



Antimicrobial Resistance of *Neisseria gonorrhoeae* Isolated from Out Patients Presenting with Urethral and Vaginal Discharges at Mbarara Regional Referral Hospital

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Authors' contributions

This work was carried out in collaboration between both authors. Author JO was involved in the study conception, design, data collection and data analysis, report writing and drafting the manuscript while author JB was involved in technical advice and review of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Gonococcus is one of the most common sexually transmitted diseases in developing countries and it has become a global health burden, hence a need for effective treatment. However, there is growing trend of antimicrobial resistant strains, in many parts of the world, to the previously effective antimicrobials thus creating serious health concerns.

Setting: Mbarara Regional Referral Hospital -South-Western Uganda.

Objectives: 1) To determine the prevalence of *gonococcus* among out patients presenting with urethral and vaginal discharges at MRRH.

2) To determine the level of antimicrobial resistance of *gonococcus* based on phenotypic methods at MRRH.

Design: The study was cross sectional and enrolled 189 participants presenting with urethral and vaginal discharges. The urethral and endo cervical swab samples collected were cultured on

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Chocolate media supplemented with 5%-10% carbondioxide in candle jar (inoculated plates were placed in a jar and a burning candle placed in the same jar, then closed, by the time the candle went off, that 5%-10% carbondioxide atmosphere would have been created). Isolates obtained were identified according to the laboratory standard operating procedures. Drug Sensitivity Test (DST) on confirmed *Neisseria gonorrhoeae* isolates was performed using the Kirby Bauer technique. The colonies of the test organism were emulsified in peptone water and then inoculated on prepared sterile chocolate agar and the following discs were applied to it (Ceftriaxone discs (30µg), Erythromycin (15ug), Ciprofloxacin (10ug) and Penicillin (10IU). The plates were incubated at 37°C for 24- 48 hours under 5% carbon dioxide atmospheres. The Zone of inhibition was seen around an antibiotic disc to which the organism was sensitive.

Results: Out of the 189 participants whose urethral swabs and Endo cervical swabs were cultured, 89 were positive cultures (47%), out of which 25 (28%) were found to have gonococcal infection, 64 (72%) patients had other micro-organisms. The prevalence of *Neisseria Gonorrhoeae* was 13%. In total, 4% of the isolates were resistant to Ceftriaxone, 28% to Ciprofloxacin, 68% to Erythromycin and 80% to Penicillin. A high percentage of resistance was observed against Penicillin (80%) and Erythromycin (68%).

Conclusion: Adults aged 18 years and above who present at Mbarara Regional Referral Hospital with urethral or vaginal discharges are more likely to have a *Neisseria gonorrhoeae* which is resistant to Penicillin and Erythromycin.

Keywords: *Neisseria gonorrhoeae*; gonococcus; antimicrobial; resistance; prevalence; Mbarara.

1. INTRODUCTION

Neisseria gonorrhoea a causative agent of gonorrhoea is a major public health concern globally and the second most prevalent bacterial infection sexually transmitted, with 106 million new cases annually [1]. Human beings are the only host for the causative agent *Neisseria gonorrhoeae*. Its incidence in both undeveloped and developing countries is high especially in vulnerable populations and marginalized ethnicities [2]. Untreated and poorly managed gonorrhoea can result into epididymitis, pelvic inflammatory disease (PID), ectopic pregnancy, and infertility which are serious complications [3]. For the past 70-80 years, gonorrhoea has been successfully treated using antimicrobials. *Neisseria gonorrhoeae* has a high ability to develop resistance to antimicrobials, resulting in the progressive loss of cheap and effective treatments and the need to use more expensive drugs that are inaccessible in many countries.

Neisseria gonorrhoea antimicrobial resistance is significantly becoming a public health problem worldwide [4] and currently there are no vaccines available for *Neisseria gonorrhoea*, hence antimicrobial treatment is essential to control the disease [5]. However, resistance to sulphonamides, penicillins, Tetracyclines, Macrolides and Fluroquinolones has emerged in most parts of the world [6].

At International level a high prevalence of *Neisseria gonorrhoea* resistant strains to

originally used antimicrobials to treat gonorrhoea has occurred. Medical literature has shown failure to treatment of gonorrhoea with extended spectrum cephalosporins-(ESCs), cefixime and ceftriaxone [7]. Still the emergency of gonococcal strains exhibiting high level of clinical resistance to all ESCs together with resistance to nearly all other available antimicrobials have resulted into a great public health concern [7].

In developed countries like United States, United Kingdom and other European countries, recommendations to use dual antimicrobial therapy especially ceftriaxone and Azithromycin have been introduced in management of *Neisseria gonorrhoea* [8]. Unfortunately, the susceptibility of gonococcal isolates to ceftriaxone has been decreasing globally and in many settings, resistance to Azithromycin is already prevailing [6]. This resistance constitutes an epidemiological situation that prevents healing and increases the likelihood of the occurrence of serious consequences associated with gonorrhoea [9]. The increasing public health crisis of gonococci should not be under estimated.

This is because treatment regimens will most certainly become more expensive due to treatment failures and associated costs to manage complications that compromise the general and reproductive health of infected individuals [1]. According to a study by Amito et al, 2012, carried out in Uganda, the prevalence of *Neisseria Gonorrhoea* was at 59% and all of

these had symptoms of pus discharges and frequency suggesting gonococcal urethritis.

The study also demonstrated that there was significantly reduced sensitivity to Ampicillin 23.4%, Ciprofloxacin 23.3%, Tetracycline 17.2% and Erythromycin 17.2%.

The high rate of resistance to panel of antibiotics like Ampicillin, ciprofloxacin, Tetracycline and erythromycin may preclude the use of these antibiotics as the empiric treatment of *Neisseria gonorrhoea* in Uganda.

2. METHODS

2.1 Study Design, Setting and Population

A cross-sectional study was conducted at Mbarara Regional Referral Hospital (MRRH) South western Uganda from January 2017 to April 2017. A total of 189 patients aged 15-69 years who attended the outpatient department clinic and presented with urethral and vaginal discharges were recruited from outpatient departments (OPD) of Antiretroviral Therapy (ART) Clinic, general OPD and Sexually Transmitted Infections (STI) Clinic. MRRH is located in Mbarara municipality that is 266km (165 miles) South Western of Kampala, the capital city of Uganda. MRRH is a public, teaching and research hospital.

2.2 Data Collection

Urethral and endocervix swabs were collected from participants who were presenting with urethral and vaginal discharges. Two swabs were collected one for Gram stain and the other for culture and sensitivity from both male and female clients.

The collected swabs were transported in a cool box in Stuart media. The swab for culture was inoculated immediately on appropriate media in Microbiology laboratory at Mbarara University of science and technology (MUST). The total number of participants enrolled was 189. Samples cultured were 189, of which 25 were of *Neisseria Gonorrhoea*, 64 for other organisms and 100 samples did not show any bacterial growth or any significant growth.

2.3 Laboratory Procedures

2.3.1 Microscopy

A direct smear for Gram staining was performed immediately the samples arrived in the

laboratory. The swab was rolled gently onto the slide to make a smear which was heat fixed. The heat fixed smear was covered with crystal violet (purple dye). After 2-3 minutes, the purple dye was washed off with water and covered with Lugo's Iodine solution. After 2-3 minutes, the slide was washed with an alcohol acetone solution.

The alcohol was rinsed off, and the slide was then stained with safranin (basic red dye). After 3 minutes the smear was washed again with water and blotted to dry. And examined under oil immersion (1000× magnification). Presence of intracellular Gram-negative kidney-shaped diplococci in polymorph nuclear leukocytes, were required for the presumptive diagnosis of gonorrhoea.

2.3.2 Culture

The pre warmed Chocolate agar medium was inoculated with urethral or endocervix swab containing the test organism and incubated at 37°C in a moist atmosphere enriched with carbon dioxide 5%-10% using a candle jar for 24 hours.

2.3.3 Identification

All positive cultures were identified by their characteristic appearance on the media. The colonies appeared pinkish-brown and were translucent; they exhibited a smooth consistency and defined margins. Biochemical tests including oxidase test to which *Neisseria gonorrhoea* was positive was also performed. Carbohydrate fermentation reaction was also performed on the isolates for the identification of *Neisseria gonorrhoea*.

2.4 Antimicrobial Susceptibility Test

McFarland Standards were used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard of 0.5 was prepared using a chemical solution of barium chloride and sulphuric acid to form a fine precipitate of barium sulphate.

Prior to using the McFarland Standard it was shaken up well and aliquoted into test tubes identical to those used to prepare the inoculum suspension. The aliquoted tubes were tightly sealed to prevent evaporation from occurring. Before each use, it was shaken well to ensure

that the barium sulphate was distributed evenly throughout the solution.

2.5 Procedures

The McFarland Standard was mixed on a vortex mixture prior to examination. A test suspension was prepared by obtaining a fresh, pure culture of the test organism and inoculating it on a suitable broth. In the presence of good lighting, the turbidity of test suspension was visually compared with that of the McFarland standard by comparing the clarity of the lines on the Wickerham card. Drug Sensitivity Test (DST) on confirmed *Neisseria gonorrhoeae* isolates was performed using the Kirby Bauer technique. The colonies of the test organism were emulsified in peptone water and then inoculated on prepared sterile chocolate agar and the following discs were applied to it (Ceftriaxone discs (30µg), Erythromycin (15µg), Ciprofloxacin (5µg) and Penicillin (10IU). 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. The plates were incubated at 37°C for 24- 48 hours under 10% carbon dioxide atmospheres. The Zone of inhibition was seen around an antibiotic disc to which the organism was sensitive. The standard control used was ATCC49226 obtained from Medical Research Centre Entebbe.

2.6 Data Analysis

Data collected from the study was entered into Excel data base and exported into STATA software where it was analysed. Analysed data was presented using tables, pie charts and graphs. Prevalence and antimicrobial resistance of *Neisseria gonorrhoea* was calculated as proportions of all the isolates detected from all samples examined.

3. RESULTS

The mean age of participants was 28years. Majority of the participants were females (71%) and most of the participants with a primary level of education (41.8%). Most (47.6%) participants were married and Protestants by religion (41.3%).

Out of the 189 participants whose urethral swabs and Endo cervical swabs were cultured, 89 were positive cultures (47%), out of which 25 (28%) were found to have gonococcal infection, 64

(72%) patients had other micro-organisms (Table 1).

3.1 Study Participants Description

This cross sectional study enrolled 189 participants presenting with urethral and vaginal discharges.

Table 1. Description of study participants who met the inclusion criteria

Characteristic	Number /Percentage (N=189)
Mean age (S.D)	28.8 (26.5-29.7)
Age range	15-69years
Gender	
Male	55 (29)
Female	134 (71)
Education level	
No formal education	15(7.9)
Primary	79(41.8)
Secondary	57(30.2)
Tertiary	36(19.0)
Unknown	2(1.1)
Marital status	
Single	64(33.9)
Married/living with a partner	90(47.6)
Divorced/ separated	19(10.1)
Widowed	1(0.5)
Unknown	15(7.9)
Religious affiliation	
Catholic	61(32.3)
Muslim	27(14.3)
Pentecostal	14(7.4)
Protestant	78(41.3)
SDA	2(1.0)
Unknown	7(3.7)

3.2 Prevalence of *Neisseria gonorrhoeae*

There were 25(13.2%) clinical isolates of *N. gonorrhoeae* (prevalence of *N. gonorrhoeae*) and were processed for susceptibility testing during the study period.

3.3 Characteristics of Participants who had *Neisseria gonorrhoeae*

Of 189 samples tested, 25(13.2%) were of *Gonococcus* and males (90.5%), with a mean age of was 29 years. Most of them were divorced/ separated (47.6%) and SDA's or Catholics (38.1%).

3.4 Antimicrobial Susceptibility Pattern of the *N. gonorrhoeae* Isolates

In total, 4% of the isolates were resistant to Ceftriaxone, 28% to Ciprofloxacin, 68% to

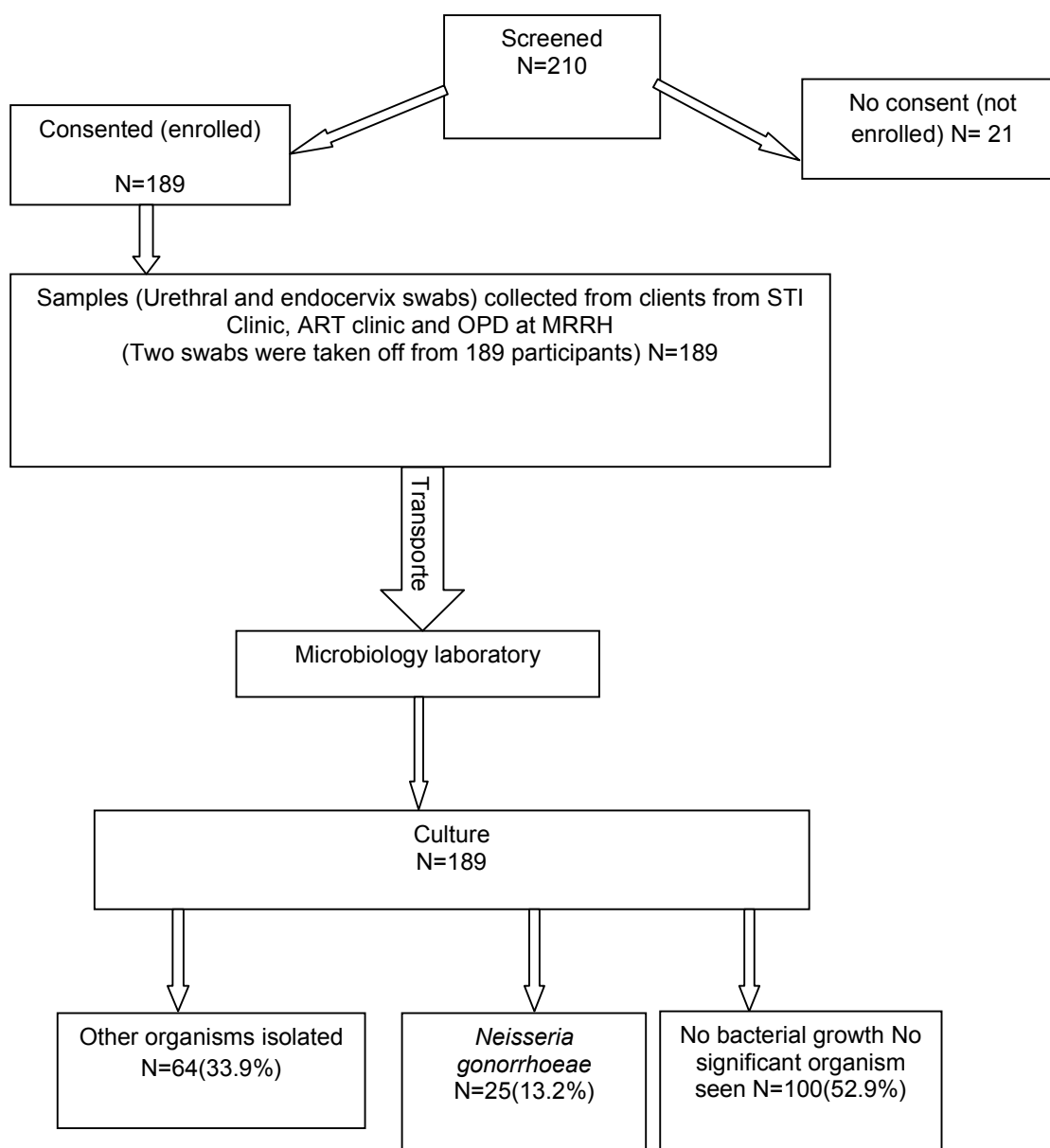


Fig. 1. Study profile

Erythromycin and 80% to Penicillin. A high percentage of resistance was observed against Penicillin (80%) and Erythromycin (68%) (Table 2).

4. DISCUSSION

Gonorrhoea is one of the most common sexually transmitted infections (STIs) in developing countries, and its control remains a major public health concern globally and clearly also in Mbarara, Uganda. The emergence of resistance to antimicrobial agents in *Neisseria gonorrhoeae*

is a major obstacle in the control of gonorrhoea. This makes it necessary to have regular monitoring of antimicrobial susceptibility of *N. gonorrhoeae* for the early detection of emergence of drug resistance.

In this study it was also found that there is a high frequency rate (13%) of gonococcal infection in patients seen at Mbarara Regional Referral Hospital. This is lower than what was observed in a study carried out in a slightly similar setting in 2018 which found out a 4.9% prevalence rate of gonococcal infection [10]. In a study that was

Table 2. Antimicrobial susceptibility patterns of *N. gonorrhoeae*

Antimicrobial agents	Susceptibility (%)	Intermediate (%)	Resistance (%)
Ceftriaxone	96	00	4
Ciprofloxacin	72	00	28
Erythromycin	32	00	68
Penicillin	20	00	80

done in Iran by Afrasiabi et al., 2014, which looked at *Neisseria gonorrhoeae* antimicrobial susceptibility patterns in Kashan, Iran, the prevalence of *Neisseria gonorrhoeae* was found at 2.38%. This was a bit lower probably due to the fact that the study focused on women subjects only.

However, these study results are much lower than what was observed in a study carried out in Harare, Zimbabwe where the prevalence was found at 82.8% [11]. The high rate of infection suggests that there is increased un protective sexual behaviour in this setting hence suggesting further interventions from the health policy makers.

More so, on the antimicrobial sensitivity pattern, the study showed that there was very high resistance to Penicillin 80%, Erythromycin 68%, Ciprofloxacin 28%, and Ceftriazone 4% which was less resistant. This is similar to a study by Amato et al, 2012 who found out that Ciprofloxacin 23.3% and Erythromycin 17.2% were resistant.

The reasons for this outbreak of *Neisseria gonorrhoeae* strains with reduced sensitivity to penicillin, Erythromycin and Ciprofloxacin could be due to increasing self prescription of the antibiotics (drugs over the counter) as the empirical first-line treatment for *Neisseria gonorrhoeae* for the last several years, and also re-infection due to the presence of a large reservoir of asymptomatic carriers that unknowingly transmit the disease to their sexual contacts. *Neisseria gonorrhoeae* has a well-recognized potential to rapidly develop resistance to antibiotics. The organism's ability for genetic recombination and phenotypic diversity increases transmission and evasion of host immune systems which are necessary for its survival in humans [12]. It is known that some of the things that can be adopted in order to control this gonococcal disease include sexual behaviour change, diagnostic ability, sufficient surveillance to the condition, appropriate antibiotic treatment to mention but a few might lead to successful disease control and prevention.

5. CONCLUSION

A high proportion of *N. gonorrhoeae* isolated from genital specimens in out patients showed resistance to both the old and new generation antibiotics.

CONSENT

After getting signed informed consent /assent from the participants; we administered a questionnaire to them face to face collecting data on socio-demographic characteristics, and then proceeded to collect the sample (endocervix swab for females and urethral swab for males).

ETHICAL APPROVAL

The study was approved by Mbarara University of Science and Technology Institution Research Committee (MUST IRC), the participants were told their participation was on a voluntary basis and no incentives to participate were provided. The potential benefits for participation included complete clinical assessment and referral for treatment in case their tests were positive.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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