the fifteen isolates was 0.19µg/ml (±0.28). There are no established MIC cut-off for intermediate and resistant categories. According to CLSI guidelines (2015), ceftriaxone MIC of 0.25µg/ml is considered as sensitive. (10) Among fifteen isolates, two isolates which were sensitive to ceftriaxone in disk diffusion method (zone size 35mm) had MIC of 0.75µg/ml and 1 µg/ml respectively. Rest of the isolates had mean MIC of (0.085µg/ml (±0.056)).

The antimicrobial susceptibility profile of *Neisseria gonorrhoeae* isolates using disk diffusion method were shown in

Table 1 Antimicrobial susceptibility profile of *Neisseria gonorrhoeae* isolates

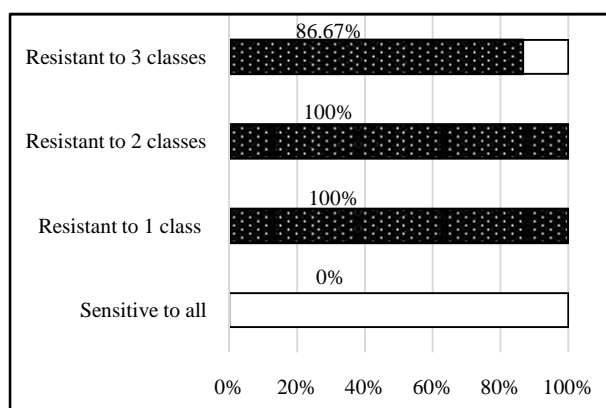
	Resistant	Intermediate	Sensitive
Penicillin (n=15)	53.33%	46.67%	0%
Tetracycline (n=15)	66.67%	33.33%	0%
Ciprofloxacin (n=15)	20%	66.67%	13.33%
Ceftriaxone (n=15)	0%	0%	100%
Cefixime (n=15)	0%	0%	100%

and summary of MIC values for penicillin and ceftriaxone is shown in Table 2.

Among the study isolates, 87% of the isolates were non-susceptible to three of the four drug classes currently approved for treatment. The incidence of PRNG, TRNG and QRNG were 53.33%, 66.67% and 20% respectively. The multi-drug resistance of the study isolates were shown in Figure 1.

Table 2 Minimum inhibitory concentration (MIC) of penicillin and ceftriaxone for the study isolates

Isolate No	Penicillin		Ceftriaxone	
	Disk diffusion	MIC	Disk diffusion	MIC
1	R	3 µg/ml	S	0.19 µg/ml
2	I	0.75 µg/ml	S	0.125 µg/ml
3	I	1.5 µg/ml	S	0.064 µg/ml
4	I	1 µg/ml	S	0.032 µg/ml
5	R	4 µg/ml	S	0.032 µg/ml
6	R	2 µg/ml	S	0.064 µg/ml
7	R	2 µg/ml	S	0.064 µg/ml
8	I	1 µg/ml	S	0.032 µg/ml
9	I	1.5 µg/ml	S	0.19 µg/ml
10	R	2 µg/ml	S	0.064 µg/ml
11	R	2 µg/ml	S	1 µg/ml
12	I	1.5 µg/ml	S	0.064 µg/ml
13	I	1.5 µg/ml	S	0.125 µg/ml
14	R	4 µg/ml	S	0.75 µg/ml
15	R	3 µg/ml	S	0.064 µg/ml

**Figure 1** Multidrug resistance: percentage of isolates showing resistance to one or more classes of drugs (cumulative %)

DISCUSSION

There is growing global concern about antimicrobial resistance in *N. gonorrhoeae* as the last available class of antibiotics to treat this condition, currently has become the first line treatment. Thus, there is an urgent need to contain the threat of untreatable gonorrhoea and this can be achieved only through continuous surveillance and appropriate management. (4)

Though, the actual incidence of gonorrhoea has not reduced, the number of samples received were less and this can be attributed to the fact that many cases are managed in peripheral and private centers using syndromic approach management for sexually transmitted infections. However, continuous monitoring of the antimicrobial pattern is very essential as the above mentioned approach needs periodic updates from the surveillance programmes.

Gram stain smear of urethral specimen from a symptomatic male has high specificity (>99%) and sensitivity (>95%) for diagnosis of gonorrhoea. (1) In our study, out of 18 samples received, 17 samples (94.44%) showed pus cells with intracellular gram-negative diplococci which is pathognomonic of gonorrhoea.

The antimicrobial resistance among our study isolates was evaluated and compared with the data available from other centres in our country. In our study we reported 53.33% and 66.67% of PRNG and TRNG isolates respectively. Similar higher resistance patterns have been seen in many centers from India as shown in a 4 year surveillance study. Centers from Chennai, Hyderabad, Kolkata, Nagpur, Delhi (UCMS) and Pune reported penicillin resistance of 80%, 16.12%, 26.31%,

60%, 27.17% and 25% respectively. But in most of these centers, the TRNG isolates were seen between 20-50% and we had a higher incidence of TRNG isolates. (7)

The reported proportion of quinolone resistance was very high in few centers like AIIMS, New Delhi (93.6%), NARI, Pune (100%), SJH_RRL, Delhi (97.07%), Nagpur (80%) and low in few centers like UCMS, Delhi (38.04%) and Hyderabad (35.4%). Though the proportion of QRNG isolates were less in our study (20%), majority of the rest (66.7%) were less susceptible to the quinolone tested and only 13.33% were susceptible.

Decreased susceptibility to ceftriaxone resistance has already been reported in many of the above centers which is alarming. Out of 79 isolates tested in AIIMS, New Delhi 7.5% were less susceptible to Ceftriaxone and similar pattern was seen in Chennai (9.5% of 21 isolates) and SJH-RRL, Delhi (5.8% of 171 isolates). (7) In our study all the isolates showed sensitivity in disk diffusion method. However, two isolates (13.33%) showed decreased susceptibility to ceftriaxone when MIC was tested using E-test method. The emerging strains with decreased susceptibility to ceftriaxone warrants continued surveillance and evaluation of clinical efficacy and outcomes in these cases is essential.

CONCLUSION

In our study we have reported high-level resistance to penicillin, tetracycline and ciprofloxacin along with the strains showing decreased susceptibility to ceftriaxone, emphasizing their future unsuitability for use. The emergence and dissemination of antimicrobial resistance in *N. gonorrhoeae* are of great concern and they stress the need for appropriate usage of available antibiotics as well the need for research into new options for treatment.

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