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## Original Research Article

# Prevalence and antimicrobial susceptibility patterns of *Neisseria gonorrhoea* among the Symptomatic Patients attending Outpatient Department in Lyatonde District Hospital Southwestern Uganda

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### ABSTRACT

**Background:** *Neisseria gonorrhoea* is one of the neglected diseases of public health importance causing symptomatic, suppurative discharge in males and being asymptomatic in females. This cross-sectional study was aimed at determining the prevalence and susceptibility pattern of *Neisseria gonorrhoea* to the commonly used antibiotics among symptomatic patients attending outpatient department in Lyatonde district hospital.

**Methods:** Urethral and endocervical swabs were collected by the attending medical laboratory technologists. The presence of gonorrhoea was confirmed by culture, Gram staining and biochemical tests. Antimicrobial sensitivity test was performed using the disc diffusion method and the result was interpreted using the National Committee for Clinical Laboratory Standards guidelines.

**Results:** Overall prevalence of *N. gonorrhoea* was 4.9% with high prevalence rates of 7.7% among the young age group of 15-25 years. Low level of antimicrobial susceptibility to cefuroxime (50%) followed by erythromycin and gentamycin both at 25% was observed. An alarming resistance to ceftriaxone and ciprofloxacin at 100% followed by penicillin 75% was exhibited by the colonies.

**Conclusions:** The high resistant rate to ceftriaxone and ciprofloxacin obviates their use as the first line of syndromic treatment of gonorrhoea in Southwestern Uganda. The use of laboratory culture for diagnosis and management of *N. gonorrhoea*, especially with cefuroxime therapy is highly recommended.

**Keywords:** Antibiotic susceptibility, *Neisseria gonorrhoea*, Prevalence, Uganda

### INTRODUCTION

Gonorrhoea is a sexually transmitted infection caused by the bacteria *Neisseria gonorrhoea* for which man is the only natural host and is of a major public health concern.<sup>1</sup> It is the second most prevalent bacterial infection sexually transmitted, with estimated 106 million new cases annually.<sup>2</sup> After transmission, *N. gonorrhoea* causes urethritis in males and cervicitis in females. Relatively few males ( $\leq 10\%$ ) but a large proportion of females ( $\geq 50\%$ ) can have an asymptomatic urogenital infection. Rectal and

pharyngeal gonorrhoea is commonly asymptomatic in both genders.<sup>3</sup> These infections are most frequently identified in men who have sex with men (MSM), but dependent on sexual practice they can be encountered in both genders in many settings.<sup>2</sup> The urogenital infections, if untreated, might ascend to the upper genital tract and result in severe reproductive complications (mostly, but not only, in females), such as pelvic inflammatory disease or even loss of life through life threatening ectopic pregnancy and at times sepsis can ensue. Epididymitis, although rare in men can result in infertility.<sup>2</sup>

Gonococcal infections also facilitate the transmission and acquisition of HIV.<sup>2,4-8</sup> *N. gonorrhoea* may also cause conjunctivitis, mostly in neonates (ophthalmia neonatorum) infected from their mother during delivery, but also in adults. Conjunctivitis may, if untreated, can lead to total blindness. Disseminated gonococcal infection (DGI) is an uncommon complication of gonococcal infection and, although rare, this can lead to, arthritis, meningitis and endocarditis.<sup>4,8-10</sup> *N. gonorrhoea* was initially highly susceptible to many antimicrobials. However, since the introduction of sulphonamides in 1936 and penicillin in 1942 for treatment of gonorrhoea, *N. gonorrhoea* has repeatedly shown an extraordinary capacity to develop resistance to all antimicrobials introduced for treatment during the past 70-80 years.<sup>11</sup> The drug resistance of *N. gonorrhoea* varies greatly. Therefore having prevalence's data as well as the drug susceptibility patterns within consecutive years is important especially for gonorrhoea, the highly drug resistant bacteria. Estimates of global prevalence of gonorrhoea among women aged 15-49 was 0.8% and among males aged 15-49 was 0.6% translating into 78 million (53-110 million) new cases of gonorrhoea.<sup>12</sup> Sub Saharan Africa has an STI incidence of 240/1000 cases which is the highest in the world.<sup>12</sup> *Neisseria gonorrhoea* is the second most prevalent bacterial infection sexually transmitted with estimated 106 million new cases annually.<sup>12</sup>

The sexual transmitted infection (STI) surveillance system in Uganda is weak and besides, Uganda is not a participating member of WHO Global Gonococcal Antimicrobial Surveillance Program (GASP) which was launched in 2009. There has been under reporting of STI cases including gonorrhoea in most parts of the country. Except for the adult prevalence of HIV (6.2%), HBV (4.1%) and syphilis (2.6%) which was a report contained in survey conducted by the Uganda Population Base HIV Impact Assessment in 2016.<sup>13</sup> In the absence of gonococcus screening program in the study area, little is known about the prevalence of gonococcal infection in Lyatonde district, Uganda. There are limited data on the antimicrobial susceptibility of *N. gonorrhoea* in Uganda. This is mainly because there are very few diagnostic microbiology laboratories in the country including Lyatonde district hospital Southwestern Uganda. Therefore, the aim of this study was to assess the prevalence of *Neisseria gonorrhoea* and the antimicrobial susceptibility patterns among the symptomatic adult patients attending outpatient department in Lyatonde district hospital. We also explored the factors associated with prevalence rates of *Neisseria gonorrhoea* among the study population.

## METHODS

A cross-sectional study was conducted at Lyatonde district hospital Southwestern Uganda from February 2018 to March 2018. A total of 82 patients aged 15-65 who attended the outpatient department clinic and had

symptoms for STIs and consented/assented for were recruited on to the study using consecutive sampling technique until the required 82 samples were collected. Patients who were on antibiotic treatment by the time of the study were excluded. The screening of patients and sociodemographic information were extracted from the research subjects by the attending Clinicians. Meanwhile the laboratory samples (females; endocervical swabs and males; urethral swabs) were collected by the medical laboratory technologists. One swab from each research subject was used for Gram stain meanwhile the remaining swab was used for culture and sensitivity. Two sets of swabs were taken from every research participants and were transferred into Amie's transport media (Oxoid, Basingstoke, and Hampshire, UK, England) used for transportation to Microbiology Laboratory, at Mbarara University of Science and Technology. The samples taken to the Microbiology Laboratory were on arrival inoculated onto non selective chocolate agar and selective agar modified Thayer Martin medium (Oxoid Basingstoke, and Hampshire, UK, England). Some fastidious strains, such as the arginine-hypoxanthine and uracil requiring strains are susceptible to the concentration of vancomycin or trimethoprim used in the selective media which can grow on non selective chocolate agar. The inoculated plates were incubated at 37°C in a moist atmosphere enriched with carbondioxide 5% using candle jar. *N. gonorrhoea* produce small raised grey shining colonies on modified Thayer-Martin medium after overnight incubation.<sup>14</sup> *Neisseria gonorrhoea* was differentiated from other *Neisseria* species, *Moraxella* species, *Kingella* species, and other commensal based on the production of acid from glucose only and not from maltose, lactose, sucrose and fructose. Accordingly, the carbohydrate utilization test was done using API NH identification kit strip (Oxoid, Basingstoke, and Hampshire, UK, England). In general all positive culture were identified by their characteristic appearance on the media, Gram staining reaction and confirmed by the patterns of biochemical reactions using the standard methods.

Antimicrobial susceptibility was tested by standard disc diffusion method (Kirby-Bauer test). Gonococcal specimens were subcultured from the selective primary medium to a chocolate agar without the selective supplement to obtain a pure culture of the specimen. From a pure culture, 3-5 selected colonies of bacteria were transferred into a tube with a straight wire and prepared a suspension in 2.5 ml normal saline and incubated at 37°C until the turbidity of the suspension become adjusted to a McFarland 0.5. Sterile swab was used to distribute the bacteria evenly over the entire surface of chocolate agar. The susceptibility of the following antimicrobial agents (Oxoid) were assessed; Penicillin (P 10 IU), Cefuroxime (CFM 5 ug), Ciprofloxacin (CF 10 ug), Gentamycin (GN 10 ug), Ceftriaxone (CT 30 ug) and Erythromycin (E 30 ug). The criteria that was used to select the antibiotics/antimicrobial agents was based on the

availability and frequent prescriptions for the empiric management of gonococcal infections. Standard reference strain of *Neisseria gonorrhoea* ATCC29226 was also used as recommended by the CLSI for susceptibility testing of gonococcal isolates.<sup>15</sup> Reference strains from Mulago National Referral Hospital Uganda were used as quality control strains throughout the study for culture and antimicrobial susceptibility testing. The bacteria pathogen was regarded as sensitive, intermediate or resistant according to the Clinical and Laboratory Standard Institute (CLSI) disc diffusion standards current at the time.<sup>15,16</sup>

### Data analysis

Data was first entered into Epidata, cleaned, validated and exported to STATA version 13.0 for analysis. Prevalence rates was calculated for positive cases of examined subjects and separately by age groups and sex. Logistic regression and Fishers exact test analysis were used to estimate the association between the dependent variable and the independent variables. The level of significant difference with 95% confidence.

## RESULTS

### Sociodemographic characteristics of the 82 patients investigated for gonorrhoea infection

A total of 82 patients who attended outpatient department (OPD) were recruited onto the study to investigate them for gonococcal infection, with majority 59 (72%) being females and the age groups 26-35 years and 36-45 years having prevalence of 34.2%. Most 44 (53.7%) had attended primary education and the married participants constituted the largest proportion of the study participants 55 (67.1%). Farming 41 (50%) was the main occupation of the study participants with 45 (54.9%) belonging to Baganda ethnic group. Most of the participants belonged to Protestant religion, meanwhile about 54 (65.9%) lived in rural setting (Table 1).

### Prevalence of *Neisseria gonorrhoea* infection and socio-demographic characteristics of 82 patients investigated for gonococcal infection at Lyatonde district hospital.

The prevalence of *Neisseria gonorrhoea* was 4.9% in the whole study population. Prevalence of *N. gonorrhoea* was higher among males 13% (95% CI: 4.1-34.6), age group 15-25 years 7.7% (95% CI: 1.8-12.5), and those attending secondary education 11.5% (95% CI: 3.6-31.2), although with no statistical significance ( $p>0.05$ ). *N. gonorrhoea* infection was observed in 4.9% (95% CI: 1.1-18.1) among farmers and those population dwelling in urban residences 7.1% (95% CI: 1.7-25.4). There is a statistical significance association of having *N. gonorrhoea* infection and belonging to Basoga ethnic group ( $p=0.022$ ) (Table 2).

**Table 1: Description of the demographic data of 82 patients investigated for gonococcal infection at Lyatonde district hospital, Southwestern Uganda (February 2018-March 2018).**

Socio-demographic characteristics	Category	Frequency (%)	95% CI
Age group (in years)	15-25	26 (31.71)	22.4-42.8
	26-35	28 (34.15)	24.6-45.2
	36-45	28 (34.15)	24.6-45.2
Gender	Male	23 (28.1)	19.3-38.9
	Female	59 (72)	61.1-80.8
Education level	None	6 (7.32)	3.3-15.6
	Primary	44 (53.7)	42.6-64.3
	Secondary	26 (31.7)	22.4-42.8
	Tertiary	6 (7.32)	3.3-15.6
Marital status	Married	55 (67.1)	55.9-76.5
	Divorce	6 (7.3)	3.3-15.6
	Single	19 (23.2)	15.2-33.7
	Widowed	2 (2.4)	0.6-9.4
Occupation	Bodaboda	1 (1.22)	0.2-8.5
	Business man	5 (6.10)	2.5-14.1
	Business woman	5 (6.10)	2.5-14.1
	Civil servant	11 (13.41)	7.5-22.9
	Farmer	41 (50)	39.1-60.8
	Hotel attendant	1 (1.22)	0.2-8.5
	Housewife	7 (8.50)	4.1-17.1
	None	1 (1.22)	0.2-8.5
	Student	9 (10.9)	5.7-20
	Teacher	1 (1.22)	0.2-8.5
	Ethnicity	Bafumbira	2 (2.44)
Banyankole		19 (23.2)	15.2-33.7
Bakiga		6 (7.3)	3.3-15.4
Baganda		45 (54.9)	43.8-65.5
Banyarwanda		7 (8.54)	4.1-17.2
Basoga		1 (1.22)	0.2-8.5
Batoro		2 (2.44)	0.6-9.5
Settings	Rural	54 (65.9)	54.7-75.5
	Urban	28 (34.2)	24.5-45.3
Religion	Catholic	28 (34.5)	24.6-45.3
	Muslim	8 (9.76)	4.9-18.6
	Pentecostal	10 (12.20)	6.6-21.4
	Protestant	30 (36.59)	26.7-47.7
	SDA	6 (7.32)	3.3-15.6

### Antimicrobial susceptibility testing

After culturing, antibiotic sensitivity testing was carried out on the positive *N. gonorrhoea* isolates using different antibiotics commonly prescribed in the health facilities and the results of the antibiogram are shown in Table 3.

Rates of resistance to ceftriaxone and ciprofloxacin was 100% and penicillin was 75%. Meanwhile, cefuroxime showed the highest sensitivity of 50%, followed by

erythromycin and gentamycin both with sensitivity of 25%.

**Table 2: Association between prevalence of *N. gonorrhoea* infection and Socio-demographic characteristics of 82 patients investigated for gonococcal infection at Lyatonde district hospital Uganda (February 2018 to March 2018).**

Socio-demographic characteristics	Category	<i>N. gonorrhoea</i> (n)	95% CI	Fisher's exact test
<b><i>N. gonorrhoea</i> positive</b>	Number	4 (4.9%)	1.8-12.5	
<b>Age group (years)</b>	15-25	2 (7.7)	1.8-12.5	0.685
	26-35	1 (3.6)	0.4-22.6	
	36-45	1 (3.6)	0.47-22.6	
<b>Gender</b>	Female	1 (1.7)	0.02-11.5	0.07
	Male	3 (13.0)	4.1-34.6	
<b>Setting</b>	Rural	2 (3.7)	0.9-14.1	0.493
	Urban	2 (7.1)	1.7-25.4	
<b>Education level</b>	None	0		0.381
	Primary	1 (2.3)	0.3-15.1	
	Secondary	3 (11.5)	3.6-31.2	
	Tertiary	0		
<b>Ethnic group</b>	Bafumbira	1 (5.0)	1.8-9.9	0.022
	Baganda	1 (5.26)	0.7-31.2	
	Banyankole	1 (2.22)	0.3-14.8	
	Basoga	1 (100)	-	
<b>Occupation</b>	Business man	1 (20)	1.03-75.0	0.419
	Business woman	1 (20)	2.03-75.0	
	Farmer	2 (4.9)	1.1-18.1	
<b>Religion</b>	Catholic	2 (7.41)	1.7-25.4	1.000
	Protestant	2 (6.67)	1.5-23.9	
<b>Marital status</b>	Married	3 (5.45)	1.7-15.9	1.000
	Single	1 (5.26)	0.7-31.2	
<b>Alcohol consumption</b>	Yes	1 (14.3)	1.6-62.9	0.305
	No	3 (4.0)	1.3-11.9	
<b>Condom use</b>	Yes	1 (11.1)	1.31-53.9	0.378
	No	3 (4.11)	1.3-12.3	

**Table 3: Antimicrobial susceptibility patterns of *N. gonorrhoea*.**

Antimicrobial agents	Number of isolates (%)		
	susceptible	Intermediate	Resistant
<b>Cefuroxime</b>	2 (50)	1 (25)	1 (25)
<b>Gentamycin</b>	1 (25)	0 (0)	3 (75)
<b>Erythromycin</b>	1 (25)	0 (0)	3 (75)
<b>Ciprofloxacin</b>	0 (0)	0 (0)	4 (100)
<b>Ceftriaxone</b>	0 (0)	0 (0)	4 (100)

## DISCUSSION

Gonococcal infection has a devastating impact on the health of adult males and females including children and if not diagnosed early and appropriately treated with sensitive antibiotics. Performing laboratory surveillance of antimicrobial resistance in *N. gonorrhoea* infection is of

great importance in order to assess the effectiveness of locally recommended therapies. At population level, surveillance is key for monitoring of local, regional and international trends in antimicrobial resistance, which can help inform and shape public health policy.

The study population comprised of 82 symptomatic adults who consented with the majority of patients being females, manifesting the health seeking behavior of females in an African setting. The overall yield of *N. gonorrhoea* isolates of 4.9% in the whole study population is lower than that found in studies conducted elsewhere in some African countries; 11.3% and 5.1% in Ethiopia, 8.4% in Tanzania, 42% in Bangladesh, 59% in Uganda and 80% Malawi.<sup>17-21</sup> The variation in prevalence rates in those other studies compared to the current study may be due to differences in the study population where pregnant women with urethral discharges attending antenatal clinics in Uganda, the antiretroviral therapy follow up cases in Ethiopia, patients only with urethral discharge in STI clinics in Malawi, and commercial sex workers in Bangladesh.

The use of culture in testing urogenital samples in this current study enabled the confirmation of *N. gonorrhoea* isolates including their antibiogram that wouldn't have been possible by microscopy alone. It is well known that microscopy of *N. gonorrhoea* has a sensitivity of 95% in males but only 40-60% in females compared to 85-100% for culture.<sup>15,22</sup> The prevalence of *N. gonorrhoea* obtained in our study is higher compared to other studies 2.2% in Jordan, 0.7% Vietnam and 0.4% in Democratic Republic of Congo.<sup>23-25</sup> The most plausible reasons for this discrepancy is due to lack of differential diagnosis, which can lead to an increase in numbers of untreated patients.

Although socio-demographic factors have great influence in the prevalence of STDs in which most studies have documented, in our current study the distribution of gonococcal infection to most socio-demographic factors have no statistical significant association,  $p$  value  $>0.05$ .<sup>26</sup> Higher prevalence of *N. gonorrhoea* was observed among the males compared to females. This finding is in concordance with studies conducted in some parts of Africa.<sup>17,18</sup> Although we never looked for factors influencing propensity to *N. gonorrhoea* infection, some studies have reported sexual promiscuity of males as they tend to have more sexual partners compared to their female sexual counter parts.<sup>27</sup> Besides, by nature males are more symptomatic for the infection than their female counterparts.

Despite there being no statistically significant difference in the frequency of gonococcal infections among different age groups, gonococcal infection was more frequent in the age group 15-25 years (7.7%). As documented by other studies sexually active age groups are at high risk of acquiring STIs and are more prone to sexual promiscuity and unsafe sex practices.<sup>28</sup> Similarly, this finding is in concordance with high STI prevalence observed among young people worldwide.<sup>29</sup> The other reason for the high burden of gonorrhoea among this age group is the lack of health information necessary for recognition of disease symptoms among these age category.<sup>30</sup> There was high prevalence of *Neisseria gonorrhoea* among the secondary

school going children than other categories of the education sector. This might be due to aforementioned risky sexual practices by the youths attending secondary school and thus calls for global concerted efforts to improve sexual and reproductive health in this target population.

Gonococcal and drug resistance vary greatly among countries and in regions even in sub-regions in developing world, because of socio-demographic factors, the treatment algorithm and the way cases are diagnosed and treated in every region. This study found high level of antibiotic resistance to ceftriaxone, ciprofloxacin and penicillin to gonococcal infection in the study area. The high level of resistance to penicillin and ceftriaxone found in our study has been widely reported throughout the world, USA, Australia and Romania due to emergence of penicillin resistant beta-lactamase producing strains.<sup>26,31,32</sup> Clinically confirmed treatment failures to cefixime and ceftriaxone by *N. gonorrhoea* isolates had been described in North America and Europe by 2010.<sup>33</sup>

In other studies conducted in Uganda on *N. gonorrhoea*, lower rates of resistance to ciprofloxacin, erythromycin and gentamycin only was observed as opposed to the findings observed in our study. A less resistance rate to ciprofloxacin, erythromycin and gentamycin antibiotics was also observed by Vandepitte et al, among the *N. gonorrhoea* isolates got from sex workers in Kampala.<sup>34</sup> The reasons for these high resistance rates could be due to the intense use of antibiotics, easy availability and indiscriminate use of these antibiotics outside the health facilities and many antibiotics are available over the counter for self medication. The alarming percentage of resistance against ceftriaxone, ciprofloxacin and penicillin in this current study could significantly challenge the current use of this antibiotics in the syndromic management package of gonococcal infections in Uganda. Therefore, it is imperative that the laboratory based diagnosis and antibiogram determination of the commonly used antibiotics for the treatment of

*Neisseria gonorrhoea* is in place instead of the current syndromic option for the management of gonorrhoea infection in Uganda.

The study findings revealed antibiotic sensitivity to cefuroxime of 50% followed by erythromycin and gentamycin, all at 25%. The 50% sensitivity to cefuroxime, a cephalosporin belonging to group three, was the best among all the drugs tested for *Neisseria gonorrhoea* susceptibility testing. The reasons for this could be that this drug is rather expensive, not intensively used and not easily available outside the hospitals. Besides these drugs are newer compared to the others. The fifty percent sensitivity to this group three cephalosporin (cefuroxime) in our study make this drug a better choice as first-line treatment for *N. gonorrhoea* in this country.

## CONCLUSION

The prevalence of *N. gonorrhoea* was 4.9% among the study population with prevalence of 13% among males compared to 1.7% in females. The age group 15-25 years was the group with most burden of *N. gonorrhoea* infection. The high-prevalence resistance to penicillin, tetracycline and ciprofloxacin by the circulating strains of *N. gonorrhoea* among the study participants, obviates the use of these agents in empiric therapy guidelines for syndromic management in Uganda.

### Recommendation

Cefuroxime had the highest sensitivity and is recommended for the first line treatment for *N. gonorrhoea* in this country. Future studies to assess the resistance trends in Uganda and to allow timely revision of treatment protocols are highly recommended.

### Limitation

There were a number of limitations of this study, particularly the small sample size and the fact that it was only done in one health centre from one region of the country, and so it is unknown whether significant regional variations in gonococcal susceptibility occur throughout Uganda. The smaller sample size of 82 provided less precision for prevalence estimates and less power to investigate factors associated with gonorrhoea infection. Due to financial constrain, the disc diffusion method (Kirby Bauer) was used for the determination of antimicrobial susceptibility to gonorrhoea isolates instead of the quantitative methods (E-Test or agar dilution test) recommended by WHO (2012). Nonetheless, the use of disc diffusion test for the determination of antibiotic susceptibility to *N. gonorrhoea* following CLSI standard in our study still suffice, especially where strains of *N. gonorrhoea* displaying borderline susceptibility patterns are rare. Furthermore, the relative high cost of E-Test procedure (list price approximately \$ 2.50 per strip) precludes the use of it for routine use especially in the third world countries like Uganda.

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