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Prevalence, Molecular Identification and Antibiogram Profile of *Neisseria gonorrhoeae* among Individuals in Yenagoa, Bayelsa State, South South, Nigeria

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Abstract

Neisseria gonorrhoeae (*N. gonorrhoeae*), the bacterium causing gonorrhea, is a global public health concern, particularly in Nigeria, due to its increasing antibiotic resistance. Its often asymptomatic nature leads to under diagnosis, continued transmission, and severe complications like infertility and pelvic inflammatory disease. The objectives of this study are to investigate the prevalence, molecular identification and antibiogram profile *N. gonorrhoeae* among individuals in Yenagoa, Bayelsa State. A cross- sectional study was conducted with 288 participants from Federal Medical Centre and Silhouette Diagnostics Consultants, both in Yenagoa. Urethral swab from males and endocervical swab from females were collected for identification of the organism. Antibiotic susceptibility was tested using the Kirby-Bauer method. The results showed a 2.78% prevalence, with higher prevalence in males (4.76%) than in females (1.64%). Antibiogram results demonstrated high resistance to penicillin (100%), tetracycline (75%), and erythromycin (62.5%), while susceptibility was highest to ceftriaxone (100%), cefixime (87.5%), and azithromycin (62.5%). Molecular analysis identified the *PorA* gene in all eight isolates. Key risk factors included previous sexually transmitted infections, multiple sexual partners, and

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transactional sex. The conclusion emphasizes the need for new antibiotics and vaccines, improved diagnostics, safer sexual practices, and routine molecular surveillance to combat resistant strains. It recommends updating treatment protocols based on current antibiogram data and enhancing public health campaigns to raise awareness and prevent gonococcal infections.

Keywords: Antibiogram, Molecular Identification, *Neisseria gonorrhoeae,* Prevalence, Yenagoa

INTRODUCTION

Neisseria gonorrhoeae (*N. gonorrhoeae*) is responsible for causing gonorrhea, which ranks as the world's second most prevalent sexually transmitted infection (STI) worldwide (Guerrero-Torres et al., 2019; Springer & Salen, 2023). It frequently infects the human urogenital tract, resulting in conditions like urethritis, epididymitis, and prostatitis in men, as well as cervicitis, pelvic inflammatory disease (PID), and salpingitis in women (Shim, 2011; Springer & Salen, 2023). The bacterium is also capable of colonizing extragenital locations, such as the rectum, pharynx, and conjunctiva (Belga et al., 2019; Dolange et al., 2018; Mahapure & Singh, 2023). In severe instances, *N. gonorrhoeae* may spread to other organs, leading to complications such as arthritis, deep vein thrombosis, and eye damage (Dolange et al., 2018), particularly in women of childbearing age.

The lack of lasting protective immunity against *N. gonorrhoeae* underscores the importance of sustained prevention efforts. (Belcher et al., 2023; Wang et al., 2024). However, the rapid development of antimicrobial resistance (AMR) complicates treatment, with resistant strains increasingly limiting therapeutic options. Effective management of gonococcal infections depends on timely diagnosis and the use of appropriate antibiotics guided by local resistance patterns.

Ceftriaxone, a member of the cephalosporin class, is the preferred first-line treatment for gonorrhea. Nevertheless, reports of strains resistant to ceftriaxone have been on the rise (Belcher et al., 2023), which spread rapidly due to delayed diagnosis, ineffective treatment, and inadequate surveillance systems. Misuse of antibiotics, such as unnecessary prescriptions or incomplete courses of treatment, further exacerbates resistance.

The rise of resistant strains emphasizes the necessity for both global and local initiatives aimed at controlling the spread of *N. gonorrhoeae* and mitigating antimicrobial resistance

(AMR) (Golparian et al., 2018; Unemo et al., 2012). Public health initiatives, including education, monitoring, and antimicrobial stewardship, are essential to address these challenges.

In Nigeria, particularly in Bayelsa State's Yenagoa metropolis, the prevalence and resistance profile of *N. gonorrhoeae* remain poorly documented. This knowledge gap hinders effective control and management strategies for combating gonorrhoea within the region. This study aims to fill this gap by determining the prevalence of *N. gonorrhoeae* and its antibiotic resistance profile in Yenagoa, providing critical data to inform treatment protocols and public health interventions.

The findings of this study will have profound implications for public health policies, treatment guidelines, and future research. They will inform targeted interventions and prevention strategies to reduce gonorrhoea prevalence and transmission, enabling educational campaigns to promote safe sexual practices and early detection. Insights into the antibiotic resistance patterns of *N. gonorrhoeae* within the study population will support clinicians in prescribing effective treatments and help shape antibiotic stewardship policies, updating treatment protocols to improve patient outcomes. Additionally, this research will bolster surveillance programs to monitor the spread and antibiotic resistance of *N. gonorrhoeae*, and provide region-specific data from Bayelsa, enriching global scientific knowledge.

MATERIALS AND METHODS

Study Design and Population

This cross-sectional study was conducted in Yenagoa metropolis, Bayelsa State, Nigeria. 288 individuals presenting with symptoms of sexually transmittable infections were recruited from Federal Medical Centre and Silhouette Diagnostic Consultants, both in Yenagoa. Informed consent was obtained from all participants before sample collection.

Sample Size

Sample size was calculated using Fisher's equation of

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where:

n = the required sample size,

Z = the Z-score corresponding to the desired confidence level (we desired 95%) = 1.96

p = the estimated proportion of the population – obtained from previous studies (25%), (Okonko et al., 2011)

d = level of precision (5%)

n = 288

Inclusion and Exclusion Criteria

The study included individuals from age 16 to 35, permanent residents of Bayelsa State or individuals who have lived in Bayelsa State for at least the past six months, individuals who provided informed consent to participate in the study, individuals who presented with symptoms suggestive of gonorrhea or those who have been diagnosed with gonorrhea by a healthcare provider, as well as individuals seeking treatment at Federal Medical Centre, Yenagoa for STIs. Most importantly, Participants who agreed to provide clinical samples for laboratory analysis were included.

Exclusion criteria included individuals under 16 years of age, individuals who are not residents of Bayelsa State and have not lived in the state for the past six months, individuals who did not provide informed consent or who withdrew their consent at any point during the study, individuals who had received antibiotic treatment for gonorrhea or other bacterial infections in the past month, as this could affect the accuracy of expected outcomes, individuals with severe co-morbid conditions that could interfere with participation in the study, such as advanced HIV/AIDS, active tuberculosis, or other serious systemic infections

Sample Collection and Identification of *Neisseria gonorrhoeae*

Samples were collected from urethral swabs in males and endocervical swabs in females. The swab was immediately used to inoculate the culture medium. Where it was not possible to inoculate immediately, the samples were transported to the laboratory using Amies transport media. Isolation of *N. gonorrhoeae* was performed using Modified Thayer-Martin agar and identified based on colony morphology, Gram staining, and biochemical tests, including oxidase, catalase activity, nitrate reduction test and glucose fermentation.

Molecular Identification of the Porin A (*PorA*) Gene

Deoxyribonucleic Acid (DNA) Extraction: Genomic DNA was extracted from purified *N. gonorrhoeae* cultures using the Thermo Fisher PureLink Genomic DNA Kit. Bacterial cells were lysed to release DNA, which was then purified using column-based methods and ethanol washes to remove contaminants, yielding pure DNA.

Polymerase chain reaction (PCR) Amplification: The *PorA* gene (821 base pairs) of *N. gonorrhoeae* was amplified using PCR with specific primers (*PorA* forward 5'-ATGAAAACTTATGAAAAAGA -3'; *PorA* reverse 5'- TCATTTAGCATTTTTGCTT -3'). Each 25 microlitre (μL) PCR reaction contained 12.5 μL of Taq polymerase mix [including deoxynucleoside triphosphates (dNTPs) and buffer], 1 μL of DNA template with forward and reverse primers, and PCR-grade water. The thermal cycling conditions were: initial denaturation at 95°C for 5 minutes, followed by 30–35 cycles of 30-second denaturation at 95°C, 30-second annealing at 55–60°C, and 1-minute extension at 72°C per kilo base of the amplicon, with a final extension of 7 minutes at 72°C.

Visualization: PCR products were analyzed by agarose gel electrophoresis. A 2% agarose gel in 1X Tris-Acetate-Ethylenediaminetetraacetic acid (TAE) or Tris-Borate-Ethylenediaminetetraacetic (TBE) buffer was loaded with the PCR products and a 100 base pair DNA ladder. The gel was electrophoresed for 30–60 minutes at 100–120 V, stained with ethidium bromide, and visualized under ultra-violet light to confirm the presence of the 821 base pair *PorA* gene amplicon.

Antibiotic Susceptibility Testing

The antibiotic susceptibility of *N. gonorrhoeae* isolates was evaluated using the disk diffusion method on MTM agar plates, chosen for its simplicity, cost-effectiveness, and reliability. Pure cultures of *N. gonorrhoeae* were suspended in sterile saline to match a 0.5 McFarland standard for consistent bacterial concentration. The bacterial suspension was spread on MTM agar plates using sterile swabs. Antibiotic disks (ciprofloxacin, ceftriaxone, cefixime, azithromycin, penicillin, tetracycline, erythromycin, gentamicin) were placed on the plates, spaced 24 mm apart to prevent overlapping inhibition zones. The plates were incubated at 37°C in 10% CO_2 for 48 hours. After incubation, the inhibition zones around the disks were measured and compared to Clinical and Laboratory Standards Institute (CLSI) guidelines to classify isolates as sensitive, intermediate, or resistant.

Data Analysis

Prevalence was calculated as the percentage of positive cases among the total samples tested. Resistance profiles were analyzed and compared across different antibiotics, and the multi-drug resistance (MDR) rate was determined. Data were analyzed using Minitab version 18 and statistical significance was set at $p < 0.05$.

RESULTS

Data Presentation

Table 1: Morphological and biochemical characteristics of *N. gonorrhoeae*

Culture media	Morphological characteristics	Gram stain	Biochemical Tests				Isolate
			CAT	OX	NR	G	
Modified Thayer-Martin (MTM) agar	Typically appears amsmall, translucent, greyish-white colonies with a smooth, convex morphology showcasing its fastidious growth requirements and selective properties for isolation in clinical microbiology.	Negative diplococci	+	+	-	+	***N. gonorrhoeae***

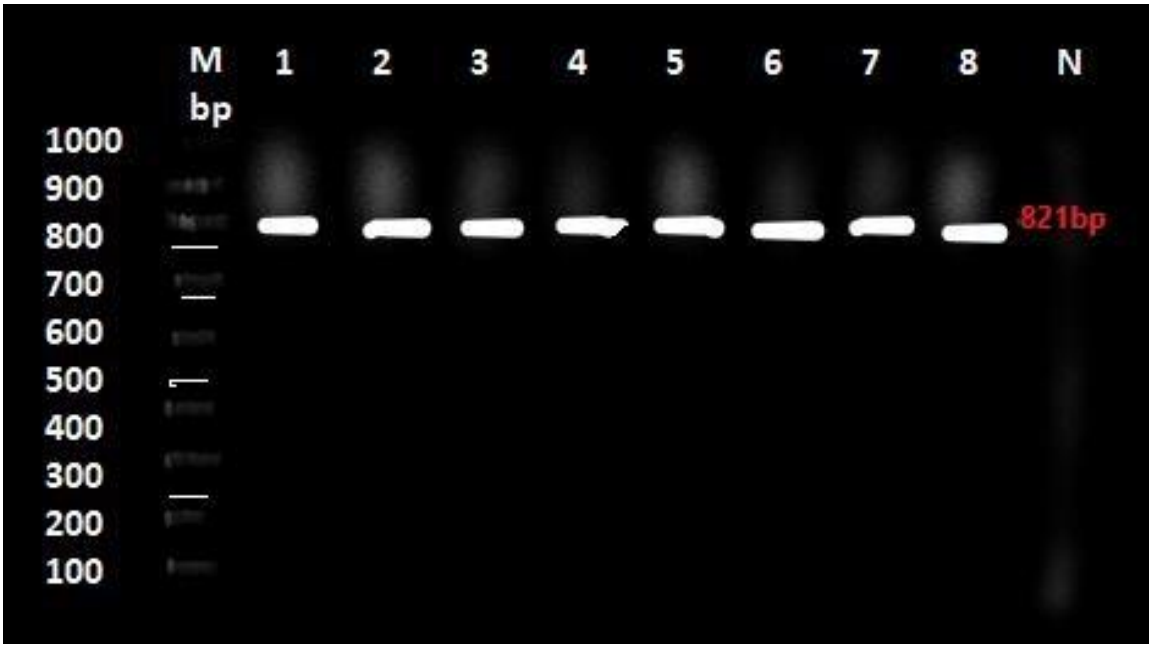


Figure 1: Agarose gel electrophoresis showing PCR amplification products of 821 bp PorA gene in N. *gonorrhoea isolates*.

Lane M, 100 bp marker; Lane 1 -5, test sample from male; Lane 6-8, test sample from female Lane N, Negative control;

Table 2: Prevalence of *N. gonorrhoeae* in Yenagoa, Bayelsa state

Age Group (In years)	Sex	Samples Collected	Positive Cases	Prevalence (%)
16-20	Male	27	2	7.41
	Female	49	0	0.00
21-25	Male	32	1	3.13
	Female	41	2	4.88
26-30	Male	21	1	4.76
	Female	51	1	1.96
31-35	Male	25	1	4.00
	Female	42	0	0.00
Overall	**Male**	**105**	**5**	**4.76**
	Female	**183**	**3**	**1.64**
	Total	**288**	**8**	**2.78**

TABLE 3: Socio-Demographic characteristics of participants attending Federal Medical Centre and Silhouette Diagnostic Consultants in Yenagoa.

Variable	Frequency, n (%)	Prevalence, n (%)	Chi Square	P-value	Remark
Sex			0.752	0.386	NS
Male	105 (36.46%)	5 (4.76%)			
Female	183 (63.54%)	3 (1.64%)-			
Age groups in years			0.921	0.820	NS
16 – 20	76 (26.39%)	2 (2.63%)			
21 – 25	73 (25.35%)	3 (4.11%)			
26 – 30	72 (25.0%)	2 (2.73%)			
31 – 35	67 (23.26%)	1 (1.49%)			
Residence			0.427	0.513	NS
Rural	95 (32.99%)	2 (2.11%)			
Urban	193 (67.01%)	6 (3.11)			
Educational Status			1.510	0.680	NS
No formal	23 (7.99%)	1 (4.35%)			
	16 (5.56%)	0 (0.0%)			

education	99 (34.38%)	3 (3.03%)			
Primary	150 (52.08%)	4 (2.67%)			
Secondary					
Tertiary					
Occupation			1.545	0.461	NS
Student	91 (31.60%)	4 (4.40%)			
Working	159 (55.21%)	3 (1.89%)			
Not working	38 (13.19)	1 (2.63%)			
Marital status			3.702	0.295	NS
Single	120 (41.67%)	2 (1.67%)			
Married	112 (38.89%)	4 (3.57%)			
Widowed	11 (3.82%)	0 (0.0%)			
Divorced	45 (15.63%)	2 (4.44%)			
HIV status					
Positive	0 (0.0%)	0 (0.0%)			
Negative	288 (100.0%)	8 (100.0%)			
History of STI			8.220	0.004	S
Yes	71 (24.65%)	6 (8.45%)			
No	217 75.35%)	2 (0.92%)			
Condom use			3.789	0.052	NS
Yes	82 (28.47%)	0 (0.0%)			
No	206 (71.53%)	8 (100.0%)			
Number of sexual partners			19.520	0.0002	S
	168 (58.33%)	0 (0.0%)			
None	99 (34.38%)	5 (5.05%)			
1 – 2	21 (7.29)	3 (14.29%)			
2>					
Traded sex			7.704	0.006	S
Yes	7 (2.43%)	3 (42.86%)			
No	281 (97.57%)	5 1.78%)			

Table 4: Antibiotic susceptibility pattern of *N. gonorrhoeae*

Antibiotics	***N. gonorrhoeae***		
	Resistant	**Intermediate**	**Sensitive**
Ciprofloxacin	1 (12.5%)	3 (37.5%)	4 (50.0%)
Ceftriaxone	0 (0.0%)	0 (0.0%)	8 (100.0%)
Cefixime	1 (12.5%)	0 (0.0%)	7 (87.5%)
Azithromycin	2 (25.0%)	1 (12.5%)	5 (62.5%)
Penicillin	8 (100.0%)	0 (0.0%)	0 (0.0%)
Tetracycline	6 (75.0%)	2 (25.5%)	0 (0.0%)
Erythromycin	5 (62.5%)	2 (25.5%)	1 (12.5%)

DISCUSSION

Table 1 summarizes the morphological and biochemical characteristics of *N. gonorrhoeae* isolates. On MTM agar, they appeared as small, translucent, greyish-white colonies with smooth, convex morphology. Gram staining confirmed Gram-negative diplococci, and biochemical tests were positive for oxidase, catalase, and glucose fermentation, but negative for nitrate reduction, confirming the isolates as *N. gonorrhoeae*. Figure 1 shows PCR amplification of the 821 base pair *PorA* gene from all 8 isolates, including samples from both males and females, marking *PorA* as a reliable biomarker. The timely identification of this gene does not only affirm the identified isolates as *N. gonorrhoeae*, it also supports efficient infection management, as echoed by previous studies (Hjelmevoll et al., 2008).

As shown in Table 2, the prevalence of *N. gonorrhoeae* in this study is 2.78%, higher among males (4.76%) than females (1.64%), aligning with other Nigerian studies (Nsofor & Eletuoh, 2017) and similar findings in the USA (Pollock et al., 2023). This aligns with global data indicating *N. gonorrhoeae* as the second most prevalent STI worldwide (Guerrero-Torres et al., 2019; Springer & Salen, 2023). Age-wise, males aged 16–20 showed the highest prevalence (7.41%), with no isolates found in females within this group. Males aged 31–35 had a prevalence of 4.00%, while males aged 26–30 (4.76%) also showed higher rates compared to females (1.96%). Risky sexual behaviors and substance abuse among young males are major contributing factors (Bozzini et al., 2021), along with poor health-seeking behaviors (Yellman et al., 2020). In contrast, females aged 21–25 exhibited higher prevalence (4.88%) than males (3.13%) in the same age bracket, likely due to increased unsupervised sexual activity and substance abuse in this group, despite general findings that females exhibit lower prevalence (Kreisel et al., 2021). Females generally engage in better preventive healthcare, influenced by societal norms and hormonal factors such as higher estrogen levels, which enhance immune response. These findings highlight the need for gender-tailored public health policies to encourage better health-seeking behavior, particularly among young males (Rodríguez-Planas et al., 2022). Further research is necessary to address sociodemographic disparities in Bayelsa.

The lack of lasting immunity against *N. gonorrhoeae* (Belcher et al., 2023; Wang et al., 2024) underscores the importance of regular surveillance and preventive measures. Chi-square tests revealed three significant risk factors for *N. gonorrhoeae* prevalence: history of STIs (p

= 0.004), multiple sexual partners (p = 0.0002), and traded sex (p = 0.006). Individuals with a history of STIs are more likely to contract *N. gonorrhoeae*, consistent with findings from studies in Port Harcourt and Ethiopia (Kahsay et al., 2023; Kennedy & Ibinabo, 2013). Previous STIs often reflect risky sexual behaviors, such as unprotected sex and multiple partners. Having multiple sexual partners significantly increases the risk of acquiring *N. gonorrhoeae,* as supported by earlier research (Kahsay et al., 2023; Kennedy & Ibinabo, 2013). The risk rises with more partners, especially when engaging in concurrent relationships, facilitating the spread of infection. Engaging in traded sex also heightens the risk due to exposure to multiple partners and limited condom negotiation, often driven by socio-economic factors like poverty and lack of education. This finding emphasizes the vulnerability of those involved in such practices.

Antibiotic susceptibility testing of *N. gonorrhoeae* in this study revealed high resistance to ciprofloxacin, penicillin, and tetracycline, aligning with global trends where these antibiotics are no longer recommended due to rapid AMR development (Golparian et al., 2018; Unemo et al., 2012). Specifically, penicillin showed 100% resistance, and tetracycline exhibited 75% resistance. However, most isolates remained susceptible to ceftriaxone (100% susceptibility) and azithromycin (62.5% susceptibility), consistent with findings from South Africa (Lewis, 2010; Takuva et al., 2014). Cefixime showed only one resistant case, reflecting a growing but contained resistance (Unemo et al., 2016). Ceftriaxone is widely recommended as the first-line treatment; however, resistance has been documented globally (Belcher et al., 2023), posing a risk of rapidly spreading strains due to delayed diagnosis and ineffective treatment protocols. This supports WHO's advocacy for combination therapy to prevent resistance escalation (Belcher et al., 2023; Golparian et al., 2018; Unemo et al., 2012).

The prevalence of *N. gonorrhoeae* in Yenagoa is higher compared to other Nigerian regions, indicating ongoing transmission. The data highlight the importance of ceftriaxone and azithromycin as effective treatment options, although emerging resistance emphasizes the need for continuous monitoring. Given the high resistance rates to older antibiotics and the potential for resistance development to newer ones, public health strategies should prioritize increasing access to diagnostic testing, promoting safe sexual practices, and updating treatment guidelines in Bayelsa State to combat the spread of *N. gonorrhoeae* effectively (Gobezie et al., 2024).

CONCLUSION

In conclusion, the findings of this study highlight the significant prevalence of *N. gonorrhoeae* in the Yenagoa metropolis and the alarming rise of antibiotic resistance, particularly to older drugs such as ciprofloxacin, penicillin, and tetracycline. While ceftriaxone and azithromycin remain largely effective, emerging resistance underscores the need for ongoing surveillance and adaptation of treatment protocols. The identification of multi-drug-resistant strains further emphasizes the urgency of updating public health strategies in Bayelsa State to focus on comprehensive diagnostic testing, effective combination therapies, and education on safe sexual practices. These measures are critical to controlling the spread of gonorrhoea and mitigating the threat of drug-resistant strains in this region.

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