Section: Microbiology

Original Research Article

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NEISSERIA GONORRHOEAE ISOLATES FROM PATIENTS ATTENDING AT STI CLINIC, HYDERABAD, INDIA: A COMPARITIVE EVALUATION OF CDS AND CLSI DISC DIFFUSION METHODS

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Received *: 12/10/2026*
Received in revised form *: 10/11/2026*
Accepted *: 23/11/2026*

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DOI: 10.70034/ijmedph.2026.4.397

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (4); 2637-2641

ABSTRACT

Background: Gonorrhoea is a common sexually transmitted infection (STI), and Neisseria gonorrhoeae has progressively acquired resistance to every antimicrobial class used against it. Continuous surveillance is essential to guide treatment guidelines. The objective is to evaluate the trend of antibiotic susceptibility pattern of Neisseria gonorrhoeae isolates amongst the patients attending STI clinic, OGH, Hyderabad. 2.To determine the antimicrobial susceptibility pattern of N.gonorrhoeae isolates carried out following calibrated dichotomous sensitivity (CDS) and National committee for clinical laboratory standards (NCCLS) methods. adopted by all the laboratories under GASP, according to W.H.O manual

Materials and Methods: A prospective study was carried out from March to August 2015 on 100 patients (25 males, 75 females) aged 15–60 years with acute genital discharge. Urethral (males) and endocervical (females) specimens were examined by Gram stain and cultured on chocolate agar and selective medium. Isolates were identified by colony morphology, oxidase, superoxol and rapid carbohydrate utilisation tests. Susceptibility was tested by CDS and CLSI methods; β-lactamase production was detected by the nitrocefin method.

Results: Sixteen of 100 patients (16%) were culture positive: 15/25 males (60%) and 1/75 females (1.3%). The 21–30-year age group accounted for 10 of the 16 cases. All 16 isolates were resistant to ciprofloxacin and nalidixic acid. Fourteen (87.5%) were penicillin resistant (12 penicillinase-producing, PPNG; 2 chromosomally mediated) and 2 (12.5%) were less susceptible. By CDS, 6 isolates (37.5%) were plasmid-mediated tetracycline resistant (TRNG). All isolates were susceptible to ceftriaxone, spectinomycin, azithromycin and cefpodoxime/cefixime. The two methods agreed for all antibiotics except tetracycline.

Conclusion: High-level resistance to penicillin, ciprofloxacin and tetracycline was found, while third-generation cephalosporins remained effective. Judicious antibiotic use and continued surveillance are necessary to prevent treatment failure.

Keywords: Neisseria gonorrhoeae; antimicrobial resistance; CDS; CLSI; PPNG; TRNG; ceftriaxone.

INTRODUCTION

Gonorrhoea, caused by Neisseria gonorrhoeae, is one of the most common sexually transmitted infections and a global health problem.[1] It is transmitted mainly by sexual contact and can also pass from mother to infant during delivery. The organism can infect the urethra, cervix, Bartholin's glands,

anorectal canal, pharynx and conjunctivae. In women it presents as lower genital tract infection, pelvic inflammatory disease and related sequelae; in men as urethritis and epididymitis; and in both sexes as proctitis, pharyngitis, conjunctivitis and, uncommonly, disseminated infection with arthritis–dermatitis syndrome, endocarditis or meningitis. Neonates may develop ophthalmia neonatorum.[2] Gonococcal infection also facilitates the acquisition and transmission of HIV and HPV.[3,4]

In the United States, rates are highest among adolescents and young adults, particularly those aged 20–24 years, and social determinants of health contribute to the community burden.[3] The CDC reported 350,062 cases in 2014 (110.7 per 100,000 persons).[3] The World Health Organization (WHO) estimated 106 million new cases among adults worldwide in 2008, a 21% increase over 2005, with the highest numbers in the Western Pacific (42.0 million), South-East Asia (25.4 million) and African (21.1 million) regions.[5] In India, the proportion of gonorrhoea in STI clinics ranges from 4.2% to 16.2%.[6]

Over the past decades N. gonorrhoeae has developed resistance to penicillin, tetracycline and quinolones in many countries, including India.[7,8] During 2009–2012, the proportion of penicillinase-producing N. gonorrhoeae (PPNG) in the WHO South-East Asia Region varied from 0% to 88.9%, tetracycline resistance from 9.8% to 100%, and ciprofloxacin resistance from 57% to 100%.[9,10] Decreased susceptibility to extended-spectrum cephalosporins (ESCs), the current first-line therapy, has been noted in India and several neighbouring countries, although frank resistance has not been reported from the region.[11] The CDC classifies drug-resistant gonorrhoea as an urgent threat.[9] No vaccine is available, so appropriate prevention, effective diagnosis and antimicrobial treatment remain the cornerstones of control.[12] Continuous surveillance of antimicrobial resistance provides a rational basis for national treatment guidelines.[8]

The present study aimed to determine the antimicrobial susceptibility pattern of N. gonorrhoeae isolates from patients attending the STI clinic at Osmania General Hospital, Hyderabad, using the CDS and CLSI methods, according to the WHO Gonococcal Antimicrobial Surveillance Programme (GASP) manual.

Aim and Objectives

1. To evaluate the trend of antibiotic susceptibility pattern of Neisseria gonorrhoeae isolates amongst the patients attending STI clinic, OGH, Hyderabad.
2. To determine the antimicrobial susceptibility pattern of N.gonorrhoeae isolates carried out following calibrated dichotomous sensitivity (CDS) and National committee for clinical laboratory standards (NCCLS) methods. adopted by all the laboratories under GASP, according to W.H.O manual.

MATERIALS AND METHODS

Study design and setting: A prospective study was conducted from March to August 2015 at the Regional STI Training, Research and Reference Laboratory, Osmania General Hospital, Hyderabad.

Study population: A total of 100 consecutive male and female patients with acute genital discharge were enrolled.

Inclusion Criteria

Patients with acute genital discharge
Study population of Age 15–60 years
Who gave consent to participate in the study.

Exclusion Criteria

Antibiotic use in the previous 4 weeks
Age <15 or >60 years were excluded
Females during menstruation
Refused to give consent.

Specimen Collection: Urethral discharge was collected from males at least 1 hour after urination, after cleaning the meatus with normal saline; if no discharge was present the urethra was milked, or a sterile swab was inserted 2–3 cm and rotated for 5–10 seconds. Endocervical specimens were collected from females using a moistened speculum (no antiseptics or lubricants), cleaning the exocervix and rotating a swab 2 cm within the canal for 5–10 seconds. Where direct inoculation was not possible, albumin-coated swabs were sent in transport medium.

Isolation and identification: Smears were Gram stained, and specimens were inoculated directly onto chocolate agar and saponin-lysed blood agar with V.C.N.T inhibitors. Plates were incubated at 36 °C in a moist atmosphere with 5% CO_2 (candle extinction jar) for 24–48 hours. Identification was by Gram stain, typical colony morphology, oxidase test, superoxol test and rapid carbohydrate utilization test (RCUT), following standard procedures.[7] Isolates were confirmed and lyophilized at the Regional Reference Research, Training and Apex STI Centre, Safdarjung Medical College, New Delhi.

Antimicrobial susceptibility testing: Inoculum were prepared from 18–24-hour pure cultures on chocolate agar, suspended in sterile saline and adjusted to 0.5 McFarland turbidity. Disc diffusion was performed by both CDS and CLSI methods, with WHO reference strains as controls. Disc potencies are shown in [Table 1]. β-lactamase production was detected using nitrocefin discs.

Table 1: Disc potencies used in the CDS and CLSI methods

Antibiotic	CDS	CLSI
Penicillin	0.5 IU	10 IU
Ciprofloxacin	5 µg	1 µg
Ceftriaxone	0.5 µg	30 µg

Spectinomycin	100 µg	100 µg
Tetracycline	10 µg	30 µg
Nalidixic acid	30 µg	–
Cefpodoxime	10 µg	–
Cefixime	–	5 µg
Azithromycin	15 µg	15 µg

Isolates were categorised as susceptible, less susceptible/intermediate or resistant; tetracycline resistance was further classified as plasmid-mediated (TRNG) or non-TRNG (NTRNG) under CDS. Results for cefpodoxime, cefixime and azithromycin were obtained by a single method because of the lack of interpretive criteria or limited resources.

RESULTS

Of 100 patients, 25 were male and 75 female. Sixteen (16%) were culture positive for N. gonorrhoeae: 15/25 males (60%) and 1/75 females (1.3%). Non-gonococcal discharge was found in 8 males (32%) and 55 females (73%); discharge was physiological in 2 males (8%) and 19 females (25.3%) [Table 2].

Table 2: Culture positivity among the study group (n = 100)

Sex	Total	Gonococcal	Non-gonococcal	Physiological
Male	25	15 (60%)	8 (32%)	2 (8%)
Female	75	1 (1.3%)	55 (73%)	19 (25.3%)
Total	100	16 (16%)	63 (63%)	21 (21%)

Age and sex distribution: Males with urethral discharge and females with vaginal discharge were most numerous in the 21–30-year group [Table 3]. Ten of the 16 gonococcal cases occurred in this age group, and the positivity among males was highest at 15–20 years (5/5).

Table 3: Age- and sex-wise distribution

Age (years)	Males	NG positive	Females	NG positive
15–20	5	5	8	0
21–30	12	9	33	1
31–40	5	0	29	0
41–60	3	1	5	0
Total	25	15	75	1

Pattern of sexual exposure: Among males, 7 (28%) reported contact with female sex workers (FSW), 1 (4%) was an MSM, 5 (20%) reported a known partner, 5 (20%) an unknown partner and 7 (28%) gave no history. Among females, 15 (20%) were FSW, 10 (13.3%) reported contact with a spouse and 50 (66.6%) gave no history.

Co-infections and aetiology of discharge: Only 1 of the 15 positive males (6.25% of all positives) had co-infection (herpes). Syphilis, chancroid, donovanosis, trichomoniasis, candidiasis, bacterial vaginosis, chlamydia, genital warts and HIV were not detected among gonorrhoea-positive patients. Among the 84 non-gonococcal cases, Candida albicans was found in 23, Trichomonas vaginalis in 7, mixed candida and trichomonas in 4, bacterial vaginosis in 17, Chlamydia trachomatis in 9 (7 males, 2 females) and genital warts in 2; 21 had physiological discharge.

Table 4: sex wise-pattern of sexual contact

Pattern of exposure	Male	percentage	Female	Percentage
FSW	7	28(%)	15	20(%)
MSM/TG	1	4(%)		0(%)
Known person	5	20(%)	0	0(%)
Un known person	5	20(%)	0	0(%)
Spouse	0	0(%)	10	13.3(%)
Not given History	7	28(%)	50	66.6(%)
TOTAL	25	100(%)	75	100(%)

[Table 4] shows In males 7(28%) had exposure history to female sex workers, 1 (4%) MSM . In females the maximum numbers cases were no. 15 (20%) FSW and 10 (13.3%) cases had history of contact with spouse.

Antimicrobial susceptibility

Table 5: Susceptibility of 16 N. gonorrhoeae isolates

Antibiotic	CDS: S / LS / R (%)	CLSI: S / I / R (%)
Penicillin	0 / 12.5 / 87.5	0 / 12.5 / 87.5
Tetracycline	62.5 NTRNG; 37.5 TRNG	0 / 25 / 75
Ciprofloxacin	0 / 0 / 100	0 / 0 / 100
Nalidixic acid	0 / 0 / 100	0 / 0 / 100
Ceftriaxone	100 / 0 / 0	100 / 0 / 0
Spectinomycin	100 / 0 / 0	100 / 0 / 0

Azithromycin	100 / 0 / 0	100 / 0 / 0
Cefpodoxime (CDS) / Cefixime (CLSI)	100 / 0 / 0	100 / 0 / 0

S, susceptible; LS, less susceptible; I, intermediate; R, resistant.

CDS method [Table 5]. All 16 isolates were resistant to ciprofloxacin and nalidixic acid. For penicillin, 14 (87.5%) were resistant (12 PPNG, 75%; 2 chromosomally mediated, 12.5%) and 2 (12.5%) were less susceptible. For tetracycline, 6 (37.5%) were TRNG and 10 (62.5%) were NTRNG. All isolates were susceptible to ceftriaxone, spectinomycin, cefpodoxime and azithromycin.

CLSI method [Table 5]. Penicillin results were identical to CDS. For tetracycline, 12 isolates (75%) were resistant and 4 (25%) intermediate. All isolates were resistant to ciprofloxacin and nalidixic acid, and susceptible to ceftriaxone, spectinomycin, cefixime and azithromycin.

Comparison of methods: The CDS and CLSI methods showed complete agreement for penicillin, ciprofloxacin, ceftriaxone and spectinomycin. A discrepancy occurred only for tetracycline: by CDS, 37.5% were TRNG, 37.5% showed chromosomally mediated resistance and 25% were less susceptible, whereas CLSI classified 75% as resistant and 25% as intermediate.

Table 6: Results of susceptibility testing 0f 16 isolates for five antibiotics by two methods.

Method	Penicillin			Cipro floxacin			Spectino mycin			Ceftriaxone			Tetra cycline				
	S	LS	R	S	LS	R	S	LS	R	S	LS	R	S	LS	R	TRNG	NTRNG
CDS	0	12.5	87.5	0	0	100	100	0	0	100	0	0	-	-	-	37.5	62.5
CLS	0	12.5	87.5	0	0	100	100	0	0	100	0	0	-	25	37.5	37.5	0

[Table 6] shows discrepancy rate with CLSI disc diffusion method was detected for tetracycline. By CDS 37.5 % were TRNG and 37.5% were having chromosomally mediated resistance and only 25% were less susceptible.

DISCUSSION

Surveillance of in vitro susceptibility is essential for monitoring the effectiveness of recommended treatment. In this study, 16% of patients with acute genital discharge had culture-confirmed gonorrhoea, and the positivity was 60% in males and 1.3% in females. The male figure is comparable to the 65% reported by Gupta et al.[13] The low rate in women probably reflects that many were referred from gynaecology clinics after failing syndromic management and mostly lacked purulent or mucopurulent discharge. Other Indian studies likewise reported low positivity in women (0.4–3.8% and 2%),[14,15] and a molecular study in the UAE found 5.5%.[16]

Most cases occurred in the 21–30-year age group, in agreement with Bala et al,[14] and Gupta et al,[13] while Shilpee et al. observed a peak at 25–30 years.[15] Regarding exposure, 28% of males reported contact with FSW, comparable to the 38.2% reported by Bhavsar et al,[20] the proportions of MSM (4%) and FSW (20%) among attendees were within ranges reported elsewhere,[17–19] underlining that FSW remain a high-risk group for STIs. Among non-gonococcal causes, candidiasis, trichomoniasis and chlamydia were prominent, similar to other Indian clinic series.[14,21] Only one gonorrhoea patient had a co-infection, fewer than in a much larger earlier study,[14] the small number of positives here limits comparison.

Antimicrobial resistance was marked. Resistance to ciprofloxacin was 100%, in line with reports from India (94–98%),[22,23] and with the long-documented spread of quinolone resistance since the 1990s.[12,24] Penicillin resistance (87.5%), with PPNG at 75%, was higher than in other recent Indian series (PPNG 20–68%),[22,23] though regional ranges of 20–79% have been reported.[6] Tetracycline resistance was 37.5% (TRNG) by CDS, within the 9.8–100% range described for South-East Asia.[10] Resistance to penicillin, tetracycline and quinolones therefore makes these drugs unsuitable for empirical therapy.

All isolates were susceptible to ceftriaxone, spectinomycin, azithromycin and cefpodoxime/cefixime, consistent with other Indian studies.[22,23] However, ceftriaxone-less-susceptible strains have been reported from India,[8,25] and the global emergence of cephalosporin resistance parallels the earlier trajectory of quinolone resistance.[26] Current national guidance recommends ceftriaxone with azithromycin, or cefixime with azithromycin when ceftriaxone is unavailable,[27] and this dual therapy remains supported by our findings.

The CDS and CLSI methods agreed for all antibiotics except tetracycline, where CLSI classifies isolates as resistant or intermediate and does not distinguish TRNG from chromosomal resistance. Singh et al. similarly found good agreement of CDS with MIC (E-test) results by TRNG/NTRNG category, with poorer correlation for CLSI.[1]

Limitations: This was a single-centre study of short duration with only 16 isolates, so prevalence estimates are imprecise and cannot be generalised. Females were over-represented and were referred cases. MICs and molecular resistance mechanisms were not determined, and susceptibility to some agents was tested by one method only.

CONCLUSION

Gonococcal positivity was higher in males (60%) than in females (1.3%) among patients with acute genital discharge.

The 21–30-year age group accounted for most cases, and female sex workers represent a high-risk group requiring targeted STI control.

High-level resistance to penicillin, ciprofloxacin and tetracycline was observed.

Ceftriaxone, spectinomycin and azithromycin remained fully active, but judicious use and continued surveillance of circulating strains are needed to prevent treatment failure and spread of resistance.

CDS and CLSI results coincided for all antibiotics except tetracycline.

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