

## Serotype and serovar distribution of *Neisseria gonorrhoeae* isolated from high-risk populations in Bangladesh

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### Abstract

*Neisseria gonorrhoeae*, the causative agent of gonococcal infection, is known to frequently change their characteristics to evade host immune mechanism. Characterization of the clinical isolates of the organism can lead to identification of the circulating strains and often a sexual network in a community to help in designing the control strategy. Keeping in mind the above consideration, a total of 239 *N. gonorrhoeae*, isolated from high-risk populations, were characterized for serotypes and serovars by monoclonal antibodies against protein I of the organism. Majority of the serotypes were serotype B (142, 59.4%). Majority of the isolates showing resistance to at least one of the antibiotics tested were also serotype B (139, 59.2%), whereas, majority of the isolates showing resistance to any three of the antibiotics (multidrug resistant, MDR) (63%) was serotype A. A total of 41 different serovars were also identified and five of which (Arst, Bropt, Bopt, Arost, and Brop) included the highest percent (49.3%) of the isolates. Many serovars (23/41, 56.1%) were new emergent and included 58 (24.3%) of the isolates investigated. All of the new serovars were resistant to at least one of the antibiotics tested and the highest rate (40/102, 39.2%) was MDR. Serotyping and serovar determination was found contributory to understand the microepidemics of the *N. gonorrhoeae* isolates. Further studies including antibiogram and contact tracing can efficiently help in control of the disease.

### Introduction

*Neisseria gonorrhoeae*, the aetiological agent of gonococcal infection or gonorrhoea, is pathogenic for humans only and adapts easily to the moist mucous membranes of genital tract and other similar structures. It is highly capable in resisting immune attack by the antigenic variability to evade host defenses, thus persisting and often causing asymptomatic (and undetected) infections<sup>1</sup>. In addition, the organism possesses some unique ability to develop resistance to antimicrobials and rapidly become ineffective to the commonly used antibiotics<sup>2</sup>. Characterization of the clinical isolates of the organism, including serotypes and serovars distribution in a community, can exactly identify the circulating strains to design the effective control strategies.

For the purpose of characterization, the information on the structural components of *N. gonorrhoeae* is very essential. The organism possesses a typical Gram-negative outer membrane composed of

several proteins in addition to other structures. Three proteins are present in large amounts and these have been named proteins I, II, and III (PI, PII, and PIII respectively). Protein I (PI) is the predominant protein species of the gonococcal outer membrane and functionally is a porin protein<sup>3</sup>. There is extensive heterogeneity of gonococcal PI proteins between strains and this property makes PI an excellent marker for strain classification and epidemiological studies. Immunological and biochemical data have determined that there are two distinct structural variants of the porin protein, IA and IB that are further subclassified into serovars based on reactivity to a panel of PI-specific monoclonal antibodies. This information forms the basis of a serological typing system for the gonococcus<sup>4</sup>.

The gonococcal serogrouping system has proved to have important epidemiological and clinical potentials. Serogroup PIA was found to correlate with resistance to the bactericidal action of normal serum, disseminated gonococcal infection (DGI)

and asymptomatic gonorrhoea<sup>5</sup>. Whereas, serogroup PIB was found to correlate with resistance to several antimicrobial agents and with homosexually acquired infection<sup>6</sup>. Few studies with *N. gonorrhoeae* epidemiology suggest that some of the strains are likely to spread within the high-risk populations like commercial sex workers and their clients<sup>7</sup>.

In Bangladesh, situation of the disease is equally difficult and its epidemiological aspect has not been purposefully explored and gonococcal serotypes and serovar patterns have not been reported to date. The present study was, therefore, carried out to examine the diversity and distribution of *N. gonorrhoeae* serotypes and serovars of the previously isolated, identified and partially characterized *N. gonorrhoeae* strains from high risk populations in Bangladesh.

### Materials and Methods

A total of 239 strains of *Neisseria gonorrhoeae* were selected randomly from ICDDR,B pool of the organisms isolated from high-risk populations by the then RTI/STI laboratory during 2003-2006. The high-risk populations from whom the isolates were collected included female commercial sex workers (CSWs)- hotel- and street-based at Dhaka City (CSWs-Floating); CSWs- brothel-based at Gualanda, Faridpur (CSWs-bb); male individuals attending out-patients department at Dhaka Medical College Hospital (male-OPD, Dhaka); male individuals attending OPD at Sylhet MAG Osmani Medical College Hospital (male-OPD, Sylhet); long distant male truckers (male truckers) by Paricharjya, Dhaka; and male individuals having sex with male (MSMs) at Sylhet.

Each of the selected isolates of *N. gonorrhoeae*, harvested from pure subculture on GC agar medium into phosphate buffered saline (pH 7.2), was boiled for 10 minutes for antigen preparation. The serotype and serovar of the isolates were determined using an equal volume of the prepared antigen suspensions and monoclonal antibodies using Pharmacia (Ph) panel (Boule Diagnostics AB, Huddinge, Sweden) consisting of five Protein IA specific (Ar, Ao, As, At, Av) and nine Protein IB specific (Br, Bo, Bp, By, Bu, Bv, Bs, Bt, Bx) reagents.

Serotype of a strain of *N. gonorrhoeae* was defined as the type of reagents with which it reacted, as “type A” if reacted with PIA reagents and as “type B” if reacted with PrIB reagents. A serovar was assigned as the pattern of reactivity of a test strain with a given set of monoclonal antibodies specific for either PrIA or PrIB<sup>8</sup>. For example, when a *N.*

*gonorrhoeae* strain reacted with PIB set with the antisera “r”, “o”, “p”, “u” & “t”, then the serovar of the strain was recorded as “Broput”.

The selected isolates of *N. gonorrhoeae* were previously tested for antimicrobial susceptibility and plasmid profile by the same investigators and these results were used to correlate serotypes and serovars<sup>9</sup>.

### Results

Among the total 239 strains investigated, majority was serotype B (142, 59.4%), followed by serotype A (88, 36.8%) and a few showing mixed type (9, 3.8%) (Figure 1).

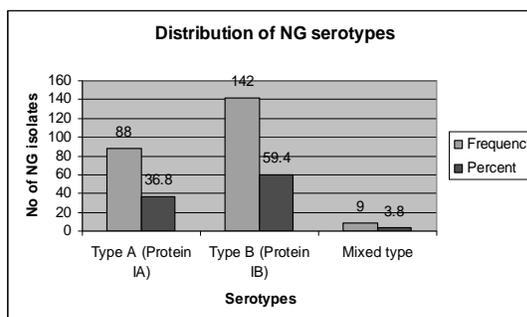


Figure 1: Distribution of NG serotypes

On distribution of the serotypes among high-risk population groups from whom the *N. gonorrhoeae* were isolated, it was found that majority of the isolates were type B among all populations, the highest rate being among the male individuals with history of exposure attending out-patient-departments (Male-OPD) (75%, 42/56), followed by male sex for male (MSMs) (66.7%, 2/3) and long-distant male truckers (GM-Truckers) (62.5%, 10/16) (Table I).

Table I: Distribution of *Neisseria gonorrhoeae* (NG) serotypes among high-risk populations

Sl No	High-risk population	source	No (%) of NG serotypes		
			Type A	Type B	Mixed
01	CSWs (n=164)		69 (42.1)	88 (53.7)	07 (4.2)
02	Male-OPD (n=56)		13 (23.2)	42 (75.0)	01 (1.8)
03	Male-Truckers (n=16)		05 (31.3)	10 (62.5)	01 (6.2)
04	MSMs (n=03)		01 (33.3)	02 (66.7)	00 (00)
Total (n=239)			88 (36.8)	142(59.4)	09 (3.8)

CSW-Commercial Sex Worker, Male-OPD- Male individuals attending outpatients department, Male-Truckers- Male-long distant truckers, MSMs- Male individuals having sex for male (homosexuals).

Considering relationship between the serotypes and antimicrobial resistance of the *N. gonorrhoeae* isolates, it was found that majority showing resistance to any one or two of the antibiotics tested was serotype B (59.3% of the isolates showing resistance to any one the antibiotics and 83.3% of

the isolates showing resistance to any two of the antibiotics), whereas, majority of the isolates showing resistance to three of the antibiotics (multi-drug-resistant, MDR) were type A (63.0%). Of the total 235 NG isolates showing resistance to at least any one of the antibiotics tested, majority (139, 59.2%) were serotype B (Table II).

**Table II:** Distribution of *Neisseria gonorrhoeae* (NG) serotypes into categories of resistance of the isolates

Sl No	Pattern of resistance	No (%) of NG isolates in each serotype		
		Type A	Type B	Mixed
01	Resistance to any one antibiotic (n=27)	10(37.0)	16(59.3)	1(3.7)
02	Resistance to any two antibiotics (n=108)	14(13.0)	90(83.3)	4(3.7)
03	Resistance to three (Pen, Tet & Cip) MDR (n=100)	63(63.0)	33(33.0)	4(4.0)
Total (n=235)		87(37.0)	139(59.2)	9(3.8)

On distribution of *N. gonorrhoeae* serotypes among plasmid-mediated resistant isolates, it is found that majority of the PPNG (Penicillinase producing *N. gonorrhoeae*) (5/10, 50.0%), and PPNG-TRNG (tetracycline-resistant *N. gonorrhoeae*, plasmid-mediated) (59/71, 83.10%) strains were type A and majority of the TRNG (92/113, 81.42%) were type B (Table III).

**Table III:** Distribution of *N. gonorrhoeae* (NG) serotypes among plasmid-mediated resistant isolates

Plasmid-mediated resistant types	Serotypes & no (%) of NG isolates		
	Type A	Type B	Mixed type
PPNG (n=10)	05 (50.0)	04 (40.0)	01 (10.0)
TRNG (n=113)	19 (16.81)	92 (81.42)	02 (1.77)
PPNG-TRNG (n=71)	59 (83.10)	09 (12.68)	03 (4.22)
Non-PPNG-Non-TRNG (n=45)	5 (11.11)	37 (82.22)	3 (6.67)
Total (n=239)	83(34.73)	105(43.93)	06(2.51)

Note: PPNG- Penicillinase Producing *N. gonorrhoeae*; TRNG- Tetracycline Resistant (plasmid-mediated) *N. gonorrhoeae*

A total of 41 different serovars were identified of which the highest *N. gonorrhoeae* isolates were

“Arst” (46/239, 19.2%) followed by “Brop” (22/239, 9.2%), “Bopt” (18/239, 7.5%). Nine major serovars included equal or more than 10 *N. gonorrhoeae* strains and represented 69.04% (165/239) of the isolates investigated. Whereas, 32 of the serovars were found to include less than 10 isolates and included 74 (30.9%) of the isolates tested. (Table IV) Two small clusters of the serovars of mixed serotype (AvBx and AstvBx) were also found (not shown in the table).

On distribution of the major *N. gonorrhoeae* serovars among high-risk source populations, it was found that the most frequently occurring serovar among floating commercial sex workers (CSWs-Floating) was “Arst” (35/156, 22.4%) followed by “Arost” (13/156, 8.3%). Among the male individuals attending OPD at Dhaka Medical College (Male-OPD, Dhaka), the most frequently occurring serovar was “Brop” (4/32, 12.5%) followed by “Arst” and “Byvut” (3/32, 9.4% each) and among males attending OPD at Sylhet MAG Osmani Medical College (Male-OPD, Sylhet), the most frequently occurring serovar was “Brop” (6/24, 25.0%) followed by “Arst” (3/24, 12.5%). Among the male truckers, “Arst” (3/16, 18.7%) was the highest occurring serovar. Serovars of the 3 *N. gonorrhoeae* isolated from MSMs were: “Arst”, “Bropyt” and “Bopt” (Table IV).

Many of the serovars identified (23/41, 56.1%) were not reported previously and are therefore, designated as newly emerged serovars. The new serovars included 58 (24.3%) of the isolates investigated and among them “Ast” as a single new emergent included highest number of isolates (12, 5.0%). (Table V) All of the new serovars were resistant to at least one of the antibiotics tested and the highest number of them (40/58, 69.0%) were resistant to any three of the antimicrobial agents (MDR). (Table VI)

**Table IV:** Major *Neisseria gonorrhoeae* (NG) serovars and their distribution among the source population

Sl No	NG serovars	No (%) of NG isolates distributed into the source population						
		CSW-Floating	CSW-bb	Male-OPD Dhaka	Male-OPD Sylhet	MSM	Male-Truckers	Total
1	Arst	35 (22.4)	1 (12.5)	3 (9.4)	3 (12.5)	1 (33.3)	3 (18.7)	46 (19.3)
2	Brop	10 (6.4)	2 (25.0)	2 (6.3)	6 (25.0)	0	2 (12.4)	22 (9.2)
3	Bopt	11 (7.1)	1 (12.5)	2 (6.3)	1 (4.2)	1 (33.3)	2 (12.4)	18 (7.5)
4	Arost	13 (8.3)	1 (12.5)	2 (6.3)	0	0	1 (6.3)	17 (7.1)
5	Brop	11 (7.1)	0	1 (3.1)	1 (4.2)	0	2 (12.5)	15 (6.3)
6	Bopyt	10 (6.4)	0	1 (3.1)	2 (8.3)	0	1 (6.3)	14 (5.9)
7	Ast	8 (5.1)	2 (25.0)	1 (3.0)	0	0	1 (6.3)	12 (5.0)
8	Broput	5 (3.2)	0	4 (12.5)	1 (4.2)	0	1 (6.3)	11 (4.6)
9	Byvut	6 (3.9)	0	3 (9.4)	0	0	1 (6.3)	10 (4.1)
10	Others (32 serovars)	47 (30.1)	1 (12.5)	13 (40.6)	10 (41.6)	1 (33.4)	2 (12.5)	74 (31.0)
Total		156(100.0)	8 (100.0)	32(100.0)	24(100.0)	3(100.0)	16(100.0)	239(100.0)

Note: CSW- Floating: Commercial Sex Workers engaged in unlawful commercial sex in hotels and streets, CSW-bb: Brothel-based CSWs; Male-OPD, Dhaka: Symptomatic males attending outpatients department of Dhaka Medical College Hospital; Male-OPD, Sylhet: Symptomatic males attending OPD of Sylhet MAG Osmani Medical College Hospital, MSM- Male individuals having sex with male

**Table V:** Newly emergent serovars of the *N. gonorrhoeae* (NG) isolates investigated (n=239)

Sl no	New Serovars of NG isolates	Number	Percent
1	Ast	12	5.0
2	Arstv	6	2.5
3	Astv	5	2.1
4-5	Brpys, Brpyt (4 each)	8	3.3
6-8	AstvBx, Brpyus, Brpyvu (3 each)	9	3.8
9-11	Bopvt, Bropvu, Bopyst (2 each)	6	2.5
12-23	Aost, Arostv, Bop, Bopyvs, Bopyvu, Bot, Bpyst, Bpyt, Bropyv, Bryst, Byst, Byvutx (1 each)	12	5.0
Total		58	24.3

**Table VI:** Relationship between new serovars and antimicrobial resistance of the *N. gonorrhoeae* (NG) isolates

Resistance to number of antibiotics tested	<i>N. gonorrhoeae</i> isolates	
	Showing new serovars	Percent
One antibiotic	4	6.9
Two antibiotics	14	24.1
Three antibiotics (MDR)	40	69.0
Total	58	100.0

## Discussion

The present investigation was carried out to see the serotypes and serovars among *Neisseria gonorrhoeae* (NG) isolated from different high-risk populations from Dhaka, Sylhet and Faridpur including commercial sex workers of brothel and street- as well as hotel-based, male individuals attending outpatients departments, homosexuals and long distant male truckers.

Majority of the NG serotypes were type B corresponding to Protein IB serogroups as identified by other investigators<sup>10-12</sup>. Cross-tabulating the isolates from high-risk populations and NG serotypes, it was again found that majority of the isolates in all populations were type B. By distribution of the isolates into the sex of the source populations, it was found that majority of the NG strains isolated were serotype B. Majority of the isolates investigated in the present study showed resistance to any one or two of the antibiotics tested previously and majority of the NG isolates showing resistance to at least any one of the antibiotic tested was serotype B (139, 59.2%) and the results thus correlate well with the reported fact that that Protein IB strains are more resistant to antibiotics<sup>13</sup>.

In the present study, there were 41 different serovars infecting 5 different high-risk populations of which 9 major serovars including  $\geq 10$  NG isolates (165/239, 69.04%) were predominating. Similarly, majority of the isolates were included in 7 of the serovars (559/859, 65.08%) in a study in Stockholm<sup>12</sup>. The major serovars in the present

study were: Arst (19.2%), Bropt (9.2%), Bopt (7.5%), Arost (7.1%), Bropt (6.2%), Bopyt (5.8%), Ast (5.0%), Broput (4.6%) and Byvut (4.1%). In another study in Stockholm reported in 1993, investigators found that among the Protein IA strains, the most common were "Arost" (71%) and among the Protein IB strains, the most common serovars were "Brpyust" (20%) and "Bropt" (19%)<sup>5</sup>.

One study among the homosexuals in Edinburgh, UK over a 5 year period among 175 infections found 32 different serovars of which "Bropt" (46/175, 26.29%) and "Brpyust" (29/175, 16.57%) were predominating<sup>11</sup>. Whereas, only 3 infections were encountered among homosexuals in the present study and found one each of different serovars of "Arst", "Bropt" and "Byvut". The isolates from homosexuals of the present study are very few in number and can not represent a cohort group of homosexuals with this insignificant result.

Many of the serovars (23/41, 56.1%) have not been reported previously and therefore, were designated as new emergent. All of the newly emerging serovars were found resistant to any one of the antibiotics tested and majority of them was multi-drug-resistant (MDR). These findings suggest that the circulating strains of *N. gonorrhoeae* among high-risk populations are changing their characteristics to evade antimicrobial challenge.

The NG serovars predominating among the commercial sex workers (CSWs) were not found consistently predominating among other populations which are supposed to have acquired from the CSWs. For this ambiguity, contact tracing might be one of the important mechanisms in the line of control of gonococcal infection. Contact tracing to identify the source and route of transmission as well as antimicrobial susceptibility surveillance are recommended.

**Conclusion:** *N. gonorrhoeae* serotype Protein IB was predominating in the present study and as these strains were also found to be more resistant to the antimicrobial agents, treatment modality for gonococcal infection might be difficult in Bangladesh. Although serotyping & serovar determination was found contributory in understanding the microepidemics, these tests require other information, especially antibiogram of the circulating strains of the organism to have effective role in control of the disease. Therefore, surveillance for antimicrobial susceptibility of the gonococcal isolates along with contact tracing to understand route of transmission can help much more for designing an effective control measure in Bangladesh.

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## References

1. Todor K. The Pathogenic Neisseriae. In: Todor's online Textbook of Bacteriology. Department of Bacteriology, The University of Wisconsin-Madison. 2004. Webpage, retrieved from: "The pathogenic Neisseriae.htm" (accessed on: 20/04/2008).
2. Alam MA, Uddin MS, Chowdhury TA, et al. Antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* isolated from sexually active males: an absolute ineffectiveness of Ciprofloxacin. *Bangladesh J Dermatol Venereol Leprol* 2005; 22(1): 10-15.
3. Gotschlich EC, Seiff ME, Blake MS and Koomey M. Porin protein of *Neisseria gonorrhoeae*: Cloning and gene structure. *Proc Natl Acad Sci USA* 1987; 84: 8135-8139.
4. Knapp JS, Tam MR, Nowinski RC, Holmes KK and Sandstrom EG. Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. *J Infect Dis* 1984; 150: 647-654.
5. Bygdeman SM, Ruden AK, Jonsson A, et al. Antibiotic susceptibility, serovars and auxotypes of gonococcal isolates in Stockholm. Relation to geographical origin of the infections. *Intl J STD AIDS* 1993; 4: 33-40.
6. Reid KG and Young H. Serogrouping *Neisseria gonorrhoeae*: correlation of coagglutination serogroup VII with homosexually acquired infection. *Br J Vener Dis* 1984; 60: 302-305.
7. Moodley P, Martin IMC, Ison CA and Sturm AW. Typing of *Neisseria gonorrhoeae* reveals rapid reinfection in rural South Africa. *J Clin Microbiol* 2002; 40 (12): 4567-4570.
8. Coghill DV and Young H. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibody coagglutination reagents. *Genitourin Med* 1987; 63: 225-232.
9. Alam MA. Antimicrobial Resistance and Molecular Epidemiology of *Neisseria gonorrhoeae* in Bangladesh. Ph.D. Thesis. Faculty of Postgraduate Medicine and Research, University of Dhaka, 2010.
10. Buchanan TM and Hildebrandt JF. Antigen-specific serotyping of *Neisseria gonorrhoeae*: characterization based upon principal outer membrane protein. *Infect Immun* 1981; 32: 985-994.
11. Young H, Moyes A, Ross JDC and McMillan A. Patterns of homosexually acquired gonococcal serovars in Edinburgh 1986-90. *Genitourin Med* 1991; 67: 312-316.
12. Ruden AK. Temporal changes in the gonococcal serovar patterns in Stockholm during two years with special reference to PPNG strains. *Genitourin Med* 1994; 70: 256-261.
13. Bygdeman S, Danielsson D and Sandstrom E. Serological classification of *Neisseria gonorrhoeae* by coagglutination: a study of serological patterns in two geographical areas of Sweden. *Acta Derm Venereol (Stockh)* 1981; 61: 423-427.