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Whole genome sequencing of *Neisseria gonorrhoeae* among key populations in two cities of Bangladesh reveals genomic diversity and stable antimicrobial resistance

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Abstract

Background *Neisseria gonorrhoeae* is an obligate human pathogen causing gonorrhea the second most reported bacterial sexually transmitted infection. High level genomic plasticity of this pathogen requires whole genome sequence (WGS) analysis for epidemiological investigations. The absence of *N. gonorrhoeae* genomic data from Bangladesh represents a critical gap in South Asian gonococcal genomic epidemiology. Here we report, a genomic investigation of *N. gonorrhoeae* from Bangladesh using WGS approach.

Methods Twenty-four *N. gonorrhoeae* isolates were collected from key populations including female sex workers, male sex workers and Hijra. These belong to two studies conducted in two districts of Bangladesh; a cross-sectional bio-behavioral survey 2014 (Dhaka) and sexual and reproductive health and rights 2022–2024 (Dhaka and Jashore). Antibiotic resistance data from disc-diffusion assay were available from specific study. Recovered isolates were sequenced using Illumina NextSeq550 instrument. Sequence typing, presence of virulence genes and antimicrobial resistance genes/mutations were determined using PubMLST, Pathogenwatch, and ABRicate. Phylogenetic analysis was conducted using maximum likelihood method with globally reported selected sequences as well as WHO reference sequences.

Results Genomic analysis for MLST revealed nine sequence types, with ST7363 predominating in 50% (12/24) of isolates, particularly among male sex workers (58.3%; 7/12). Novel NG-STAR types comprised 33.3% (8/24) of isolates, while novel NG-MAST types reached 62.5% (15/24). Genomic analysis predicted resistance to ciprofloxacin (100%), sulfonamides (91.7%) and tetracycline (79.2%), while 100% sensitivity was predicted for azithromycin, ceftriaxone and spectinomycin. Phylogenetic analysis showed four intra clusters for 18 isolates and 6 were distributed separately.

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Conclusions Here we report, genomic diversity of *N. gonorrhoeae* from Bangladeshi key populations predicting relatively stable antibiotic susceptibility pattern in comparison to previous studies. These findings emphasize the urgent need for genomic surveillance and antimicrobial stewardship in Bangladesh to preserve current treatment options.

Keywords *Neisseria gonorrhoeae*, Key population, Sexual and reproductive health and rights, SRHR, Whole genome sequencing, Bangladesh

Introduction

Sexually transmitted infections (STIs) lead to substantial morbidity, mortality, and reduced quality of life for infected individuals, affecting various aspects of sexual and reproductive health. The most reported STI after *Chlamydia trachomatis* is *Neisseria gonorrhoeae* infection [1]. *N. gonorrhoeae* is the etiologic agent of gonorrhea and transmits exclusively through sexual contact. Globally, an estimated 82.4 million new *N. gonorrhoeae* infections occurred in 2020 alone [2]. Microbial culture of *N. gonorrhoeae* is crucial for diagnosing gonorrhea, as the investigation of antibiotic resistance phenotypes and genome-wide *de novo* analysis requires isolation of the bacterium. The specific nutrient requirements and reduced oxygen needs for growth make *N. gonorrhoeae* a fastidious pathogen. This affects diagnostic sensitivity, leading to routine diagnosis of gonorrhea dependent on either clinical investigation or molecular methods [3].

N. gonorrhoeae is a gram-negative diplococcus that inhabits the urogenital, rectal, and oropharyngeal areas of infected individuals. Key populations, such as female sex workers (FSW), male sex workers (MSW), Hijra (a traditional third-gender group in South Asia), adolescents, and people with diverse sexual orientations and practices, are particularly vulnerable to this infection due to the bacterial specific ecological preference to mucosal regions in human body. Notably, Hijra are considered as key population as they engage in sex work and at high risk of STIs due to frequent unprotected sexual contact. Undiagnosed and untreated infections can lead to complications ranging from epididymitis and salpingitis to pelvic inflammatory disease, ectopic pregnancy, infertility, and even newborn blindness [4, 5]. The fastidious nature of the pathogen, limited resources in low- and middle-income countries, asymptomatic infections, and the socially hidden nature of key populations all contribute to the underreporting of *N. gonorrhoeae*. Consequently, these key populations remain major reservoirs of this pathogen in the community.

At the advent of the antibiotic era beginning in the 1940s, sulfonamide, penicillin, and tetracycline were successfully used to treat *N. gonorrhoeae* infections. The pathogen has since developed resistance to a wide range of antibiotic classes, including fluoroquinolones and macrolides [6]. WHO guidelines in 2016, and Bangladesh treatment regime in 2018 included a single dose of ceftriaxone or cefixime in combination with azithromycin for gonococcal infection [7, 8]. However, azithromycin resistance has increased around 10-fold since 2013 [9]. Several countries—including Vietnam, Slovenia, Estonia, South Korea, Iran, Cambodia, and Switzerland—have reported ceftriaxone resistance above 2%, while Bangladesh, Iran, Slovenia, China, Japan, and Italy have reported cefixime resistance above 5% [10]. Given relatively lower resistance, the Centers for Disease Control and Prevention (CDC), USA recommends a single dose of ceftriaxone [9].

Against antibiotics, *N. gonorrhoeae* has evolved to possess a wide range of resistance mechanisms. These include plasmid-mediated *blaTEM* gene (which hydrolyzes penicillin) [11], mutations in *penA* and *ponA* genes (conferring resistance to penicillin and extended-spectrum cephalosporins) [12, 13], 23S rRNA mutations (resistance to macrolides) [12], *rpsE* gene mutations (spectinomycin resistance) [12], *gyrA* and *parC* mutations (quinolone resistance) [12], and *erm* gene mutations (erythromycin and azithromycin resistance) [12]. Mutations in the gene products of *porB* and *pilQ* decrease the influx of antibiotics, including penicillin and tetracycline [14]. Resistance due to *mtrR* gene mutations leads to overexpression of the *mtrCDE* efflux pump, reducing the effectiveness of penicillin, cephalosporin, tetracycline, and macrolide group of antibiotics [15]. The bacteria can also acquire resistance genes from other bacterial species, such as the *tetM* gene from *Haemophilus influenzae*, which confers resistance to tetracycline [12].

N. gonorrhoeae express multiple virulence factors, including lipooligosaccharide, Opa (opacity protein), type IV pili, and IgA proteases 1 and 2. These virulence factors protect the bacterium from the host immune system and complicate diagnosis by giving rise to diverse serotypes [16, 17]. The expression of these virulence factors are stochastically modulated due to the unique slipped-strand mispairing. This phenomenon is called phase variation, which make phenotypic characterization complex [18]. In addition, *Neisseria* spp. is naturally competent for intra- and inter-species transformation and recombination. These events lead to the emergence of antibiotic resistance and a "non-clonal" population structure. Therefore, tracking the emergence of novel variants and transmission of *N. gonorrhoeae* across different social and sexual networks, typing beyond species identification is crucial.

Genotyping is the most reliable method for comparative analysis among *N. gonorrhoeae* strains. Multilocus Sequence Typing (MLST) which uses seven housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, and *pgm*) is the most widely used scheme for genotyping [19]. *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) uses seven well characterized genes (*penA*, *mtrR*, *porB*, *ponA*, *gyrA*, *parC*, and 23S rRNA), which are associated with resistance to three classes of antibiotics for better tracking of new drug-resistant gonococcal strains [20]. *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) scheme have been also used which analyzes sequences of two hypervariable loci (*porB* and *tbpB*) [21]. In recent years, whole genome sequencing (WGS) has become more affordable, allowing for core genome sequence typing (cgST) based genotyping along with life identification number (LIN) for genomic lineage nomenclature [22, 23].

Reports on the genomic features and antibiotic resistance of *N. gonorrhoeae* from Bangladesh are limited. The last reported study included isolates from 2012 [24–26]. A study of *N. gonorrhoeae* isolates in Bangladesh from 1996 to 2006 revealed a significant rise in ciprofloxacin resistance, from 9% to 87% within study period from population with high-risk behavior and general population [25]. A cross-sectional bio-behavioral study conducted in 2014, reported resistant phenotype among key populations against ciprofloxacin (95.2%), doxycycline (90.5%), penicillin (33.3%), and cefixime (14.3%) [27]. However, genomic investigation of *N. gonorrhoeae*, particularly using WGS, is still lacking from Bangladesh.

This analysis will provide a more detailed understanding of the genomic characteristics and resistance mechanisms in *N. gonorrhoeae* circulating among key population of two sites in Bangladesh. By addressing the current gap in genomic data and recent status of antibiotic resistance from Bangladesh, we hope to contribute genomic epidemiology of regional gonococcal genomics and antibiotic resistance trends.

Methods

Sources of *N. gonorrhoeae* isolates

A total of 24 *N. gonorrhoeae* isolates from Bangladesh were available from two studies for WGS (2014 and 2022–2024). The first one was a cross-sectional bio-behavioral survey conducted in 2014 among key populations of Dhaka, including two *N. gonorrhoeae* isolates from FSW [27]. From reported 21 isolates of the 2014 study, only two *N. gonorrhoeae* were recovered. The second study focused on sexual and reproductive health and rights (SRHR) and 22 were isolated and available for WGS. This study was conducted from 2022 to 2024 and included FSW (street-based, residential, hotel-based, and brothel-based sex workers) in Jashore and MSW and Hijra population in Dhaka (study protocol available from Reza et al.) [28]. Jashore is a district of south-west part of Bangladesh sharing border with India (Additional file 1). Both studies isolated *N. gonorrhoeae* cervical swabs from FSW and anorectal and oropharyngeal swab samples from MSW and Hijra.

Recovery of stored isolates

Bacterial stocks in skim milk were inoculated onto chocolate agar plates and incubated at 37 °C in a candle jar for 24–48 h. The isolates were confirmed using an automated bacterial identification system (Vitek-2) and WGS was performed in the icddr, b genome centre, an institutional facility for next generation sequencing. Antimicrobial susceptibility testing was performed by the Kirby-Bauer method (disc diffusion), breakpoints for sensitive, intermediate, and resistant were interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines [29]. *N. gonorrhoeae* reference strain ATCC 49226 was used as a standard. Chocolate agar plates and Standard 6 mm paper discs (Oxoid, Basingstoke, Hampshire, UK) were used and inoculated plates were incubated for 24–48 h at 37 °C, in a candle jar. The antibiotics used were ceftriaxone (30 µg), cefixime (5 µg), penicillin G (10 units), azithromycin (15 µg), ciprofloxacin (5 µg), tetracycline (30 µg) and spectinomycin (100 µg).

Whole genome sequencing

Genomic DNA was extracted from *N. gonorrhoeae* pure cultures using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany; Cat no. 69504) according to the manufacturer's instructions. Briefly, bacterial cells were resuspended in lysis buffer and incubated with proteinase K for enzymatic digestion. The lysate was then purified using spin columns, and DNA was eluted in nuclease-free water.

Multiplex libraries were prepared using the Illumina DNA Prep library kit (Illumina, USA; Cat no. 20018705) following the manufacturer's instructions. Briefly, the DNA was fragmented and tagged with adapters via a single transposase enzymatic reaction, followed by amplification using an optimized, limited-cycle PCR protocol with indexing. The final library was normalized and denatured according to the instructions for the NextSeq instrument. Paired-end 2 × 150-bp indexed reads were generated on the Illumina NextSeq550 platform (NextSeq550 Mid Output kit, 300 cycles; Cat no. 20024905). The WGS are available at NCBI: raw sequencing reads under SRA accession, and genome assemblies under GenBank accession. Accession number for each sequence is available in Additional file 2.

Whole genome sequence analysis

The quality of the raw reads was assessed using FastQC v0.12.1, trimming of the adapter and low-quality reads was performed with fastp v0.23.2 [30, 31]. *De novo* assembly method of SPAdes v3.15.5 was used for the assembly of the files into draft genome with setting the parameter of coverage cutoff value to auto and using careful parameter for reducing the mismatch [32]. Contigs less than 200 bp in the draft assembly were trimmed with Seqkit v2.3.0 [33]. Assembly quality was assessed using Quast v5.2.0 [34]. Kraken2 v2.1.3 with the Standard-8 database, updated as of 1 December 2024 and Speciator v4.0.0 from Pathogenwatch was used for the species identification [35, 36]. For MLST, NG-STAR, and NG-MAST typing Pathogenwatch v22.2.0 and pyngoST were used [36, 37]. Novel alleles and allele profiles of NG-MAST were submitted to the NG-MAST database on the PubMLST platform, whereas novel NG-STAR alleles and allele profiles were submitted to NG-STAR database hosted by the Public Health Agency of Canada. The cgST and LIN code were determined using the *N. gonorrhoeae* cgMLST v2 using PubMLST [24, 39]. ABRicate v1.0.0 with default parameter was used for detection of the virulence gene using the VFDB full, and acquired resistant genes were detected using the ResFinder database [38–40]. Resistance to antimicrobial agents were predicted using Pathogenwatch v22.2.0 [36]. Concordance between the phenotypic and genomic antimicrobial susceptibility prediction was assessed using categorical agreement and McNemar's χ^2 test. Analyses were conducted in R v4.4.2 (stats v4.4.2).

Table 1 *N. gonorrhoeae*, collection year, site and specimen type data stratified by key populations

Characteristics	Total (*n* = 24)	FSW (*n* = 10)	MSW (*n* = 9)	Hijra (*n* = 5)
Isolation Year				
2014	2 (8.3%)	2 (20.0%)	-	-
2022	18 (75.0%)	6 (60.0%)	8 (88.9%)	4 (80.0%)
2023	2 (8.3%)	1 (10.0%)		1 (20.0%)
2024	2 (8.3%)	1 (10.0%)	1* (11.1%)	-
Collection site				
Dhaka	16 (66.7%)	2 (20.0%)	9* (100.0%)	5 (100.0%)
Jashore	8 (33.3%)	8 (80.0%)	-	-
Specimen type				
Anorectal swab	13 (54.2%)	-	8* (88.9%)	5 (100.0%)
Cervical Swab	10 (41.7%)	10 (100.0%)	-	-
Oropharyngeal swab	1 (4.2%)	-	1 (11.1%)	-

* The MSW from Dhaka also worked as MSM. Anorectal swab was positive for *N. gonorrhoeae*

"-" Not applicable as not part of the sampling strategy

Phylogenetic analysis of *N. gonorrhoeae* sequences

For comparative analysis with globally reported publicly available *N. gonorrhoeae* genomes, sequence data available up to 1 November 2025 was downloaded from PubMLST [41]. Genomes were selected to represent MLSTs. For STs with fewer than 10 isolates, all available FASTA sequences were included. For STs with ≥ 10 isolates, one isolate per country was included, prioritizing the most recent isolate; when multiple isolates met this criterion, one was selected at random. Counts per ST reflect PubMLST entries with allele profiles (not sequence availability) is presented in Additional file 3 and the metadata of the selected isolates are provided in Additional File 4. Additionally, fifteen WHO 2024 reference strains were also included [42]. All genomes were annotated with Prokka v1.14.6, and the resulting General Feature Format files were subjected to core genome extraction and alignment using Roary v3.13.0. The core genome alignment file was trimmed for ambiguously aligned regions with trimAl v1.4.rev15 with the -automated1 parameter [43–45]. A maximum-likelihood phylogeny was inferred with IQ-TREE3. ModelFinder was used to select the best-fit substitution model with 1,000 ultrafast bootstrap replicates [46]. Final tree was midpoint rooted, visualized and annotated in ITOL v6.9 [47].

Results

Each reported *N. gonorrhoeae* isolate was detected from individual cases. The WGS had average read coverage of 207X (minimum 75X to maximum 487X) and average GC% of 52.4 (from 52.3 to 52.6). Speciator from Pathogenwatch and Kraken-2 identification tools identified all these draft genomes as *N. gonorrhoeae*. The details of the quality of sequence reads are available at Additional file 2 and other demographic information is available in Additional file 5. Frequency of detection was 41.7% (10/24) from FSW, 37.5% (9/24) from MSW and 20.8% (5/24) from Hijra. Two cases, from 2014 bio-behavioral survey, were from FSW. Two third cases were from Dhaka city (16/24) and rest from Jashore (8/24). The bacteria were isolated from 54.2% (13/24) anorectal swabs, 41.7% (10/24) cervical swabs and 4.2% (1/24) oropharyngeal swab (Table 1).

High frequency of *N. gonorrhoeae* ST7363 was identified from MLST and extensive diversity by NG-MAST and LIN code analysis

A total of 9 different MLST were identified among these 24 *N. gonorrhoeae* isolates reported here. Most frequent MLST was ST7363, detected from 50.0% (12/24) cases. Most of these cases (83.3%;10/12) of ST7363 were from 2022 to 2024 study and from Dhaka. This MLST was detected from anorectal swabs of Hijra (*n* = 3) and MSW (*n* = 6), cervical swab of FSW (*n* = 2) and oropharyngeal

Table 2 MLST, NG-MAST, LIN code lineage and NG-STAR type of the 24 *N. gonorrhoeae* isolates from the two studies stratified by population and location of the isolates

Sequence types	Total	Key population			Location	
		FSW	MSW	Hijra	Dhaka	Jashore
		(*n* = 10)	(*n* = 9)	(*n* = 5)	(*n* = 16)	(*n* = 8)
MLST typing (9 types)						
7363	12	2	7	3	10	2
13559	3	3	0	0	0	3
8776	2	1	0	1	1	1
1579	2	1	0	1	1	1
Others*	5	3	2	0	4	1
NG-MAST typing (5 types)						
Novel	15	6	5	4	10	5
8115	3	1	2	0	2	1
15647	3	0	2	0	2	0
1407	2	1	0	1	1	1
Others*	2	2	0	0	1	1
LIN code lineages (9 types)						
0_0_52	11	2	6	3	9	2
0_2_0	4	3	1	0	2	2
0_0_85	2	2	0	0	0	2
0_18_1	2	1	0	1	0	2
Others *	5	2	3	0	4	1
NG-STAR type (7 types)						
Novel	8	6	1	1	3	5
158	7	1	4	2	6	1
3155	3	0	2	1	3	0
90	2	1	0	1	1	1
Others*	4	2	2	0	3	1

*Isolates with less than 2 observations

swabs of one MSW (*n* = 1) (Table 2). NG-MAST typing identified 5 known types in 9 isolates and rest (*n* = 15) as novel types. Among novel types, 11 isolates had previously unreported alleles and 4 had unique combination of previously reported alleles (Additional file 5). Distribution of the novel types among key populations were, Hijra (80%, 4/5), FSW (60%, 6/10) and MSW (55.6%, 5/9). From the FSW of Jashore, 5 novel NG-MAST types were detected. The known NG-MAST types were 8115 (12.5%), 15647 (12.5%) and 1407 (12.5%) detected from 24 cases. LIN code based on core genome-based clustering showed highest frequency of 0_0_52 (*n* = 11) lineage followed by 0_2_0 (*n* = 4) lineage. Lineage 0_0_52 is most frequent among MSW population from Dhaka (Table 3 and Additional file 3). All of lineage 0_0_52 was also MLST7363 and fall under diverse NG-MAST type mainly due to diverse alleles of *porB* gene (Additional file 5).

Phenotypic and genomic prediction of antibiotic resistant pattern of the *N. gonorrhoeae* isolates

Phenotypically all of the *N. gonorrhoeae* isolates were sensitive to ceftriaxone. High frequency of resistance was observed for ciprofloxacin (83.3%), tetracycline (41.7%), penicillin G (29.2%) among the 24 isolates (Table 2). Cefixime non-susceptible strain (*n* = 2) were from Dhaka in 2014 (ST1599) and 2024 (ST7363). Azithromycin resistance was observed from ST7363 collected from FSW in 2024 from Jashore (Additional file 5 & 6). Genomic analysis by Pathogenwatch predicted high resistance to ciprofloxacin (100%), followed by sulfonamides (91.7%; 22/24), and tetracycline (79.2%; 19/24). Intermediate level resistance was predicted for penicillin (70.8%, 17/24), and tetracycline (8.3%; 2/24). Genomic analysis also predicted 100% sensitivity to azithromycin, ceftriaxone, and spectinomycin. Cefixime resistance was predicted in 8.3% (2/24) isolates, discordant with phenotype (Fig. 1). Genomic prediction showed complete categorical agreement with phenotypic observations for ceftriaxone, ciprofloxacin, and spectinomycin. For azithromycin, cefixime, penicillin G and tetracycline categorical agreement varied from 95.8 to 83.3% and McNemar's p- value varied from 1.0 to 0.13 with no significant discordance between two methods (Table 3).

Table 3 Phenotypic antibiotic susceptibility of *N. gonorrhoeae* determined using disc-diffusion method and genotypic prediction of the antimicrobial susceptibility pattern from key population. The concordance between the two methods was assessed using categorical agreement and mcnemar's χ^2 test

	Phenotypic ($n=24$)			Genotypic ($n=24$)			Statistical relevance between genomic and phenotypic method[a]	
Antibiotic	**Resistant (%)**	**Intermediate (%)**	**Sensitive (%)**	**Resistant (%)**	**Intermediate (%)**	**Sensitive (%)**	**Categorical Agreement (%)**	**McNemar's χ2 (*p*-value)**
Azithromycin	4.2	0	95.8	0	0	100	95.8	0 (1.0)
Cefixime *	8.3	0	91.7	8.3	0	91.7	83.3	0 (1.0)
Ceftriaxone	0	0	100	0	0	100	100	-
Ciprofloxacin	83.3	16.7	0	100	0	0	100	-
Penicillin G	29.2	66.7	4.2	29.2	70.8	0	95.8	0 (1.0)
Spectinomycin	0	0	100	0	0	100	100	-
Sulfonamides	-	-	-	91.7	0	8.3	N/A	N/A
Tetracycline	41.7	37.5	20.8	87.5	8.3	4.2	83.3	2.25 (0.13)

* Non susceptible for cefixime due to lack of resistant break point in CLSI guideline

a, Intermediate consider as resistant

-, McNemar's χ2 (p-value) undefined due to absence of discordance

NA, Not applicable; Absence of phenotypic data

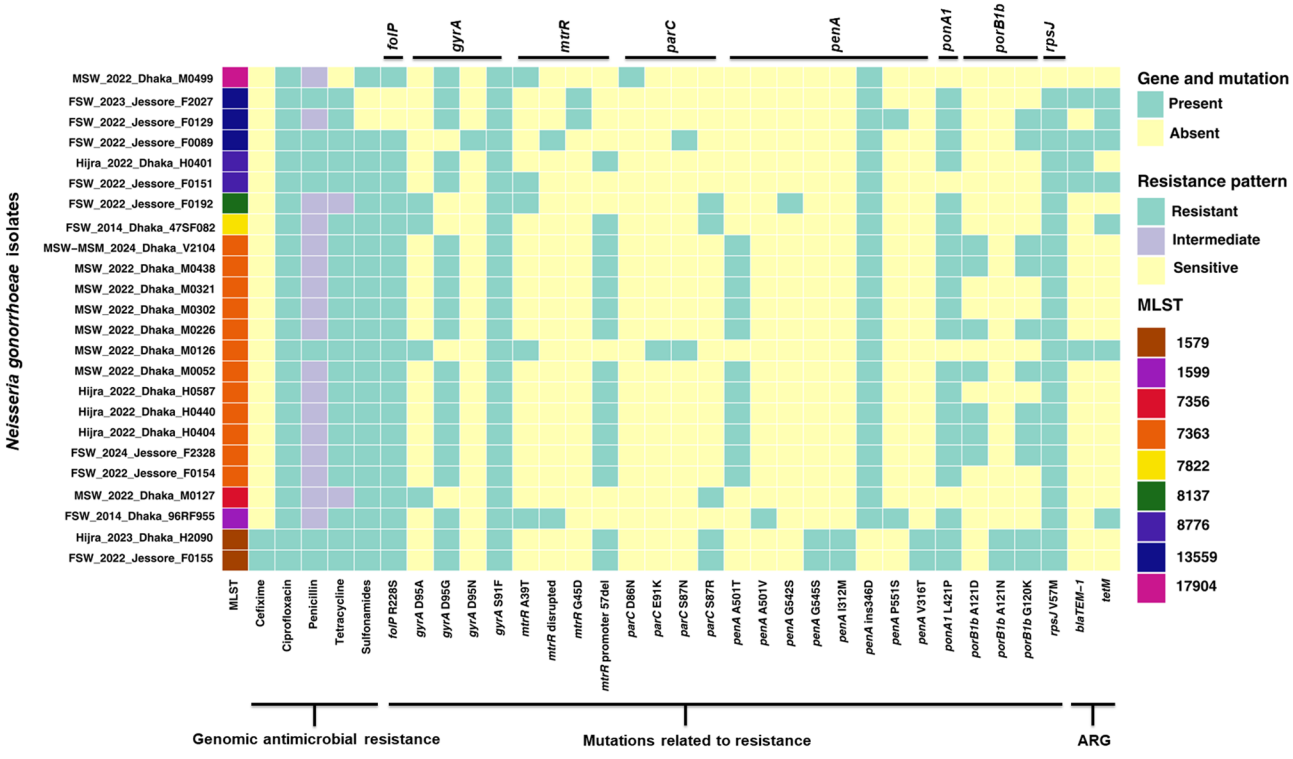

Fig. 1 Resistance to antimicrobial agents, presence of resistance genes and mutations predicted using Pathogenwatch from the draft genome of the 24 *N. gonorrhoeae* isolates. Antibiotic resistance is colored as figure legend. Presence and absence of antibiotic resistant genes and mutations are presented as either "present' or "absent" as per figure legend

Novel NG-STAR indicates high genetic diversity of antibiotic resistance in *N. gonorrhoeae* strains

NG-STAR typing scheme was able to detect 7 known sequence types. Most frequently detected NG-STAR was ST158 (29.2%, 7/24) followed by ST3115 (12.5%, 3/24). Most of the ST158 was detected (85.7%, 6/7) from Dhaka. NG-STAR scheme referred to 33.3% (8/24) isolates as novel. Frequency of novel NG-STAR types was in Jashore 62.5% (5/8) and Dhaka 37.5% (3/8) (Table 2). Novel NG-STAR types are either due to previously unreported combination of alleles ($n=2$) or previously unreported types of *penA*, *mtr* or 23S RNA alleles (Additional file 5).

Three isolates had same MLST (7363), NG-STAR (158), NG-MAST (8115), and LIN superlineage (0_0_52_1) but differed in their LIN group at the 25-allele threshold (Additional file 5). These three isolates (M0438, F2328 and V2104) were detected from both Dhaka (MSW = 2) and Jashore (FSW = 1). Between these two MSW, one (V2104) was also known to act as male having sex with male (MSM) (Table 2 and Additional file 5). Comparison of NG-STAR types and genomic prediction of antibiotic resistance showed that novel isolates had resistant trait for ciprofloxacin and penicillin G, which was similar to other detected known NG-STAR types. Cefixime resistance was predicted for two isolates, both of which were NG-STAR type ST90 (Fig. 2).

Genome analysis-based prediction of antibiotic resistance and detection of mutations and genes to confer antibiotic resistance and virulence

The isolates harbored β-lactamase resistance gene *bla-TEM-1* (20.8%, 5/24) and tetracycline resistance gene *tetM* was detected from 29.2% (7/24) isolates. Point mutations for antibiotic resistance showed presence of R228S mutation in *folP* gene (91.7%; 22/24) among sulphonamide resistant strains. All sequences had D95N/G/A and S91F mutation in *gyrA* gene noted for providing resistance against ciprofloxacin. Notably, ciprofloxacin resistance associated mutation D86N was observed in *parC* gene for one isolate. Multiple penicillin resistance mutation A501T in *penA* (45.8%; 11/24), L421P in *ponA* (83.3%; 20/24), G120K in *proB1b* (45.8%; 11/24) were also detected. Tetracycline resistance conferring mutation V57M in *rpsJ* gene was detected from 95.8% (23/24) isolates. Several other mutations known to confer resistance to multiple antibiotics including penicillin, tetracycline, cefixime and azithromycin were detected (Fig. 1). Sequence analysis for the detection of virulence genes showed most of the isolates harbor virulence genes known to confer virulence to *N. gonorrhoeae*. The frequency of all virulence genes detected is available in Additional file 5 & 7.

Phylogenetic analysis

Maximum-likelihood phylogeny analysis of the Bangladeshi isolates with globally representative *N. gonorrhoeae* sequences indicated that the bacterial isolates were distributed across multiple distinct lineages rather than a single dominant clone. Phylogenetic clustering corresponded closely with MLST types. Overall, the isolates from Bangladesh formed four intra clusters for 18 isolates and 6 were distributed separately in the phylogenetic

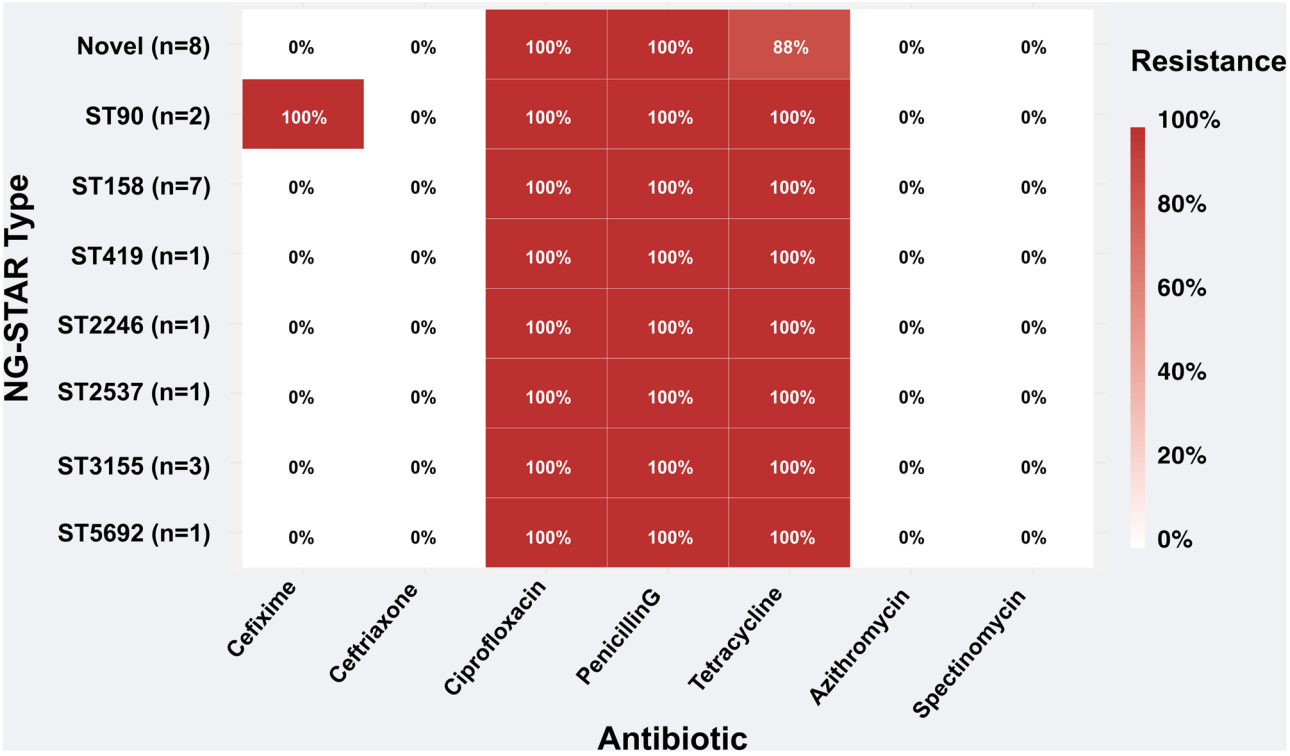

Fig. 2 Relationship with NG-STAR with the phenotypic antimicrobial susceptibility pattern detected using Kirby-Bauer method (disc diffusion), following Clinical and Laboratory Standards Institute (CLSI) guidelines of the 24 *N. gonorrhoeae* isolates. The color intensity denotes the resistance against the respective antibiotic. Novel NG-STAR types are either due to previously unreported combination of typing gene alleles ($n = 2$) or previously unreported types of *penA*, *mtr* or 23S RNA encoding alleles

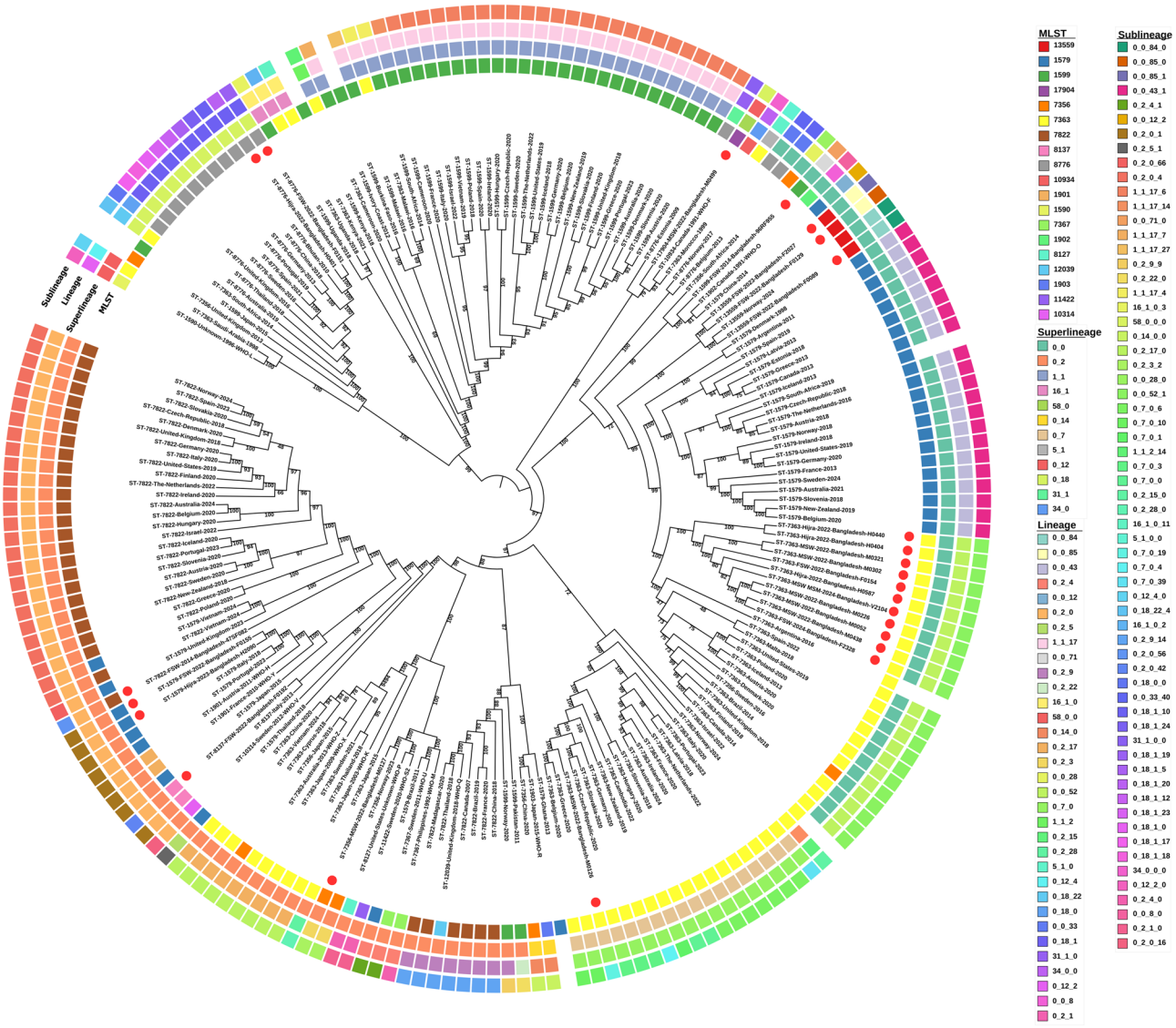

Fig. 3 Core-genome maximum-likelihood tree of 24 isolates together with globally representative isolates for the detected sequence types (STs) and WHO 2024 reference strains. Bangladeshi sequences are highlighted with red filled circles. Color-coded rings from inner to outer indicate MLST, superlineage, lineage, and sublineage

tree. The predominant sequence type ST7363 formed a distinct subclade, diverging from European strains of the same ST while one ST7363 isolate (M0126) grouped with European strains. The isolate from ST1599 differed in the superlineage level from its global representative ones and placed in different cluster. Bangladeshi isolates of ST7356, ST1579, ST13559 clustered with strains reported from Europe sharing similar core genome clonal cluster till sublineage level. ST8776 isolates clustered with strains reported from Asia and Europe, being closest with the representative sequence reported from Bhutan in 2010. The most divergent isolate was M0499 (ST17904, only isolate available in PubMLST), which separated at the superlineage level and clustered independently; this isolate was isolated from an MSW in 2022. ST7822 sequenced from an FSW of 2014 study, represented a unique sublineage compared to other representative sequences from this ST. ST8137 clustered with the only available sequence from this ST reported from Italy in 2013. (Fig. 3).

Discussion

Here we report genomic characterization of *N. gonorrhoeae* isolates available from two studies conducted in 2014 and 2022–2024, using WGS from two sites in Bangladesh. ST7363 was identified as the most dominant *N. gonorrhoeae* genotype circulating among all three key populations reported here. However, due to gonococcal extensive horizontal gene transfer the MLST based genome ancestry can be very different [48]. Using LIN

code based on core genome clustering, we observed diverse lineage and sublineage, and NG-MAST types along with NG-STAR based typing for antibiotic resistance types. These analyses represent the only available genome wide investigation of *N. gonorrhoeae* from Bangladesh to date.

The high frequency of ST7363, particularly in Dhaka among MSW from 2022–2024 study, suggests current circulation of this ST in this population. ST7363 had been reported as the dominant ST from multiple countries including China [49, 50], Thailand [51], Taiwan [52], Japan [53] and Spain [54]. MSW and MSM population from Spain were reported to be infected with this ST [54]. Anorectal swabs from MSW were main source of isolating ST7363, indicating possible dissemination through receptive anal intercourse. MSW are receptive partner of anal sex and almost half of MSW have reported to be engaged in unprotected sex in Bangladesh [55, 56].

The high proportion of novel NG-MAST types among the FSW population from Jashore suggests extensive exposure to diverse *N. gonorrhoeae* strains or sex worker's lifestyle in this population. Diversity of novel NG-MAST types indicates extensive recombination events affecting *porB* and *tbpB* genes, which encode critical surface proteins involved in antibiotic resistance and immune evasion [57]. The antigenic variation due to *porB* diversity is a challenge for vaccine development, which was found to be the main genomic determinant of novel isolates from Bangladesh [58]. The comprehensive virulence gene repertoire demonstrates complete conservation of critical pathogenicity factors across all *N. gonorrhoeae* isolates [59].

In the backdrop of genomic diversity observed here, detection of similar MLST, NG-STAR and NG-MAST type from MSW/MSM and FSW of separate locations indicate sexual network bridging. In addition, it also indicates importance of WGS for tracking transmission of a specific gonococcal strain across different risk networks and geographic locations.

Antibiotic resistant gene product TEM-1 β-lactamases had been widely reported from gonococci, which upon acquiring one or two amino acid change can turn into extended-spectrum β-lactamase [60]. Other resistant determining point mutations detected here, have been previously reported to confer resistance to ciprofloxacin, penicillin and sulfonamides globally [61, 62]. The A501T mutation in penicillin-binding protein 2 may confer intermediate-level resistance to extended-spectrum cephalosporins when present in mosaic *penA* alleles [63]. The presence of NG STAR type ST90 linked with cefixime resistance genotype is alarming as this ST has been reported previously for less susceptibility to cephalosporins and ciprofloxacin [64]. These observations indicate *N. gonorrhoeae* circulating among key populations in Bangladesh, can become resistant to currently recommended antibiotics, which demands strong monitoring. It is worth mentioning that, the *parC* D86N observed here, has been reported to confer resistance to gepotidacin, a novel triazaacenaphthylene antibiotic that received approval for medical use in March 2025 [65, 66].

In Bangladesh, ciprofloxacin resistance increased from 9% in 1997 to 87% in 2006 [25]. The cross-sectional bio-behavioral survey conducted among 1273 key populations in 2014, yielded 21 isolates which were 100% sensitive to ceftriaxone and spectinomycin [27]. From 2019 to 2021, national STI surveillance in Bangladesh reported six *N. gonorrhoeae*, isolated from urethral discharge and endocervical swab which were susceptible to ceftriaxone, cefixime and spectinomycin (Sexually Transmitted Infections Surveillance in Bangladesh (2019–2021), IEDCR, December 2021). The 2022–2024 SRHR study (isolates included here), 8.3% cefixime resistance was observed in comparison to 14.3% in 2014 [27]. In comparison to 1997–2006, antibiotic susceptibility remains relatively stable in recent decade (2014–2024). Therefore, ceftriaxone and azithromycin remain a useful antibiotic against *N. gonorrhoeae*. Over 95% susceptibility to ceftriaxone and azithromycin indicates that the AMR situation in Bangladesh might be still stable than that in other reported countries [67]. However, all these studies used disc-diffusion method for antibiotic susceptibility test due to feasibility in clinical investigations, which is not gold standard. Investigation of antibiotic susceptibility through measuring minimum inhibitory concentration using either or both agar dilution method or E-test is necessary [68, 69].

Phylogenetic analysis reveals that *N. gonorrhoeae* strains from Bangladesh are related to strains from diverse geographic regions including Europe and Asia which aligns with large number of Bangladeshi diaspora who frequently visit Bangladesh. The FSWs were from border district of Jashore, which is exposed to transportation workers from neighboring country India. Analysis of 65 isolates from India, Pakistan, and Bhutan isolated between 2007 and 2011, reported 42 novel NG-MAST STs, however it lacks WGS data [70]. These indicates the possibility of international transmission of *N. gonorrhoeae*. This is also supported by the clustering of Bangladeshi isolates with strains from both developed and developing countries across multiple continents. ST7363 isolates from Bangladeshi form a distinct phylogenetic cluster among global ST7363 isolates. The most phylogenetically divergent isolate ST17904 represents a unique evolutionary lineage clustering closely with isolates dating to 1991 from Canada (WHOF) and 1999 from Morocco. Considering the genetic plasticity of *N. gonorrhoeae*, persistence for such extended time and geographic distance with minimal genetic change is unlikely.

Indicating either lack of intermediate strains or representative of a unique Bangladeshi lineage if more sequences were available for analysis.

A major limitation of our investigation is the very limited number of isolates. Being a fastidious pathogen, the isolation of *N. gonorrhoeae* is difficult, therefore restricting the availability of isolates from specific population-based surveillance studies. National efforts to collect *N. gonorrhoeae* isolates from diagnostic facilities or long-term surveillance may serve as a good source to analyze circulating strains among the Bangladeshi population at the genomic level. In addition, complete genome sequence analysis incorporating long-read sequences will help to further confirm the genomic features of *N. gonorrhoeae* reported here. This is the first report of genomic investigation of *N. gonorrhoeae* using WGS from Bangladesh. Susceptibility to current first-line treatments provides hope for continued effective therapy. However, the presence of emerging resistance mechanisms demands vigilant surveillance and judicious antibiotic prescription.

Conclusions

This first genomic analysis of *N. gonorrhoeae* from Bangladesh demonstrates marked genetic heterogeneity, including multiple novel sequence types of NG-STAR and NG-MAST, yet resistance to key antibiotics, particularly ceftriaxone remains stable and below the WHO 5% threshold. The absence of ceftriaxone-resistant isolates is encouraging; however, the observed genetic diversity and presence of resistance determinants highlight the risk of resistance emergence. Sustained genomic surveillance and rigorous antibiotic stewardship are imperative to maintain treatment efficacy and preempt the spread of resistant gonococcal strains.

Abbreviations

cgST	Core genome sequence typing
FSW	Female sex worker
MLST	Multilocus Sequence Typing
MSM	Male having sex with male
MSW	Male sex workers
NG-MAST	*N. gonorrhoeae* multi-antigen sequence typing
NG-STAR	*N. gonorrhoeae* sequence typing for antimicrobial resistance
PID	Pelvic inflammatory disease
SRHR	Sexual and reproductive health and rights
STI	Sexually transmitted infections
WGS	Whole genome sequencing
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Author contributions

MA and ASD drafted the manuscript and guided the overall analysis. ASD, ZI, GS, and MSS generated the laboratory and epidemiological data. ZI performed the bioinformatics analysis. GS, MR, MA, MSS, SIK, and MR were responsible for conducting the studies and gathering demographic information. All co-authors reviewed and approved the manuscript. SIK and MR secured funding and supervised the overall research.

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Data availability

The whole genome sequencing data generated and analyzed in this study are publicly available in the NCBI databases. Raw sequencing reads are deposited under the Sequence Read Archive (SRA) with accession numbers SRR27726224 – SRR27726245 and SRR31069465 – SRR31069466. Genome assemblies are available in GenBank under accession numbers JBPIXA000000000 – JBPIXX000000000. Accession numbers for each sample are also provided in Additional file 2.

Declarations

Ethics approval and consent to participate

The study was conducted following the approval of the research review committee and the ethical review committee of icddr, b (Protocol number PR-22033). All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study. The consent process ensured that participants were fully informed about the purpose, procedures, risks, and benefits of the study, and their participation was entirely voluntary. Written consent forms were signed by each study participants before any study-related procedures were initiated. No animal was included in this investigation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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