A study on various *Neisseria gonorrhoeae* phenotypes circulating in Tripura

Sir,

Gonorrhea is one of the most common sexually transmitted infections in the developed world caused by the Gram-negative diplococcus *Neisseria gonorrhoeae*. Once an easily treatable disease, it has become a therapeutic problem owing to coinfections, asymptomatic infections, misdiagnosis and antibiotic misuse leading to multidrug resistance. There was lack of epidemiologic data from Tripura on the frequency of disease and drug resistance patterns, which encouraged us to conduct this study which would help to identify the circulating drug resistant phenotypes and thereby determine the drugs effective in the local population.

The study was conducted at Agartala Government Medical College, Agartala, Tripura, between July 2013 and June 2015 after receiving clearance from the Institutional Ethical Committee. Two hundred and seventy five subjects suspected to have clinically compatible features of sexually transmitted infections in the reproductive age group of 18–49 years attending the dermatology or the obstetrics and gynecology clinics were included in the study. Direct Gram-stain of urethral/cervical smear was performed followed by culturing on chocolate agar (HiMedia Laboratories, Mumbai, India). Identification was done as per standard protocol. *Chlamydia trachomatis* screening was done using polymerase chain reaction.[1] For diagnosis of human immunodeficiency virus infection, National Acquired Immune Deficiency Syndrome Control Organization guideline of strategy III was followed. Antimicrobial susceptibility testing was performed against penicillin G, tetracycline, ciprofloxacin, nalidixic acid, azithromycin, spectinomycin and ceftriaxone through E-test and Kirby–Bauer disc diffusion methods. Modified Hodge test was carried out among the resistant phenotypes.[2] Briefly, plain chocolate agar was lawn cultured with β-lactam sensitive *Staphylococcus aureus* strain (ATCC®25923™). Single penicillin disc (10 U) was placed in the center and test strain was streaked radially out from the disk. Similarly, three more streaks [Figure 1] of second test strain, positive control (β-lactamase producing strain of *N. gonorrhoeae* ATCC®31426™) and negative control (β-lactam sensitive strain of *S. aureus* ATCC®29213™) were made at 90°, 180° and 270° respective to the first strain. Liquid acidometric

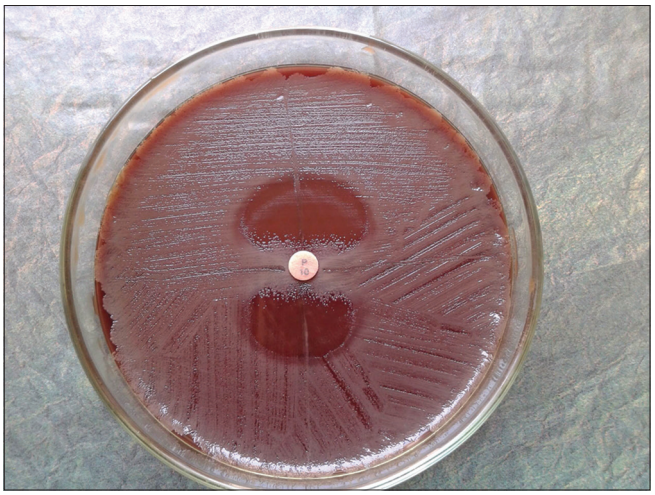

Figure 1: Penicillinase production confirmation through Hodge test. Disruption of inhibition zone of β-lactam sensitive *S. aureus* at 0° and 180° positions by β-lactamase producing strains. At 90° and 270° positions are β-lactam sensitive isolates

test was done as per standard protocol.[3] Briefly, benzylpenicillin solution (HiMedia Laboratories, Mumbai, India) containing 0.1% aqueous cresol red (HiMedia Laboratories, Mumbai, India) (pH 8.0) was aliquoted at 50 µl/well in a 96-well microtiter plate. To each well, a loop full of test culture was dispersed. A change in color from cherry red to bright yellow (pH 7.2) [Figure 2] within 30 minutes indicated positive β-lactamase production.

Among the study subjects of 275 cases, 29 (10.5%) were positive on direct microscopy of Gram-stained slides for intra- and extra-cellular Gram-negative diplococci. However, isolation of *N. gonorrhoeae* on culture could be done in 20/29 (69%) cases. The time lapse between acquisition of infection to the time of visit to the clinic ranged from 4 to 60 days with a median value of 10 days. The duration from exposure to onset of symptoms ranged between 2 and 14 days [Figure 3]. Of the 29 Gram-stain positive subjects, six were coinfected with *C. trachomatis* and one was human immunodeficiency virus seropositive. Susceptibility testing towards the standard drugs showed the highest sensitivity for ceftriaxone 20/20 (100%) and spectinomycin 19/20 (95%) while least sensitivity was for fluoroquinolones 5/20 (25%) [Table 1]. A single isolate showed multidrug resistance with resistance towards spectinomycin, fluoroquinolones, tetracyclines and azithromycin. The predominant drug resistant phenotype found was plasmid-mediated resistance to both penicillins and tetracyclines 7/20 (35%) followed by penicillinase-producing

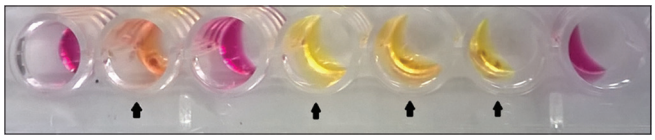

Figure 2: β-lactamase production through liquid culture method. Bright yellow/ orange coloration in wells (▲) due to β-lactamase production by *N. gonorrhoeae*, cherry red coloration due to lack of β-lactamase production

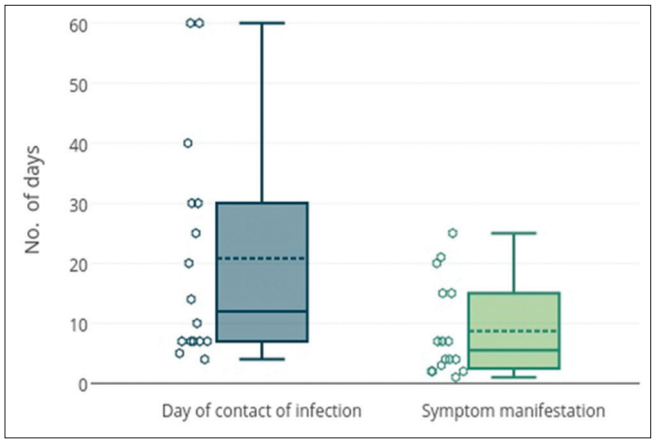

Figure 3: Box and whisker plot of days lapsed since contact of gonococcal infection to visit to clinic and days passed between contact and manifestation of symptoms

N. gonorrhoeae (15%) and chromosome-mediated penicillin-resistant *N. gonorrhoeae* 2/20 (10%) [Table 2].

Gonorrhoea accounted for 10.5% of all sexually transmitted infections in our study, which is similar to other studies from India.[4,5] As per revised category of drugs proposed for treatment of gonorrhoea, 1/20 (5%) cases were found to be resistant to Category I drugs; 17/20 (85%) were resistant to one or more drugs of Category II; and 9/20 (45%) were found to be resistant to Category III drugs [Table 1]. Among the Category II drugs, in spite of increased reports of resistance, azithromycin has been proposed for more frequent use.[6] Our study found azithromycin resistance rate to be 4/20 (20%), which is higher than the 5% failure rate criteria.[7] Whether such high resistance still qualifies it as a candidate for treatment requires a larger prevalence study.

On the basis of minimum inhibitory concentration breakpoints and simple tests for β-lactamase production, it is possible to determine the mechanism of resistance without going into molecular studies.[8] However, simple tests like β-lactamase production are not appropriate for determining the mechanism of resistance for tetracyclines and for these drugs, confirmation requires molecular testing. Our study showed that resistance was plasmid-mediated in most cases, most commonly conferring resistance to both penicillins and tetracyclines through β-lactamase producing as well as tetracycline determinant (tetM) conjugative plasmids. This was followed by β-lactamase-producing plasmids conferring resistance to penicillins. The third most abundant penicillin-resistant phenotype was resistant to penicillins through chromosomal-mediated resistance. Limitations of the study were the small sample size and low participation by female subjects due to lack of awareness and stigma attached with sexually transmitted infections. Secondly, we were unable to study the effect of HIV on gonorrhoea as our study included only a single subject harboring this coinfection. To estimate the effect of gonorrhea on human immunodeficiency virus coinfection in the community, a large-scale prevalence study is essential.

In conclusion, frequency of gonococcal infection among symptomatic subjects was similar to studies from other parts of India. There was good sensitivity to the drugs recommended currently by the Centers for Disease Control and Prevention. Most common mechanism of drug resistance was found to be plasmid mediated conferring resistance to both penicillins and tetracyclines.

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Table 1: Antibiotic susceptibility profile of revised category core drug groups.

AST results (%)	Category I		Category II			Category III
	Ceftriaxone	Spectinomycin	Azithromycin	Penicillins	Fluoroquinolones	Tetracyclines
Susceptible	20 (100)	19 (95)	16 (80)	8 (40)	5 (25)	8 (40)
Intermediate	0	0	0	2 (10)	4 (20)	3 (15)
Resistant	0	1 (5)	4 (20)	10 (50)	11 (55)	9 (45)

Table 2: Number and characteristics of isolates of each phenotype identified in the study

Phenotype	Phenotypic definition based on associated β-lactamase and MIC values	Number of isolates with specified characteristics (%)
Susceptible	β-lactamase negative Penicillin susceptible (MIC <2.0 μg/ml) Tetracycline susceptible (MIC <2.0 μg/ml)	6 (30)
Plasmid-mediated resistance to both penicillins and tetracyclines	β-lactamase positive Tetracycline resistant (MIC ≥16.0 μg/ml)	7 (35)
Penicillinase-producing *N. gonorrhoeae*	β-lactamase positive (irrespective of MIC value)	3 (15)
Chromosome mediated penicillin-resistant *N. gonorrhoeae*	β-lactamase negative Penicillin resistant (MIC ≥2.0 μg/ml) Tetracycline susceptible (MIC <2.0 μg/ml)	2 (10)
TRNG	β-lactamase negative Penicillin susceptible (MIC <2.0 μg/ml) Tetracycline resistant (MIC ≥16.0 μg/ml)	1* (5)
Chromosome-mediated tetracycline-resistant *N. gonorrhoeae*	β-lactamase negative Penicillin susceptible (MIC <2.0 μg/ml) Tetracycline resistant (MIC 2.0-8.0 μg/ml)	0*
Chromosome-mediated penicillin- and tetracycline-resistant *N. gonorrhoeae*	β-lactamase negative Penicillin resistant (MIC ≥2.0 μg/ml) Tetracycline resistant (MIC ≥2.0 μg/ml)	1 (5)

*Some isolates may show TRNG characteristics but may be TetR, the difference can only be confirmed at molecular level by determining the presence or absence of resistance-conferring plasmid tetM. MIC: Minimum inhibitory concentration, *N. gonorrhoeae*: *Neisseria gonorrhoeae*, TRNG: Tetracycline resistant *N. gonorrhoeae*, TetR: Tetracycline-resistant

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Conflicts of interest

There are no conflicts of interest.

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