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WJ conceptualized the study and participated in data collection. JB and WJ contributed to the design and conduct of the molecular assays. WJ carried out the culture and sensitivity. BJ, BA, WJ and PT contributed to study design and data analysis, JB over saw the overall running of the study while WJ and NPP contributed in writing the manuscript. All authors read and approved the final version of the manuscript.

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Antimicrobial Resistance in Clinical *Neisseria gonorrhoeae* Isolates at Regional and National Referral Hospitals in Uganda

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Abstract:

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a growing global health challenge, particularly in sub-Saharan Africa yet data in Uganda remain limited. This cross-sectional study analyzed 54 clinical isolates of *N. gonorrhoeae* collected between July 2019 and June 2021 from three referral hospitals in Uganda. Phenotypic resistance patterns were determined using the Kirby-Bauer disc diffusion method, while genotypic analysis employed PCR to detect resistance-associated mutations in the *pen*A, *gyr*A, and *par*C genes. Resistance was highest to Penicillin (100%) and Tetracycline (98.15%), followed by Ciprofloxacin (87.04%). In contrast, lower resistance rates were observed for Cefixime (33.33%) and Cefoxitin (28.26%). Genotypic analysis revealed the *pen*A gene in 66.7% of isolates, making it the predominant genetic determinant of resistance, while *gyr*A and *par*C were detected in 35.3% and 25.5% of isolates, respectively. Statistical analysis indicated that older adults (≥ 45 years) had the highest odds of infection (OR = 2.88, p = 0.045). These findings underscore the need for enhanced AMR surveillance and updates to treatment guidelines prioritizing Ceftriaxone and Cefixime for gonorrhea management.

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INTRODUCTION

Neisseria gonorrhoeae, the causative agent of gonorrhea, is a significant sexually transmitted infection (STI) in humans. The global burden of gonorrhea, with over 9 million cases annually among persons aged 15–49 years including its increasing antimicrobial resistance, poses a critical public health threat, (Whelan *et al.*, 2021; WHO, 2017). In Uganda, the prevalence of gonorrhea is estimated at 13%, with reported resistance to Ceftriaxone and Ciprofloxacin contributing to treatment failures (Kakooza *et al.*, 2023). Antimicrobial resistance in *Neisseria gonorrhoeae* has led to increased treatment failures and more complex, costly regimens, particularly in resource-limited settings.

AMR in *N. gonorrhoeae* has been linked to genetic variations, particularly mutations in genes like *pen*A (encoding β-lactamase resistance) and *gyr*A/*par*C (associated with fluoroquinolone resistance) (Kivata, 2021; Sarenje *et al.*, 2024). Despite the WHO's Gonococcal Antimicrobial Surveillance Program (GASP), existing studies on *N. gonorrhoeae* have primarily focused on phenotypic resistance patterns (Kakooza *et al.*, 2021), leaving a gap in understanding the genetic determinants underlying resistance in local strains. This study aims to address this gap by providing detailed insights into the phenotypic and genotypic resistance profiles of *N. gonorrhoeae* isolates from clinical samples collected in regional and national referral hospitals across Uganda. The findings are intended to inform national treatment guidelines and enhance antimicrobial resistance surveillance efforts in the country.

MATERIALS AND METHODS

Sample collection

This cross-sectional study analyzed 54 *N. gonorrhoeae* isolates collected from July 2019 to June 2021. Isolates were obtained from patients aged 18–65 presenting with urethral or vaginal discharge at Mbarara and Kabale Regional Referral Hospitals and Mulago National Referral Hospital. Isolates were stored at -80°C in brain heart infusion broth with 2% glycerol until analysis.

Phenotypic screening of antibiotic resistance

Isolates were sub-cultured on Thayer-Martin agar under 5% CO_2 for 72 hours. Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method and the results were interpreted according to CLSI guidelines, 2021 (Khaki *et al.*, 2014; Iqbal *et al.,* 2016). Antibiotics used included; Penicillin (10units), broad extended spectrum cephalosporins (Ceftriaxone 30µg, Cefixime 5µg, Ceftazidime 10 µg, Ceftaxime 30 µg, Cefuroxime 30µg, Cefoxitin 30 µg), Fluoroquinolones (Ciprofloxacin 5µg), Spectinomycin 100µg, and Tetracycline 10µg based on WHO recommendations and regional prescription patterns. Results were categorized as susceptible (S), intermediate (I), or resistant (R).

Genotypic screening of antibiotic resistance

DNA extraction

DNA was extracted by boiling bacterial pellets in TE buffer at 95°C for 1 hour. DNA quality and quantity were measured using a Nanodrop spectrophotometer.

Detection of *Pen*A, *Gyr*A and *Par*C of *Neisseria gonorrhoeae*

PCR targeted resistance-associated genes which included; *Gyr*A gene and *Par*C that encode for fluoroquinolone resistance (Kivata *et al.*, 2019) while *Pen*A encodes encodes β-lactamase resistance, while *Gyr*A and parC mutations are linked to fluoroquinolone resistance (Xiu *et al.*, 2020). The PCR master mix reagents for each gene mutation target was prepared as follows: 12.5µL Hot Start Taq2x master mix (M0496S)-New England Bio-labs, 1.0µL forward (10µM), 1.0µL reverse (10µM), 5.0µL DNA template and 5.5µL RNAase-Free-H_2O making up to 25.0µL final reaction volume (Table 1).

Table 1. Primers sets used in the detection of mutation in PenA, GyrA and ParC genes from the study isolates (Attram *et al.*, 2019; Bailey *et al.*, 2019)

Gene locus	Gene	Primer	Annealing temp.	Amplicon Bp
PenA (PBP2)	PenA	(F)5'CGTGATTGCGAAGGCATTGG3' (R)5'GTGCGTCAGTGCGGTATAGG3'	52°C	1026
gyrA (QRDR, gyrA subunit)	GyrA	(F)5' TCCGCCACGACCACAAATTC3' (R)5' CTGCCAGCATTTCATGTGAG3'	57℃	311
parC (QRDR, parC subunit)	ParC	(F)5' GCCATGAGCGTGGTCAAAG3' (R)5' ACCGTCCCCTGATTGATTTC3'	57℃	287

PCR amplification/Cycling

The PCR amplification was carried out in a conventional PCR Thermocycler (CLASSIC K960 Thermal Cycler), the program was initial denaturation at 95°C for 1 minute followed by 35 cycles (denaturation at 95°C for 45 seconds, annealing at 52°C for 1 minutes and elongation at 72°C for 1 minute) and the final extension cycle of 72°C for 5 min. Amplified products were visualized using 1.5% agarose gel electrophoresis.

Data analysis

Data were entered into EpiData v2.2.3, cleaned, and analyzed using STATA 15. Descriptive statistics and logistic regression assessed associations between demographics and resistance patterns.

RESULTS

Social -demographic characteristics

The mean patient age was 30 years (range: 18–65), with males comprising 56.75%. Adults aged ≥45 years had the highest odds of being sexually active (OR=2.88) (Table 2).

Table 2. Influence of socio-demographic characteristics on sexual activity of participants

Characteristics	Category	N (%)	OR (95%ci)	p-value
Gender	Male	109(56.75)	0.11(0.05-0.233)	0.001
	Female	143(43.25)	1	
Age	15-29	140(55.56)	1	
	30-44	101(40.08)	1.43(1.320-1.640)	0.001
	45 and above	11(4.37)	2.88(1.55-4.992)	0.045

Antibiotic Resistance Patterns

Phenotypic testing revealed 100% resistance to Penicillin, 98.15% to Tetracycline, and 87.04% to Ciprofloxacin. Cefixime (33.33%) and Cefoxitin (28.26%) exhibited the lowest resistance rates (Table 3).

Molecular Characterization

The *Pen*A gene was detected in 66.7% (n=34) of isolates, while *Gyr*A and *Par*C were found in 35.3% (n= 18) and 25.5% (n=13), respectively (Table 4). Resistance to Cefixime and Penicillin was primarily linked to *Pen*A mutations. The expression of resistance to antibiotics by coding genes and the proportion of isolates that expressed resistance to antibiotics per Referral Hospital are as shown in Table 5 and Table 6 respectively.

Table 3. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates.

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin	NA	NA	100.0
Ciprofloxacin	3.70	9.26	87.04
Spectinomycin	83.33	3.70	12.96
Tetracycline	NA	1.85	98.15
Cefixime	66.67	NA	33.33
Ceftaxime	31.58	NA	68.42
Ceftazidime	22.64	NA	77.36
Cefuroxime	46.30	14.81	38.89
Cefoxitin	58.70	13.04	28.26
Ceftriaxone	92.5	5.0	2.5

Table 4. Characterization of *Neisseria gonorrhoeae* by penA, gyrA and parC genes.

Encoding Genes	Positive count N (%)	Negative N(%)	Total
PEN A	34(66.7)	17(33.3%)	51
GyrA	18(35.3)	33(64.7%)	51
Par C	13(25.5)	38(74.5%)	51

Table 5. Determinants of antimicrobial resistance by encoding genes of *Neisseria gonorrhoeae* strains.

Antimicrobial (Resistance, % of isolates)	Gyra %(n)	PENA % (n)	PARC %(n)
Penicillin (100%)	None	100% (2)	None
Tetracycline (98.15)	27.4% (17)	56.5% (35)	16.1% (10)
Ciprofloxacin (87.04%)	27.4% (17)	56.5% (35)	16.1% (10)
Spectinomycin (12.96%)	27.4% (17)	56.5% (35)	16.1% (10)
Ceftazidime (77.36%)	27.4% (17)	56.5% (35)	16.1% (10)
Ceftaxime (68.42%)	31% (13)	52.4% (22)	16.7% (7)
Cefixime (33.33%)	14.3% (1)	71.4% (5)	14.3% (1)
Cefoxitin (28.26%)	26.9% (14)	55.8% (29)	17.3% (9)
Cefuroxime (38.89%)	27.4% (17)	56.5% (35)	16.1% (10)
Ceftriaxone (2.50%)	27.4% (6)	55.4 (8)	17.2 (5)

Table 6. Proportion of isolates expressing resistance against antibiotics by Referral Hospitals.

Referral Hospitals	PEN A		GyrA		ParC	
	Positive	Negative	Positive	Negative	Positive	Negative
Kabale	7(13.0%)	3(5.6%)	4(7.4)	6(11.1)	3(5.6%)	7(13.0%)
Mbarara	14(25.9%)	3(5.6%)	3(5.6%)	14(25.9%)	2(3.7%)	15(27.8%)
Mulago	14(25.9%)	13(24.0%)	10(18.5%)	17(31.5%)	5(9.3%)	22(40.7%)

DISCUSSION

This study confirms high resistance rates to Penicillin and Tetracycline in Uganda, consistent with global trends (Allan-Blitz *et al.*, 2017; Unemo and Shafer, 2014). Similarly, Adam et al. (2019) observed Tetracycline-resistant isolates (70.6 %) which correlated with the presence of *Gyr*A for quinolones (Bailey *et al.*, 2019). Similarly, studies conducted in Europe and East Asia also revealed that resistance for ciprofloxacin was essentially above 50% (Wi *et al.*, 2017). The high resistance rates observed in this study can be attributed to overuse and misuse of antibiotics to treat common infections

(Giske *et al.*, 2008; Iqbal *et al.,* 2024). Unfortunately, a medication with resistance beyond the WHO recommended level of 5% should not be recommended for therapy (Mahler *et al.*, 2021). High resistance levels reflect widespread misuse of antibiotics in Uganda, emphasizing the need for stricter prescription regulations.

The low resistance to Ceftriaxone and Cefixime suggests these remain effective first-line treatments. This is consistent with several research findings that have reported low resistance in cephalosporins (Bailey *et al.*, 2019; Lin *et al.*, 2022; Radovanovic *et al.*, 2022). This low resistance may be attributed to the structural modifications of cephalosporins that make them resistant to bacterial β-lactamases (Turner *et al.*, 2021). Furthermore, cephalosporins have high tissue penetration and maintain effective concentrations for longer periods, reducing the chances of bacterial survival and the development of resistance (Lin and Kück, 2022).

Molecular findings emphasize the critical role of the *Pen*A gene in mediating β-lactam resistance. The detection of *Gyr*A and *Par*C mutations, though less frequent, confirms their contribution to fluoroquinolone resistance. This is consistent with a study which reported 75.0% of the Ciprofloxacin-resistant isolates detected expressed a combination of mutations in the *Gyr*A and *Par*C genes that are known to promote Ciprofloxacin resistance (Allan-Blitz *et al.*, 2017). Similarly, other studies in resistance due to mutation expressed existence of correlation between the presence of *Gyr*A (70.6%) and Tetracycline resistance isolates for quinolones (Bailey *et al.*, 2019; Manoharan-Basil *et al.*, 2021). Continuous surveillance of these genetic markers is essential to anticipate and mitigate emerging resistance trends in order to improve patient outcomes. Furthermore, enhanced laboratory capacity is essential for accurate diagnosis and resistance monitoring.

This study was limited by its small sample size and its focus on three referral hospitals, which may not fully represent national resistance trends. Future research should include larger sample size and incorporate whole-genome sequencing to provide a more comprehensive understanding of resistance mechanisms.

CONCLUSION

This study highlights the high prevalence of antimicrobial resistance in *Neisseria gonorrhoeae* isolates in Uganda, particularly to Penicillin, Tetracycline, and Ciprofloxacin. The presence of *Pen*A, *Gyr*A, and *Par*C genes underscores the importance of molecular surveillance to track emerging resistance mechanisms. Cefixime and Ceftriaxone remain effective treatment options, emphasizing their priority in national guidelines. To address growing resistance, stakeholders should prioritize antibiotic stewardship, better diagnostic capability, and public education in order to improve patient outcomes and reduce the public health risk posed by resistant *N. gonorrhoeae*.

DECLARATION

Ethics approval

This is study was approved by Research and ethics committee of Mbarara University of Science and Technology (MUST-2021-181). Upon clearance, permission was sought from the regional and national hospitals to access the isolates and patient information.

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Data Availability

The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

A preprint has previously been published (Jackson *et al.*, 2024).

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CONFLICT OF INTEREST

No conflict of interest among the authors relating to the publication of this article.

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