Antimicrobial Resistance of Neisseria Gonorrhoae isolated from adults aged 18 years and above presenting with urethral and vaginal discharges at Mbarara Regional Referral Hospital

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.

Objective: Antimicrobial Resistance Neisseria Gonorrhae isolated from adults aged 18 years and above presenting with urethral and vaginal discharges at Mbarara Regional Referral Hospital.

Design: The study was cross sectional and enrolled 189 participants presenting with urethral and vaginal discharges. All the samples were cultured on 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 gonorrheae* 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.

Key words: Neisseria Gonorrhoeae, Gonococcus, antimicrobial, resistance, prevalence, Mbarara

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 (WHO, 2012). Human beings are the only host for the causative agent *Neisseria gonorrhoeae* .It's incidence in both undeveloped and developing countries is high especially in vulnerable populations and marginalized ethnicities(CDC, 2012).Untreated and poorly managed gonorrhoea can result into epididymitis, pelvic inflammatory disease (PID) ,ectopic pregnancy, and infertility which are serious complications (Tapsall et al., 2009).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 becoming significantly a public health problem worldwide (Ndowa et al., 2013) and currently there are no vaccines available for *Neisseria gonorrhoea*, hence antimicrobial treatment is essential to control the disease(Jerse and Deal, 2013). However, resistance to sulphonamides, penicillins, tetracyclines, macrolides and fluroquinolones has emerged in most parts of the world(Unemo et al., 2014).

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 (Unemo and Nicholas, 2012). 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 (Unemo and Nicholas, 2012).

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 (Bignell and Unemo, 2013). Unfortunately, the susceptibility of gonococcal isolates to ceftriaxone has been decreasing globally and in many settings, resistance to Azithromycin is already prevailing (Unemo et al., 2014). This resistance constitutes an epidemiological situation that prevents healing and increases the likelihood of the occurrence of serious consequences associated with gonorrhoea (Ohnishi et al., 2011). 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 (WHO, 2012). 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 gonorrhoeae* in Uganda.

METHODS

Study design, setting and population

The study was conducted at Mbarara Regional Referral Hospital (MRRH) 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 hospital, a

teaching and research hospital for MUST. It serves as the regional Referral Hospital for south western Uganda.

The catchment area of the hospital includes the neighbouring countries of Rwanda, Northern Tanzania and Democratic Republic of Congo and it serves people coming from nearby districts that is Isingiro, Ntungamo, Kiruhura, Ibanda ,Kamwengye, Lyantonde, Bushenyi, Sheema, and Rubirizi.

Data collection

After getting signed informed consent 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). Urethral and endocervix swabs were collected from participants who were presenting with urethral and vaginal discharges.

Laboratory procedures

Two swabs were collected one for Gram stain and the other for culture and sensitivity from both male and female clients from endo cervical canal for females and urethra for males. The collected swabs were transported in a cool box in Stuart media.The swab for culture was inoculated immediately on appropriate media in Microbiology MUST laboratory.

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.

Culture

The pre warmed Chocolate agar medium was inoculated with urethral or endocervix swab containing the test organism and incubated at 37 ^{0}C in a moist atmosphere enriched with carbon dioxide 5%-10% using a candle jar for 24 hours.

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 gonorrhoeae* was positive was also performed. Carbohydrate fermentation reaction was also performed on the isolates for the identification of *Neisseria gonorrhoeae.*

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 sulfuric acid to from a fine precipitate of barium sulfate.

Prior to using the McFarland Standard it was shaken up well and aliquoted into test tubes identical to those used to prepare the inoculums suspension. The aliquoted tubes were tightly sealed to prevent evaporation from occurring. Before each use, it was shaken well to ensure that the barium sulfate was distributed evenly throughout the solution.

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 gonorrheae* 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 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 ATTC49226 obtained from Medical Research Centre Entebbe.

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 *Nesseria gonorrhoeae* was calculated as proportions of all the isolates detected from all samples examined.

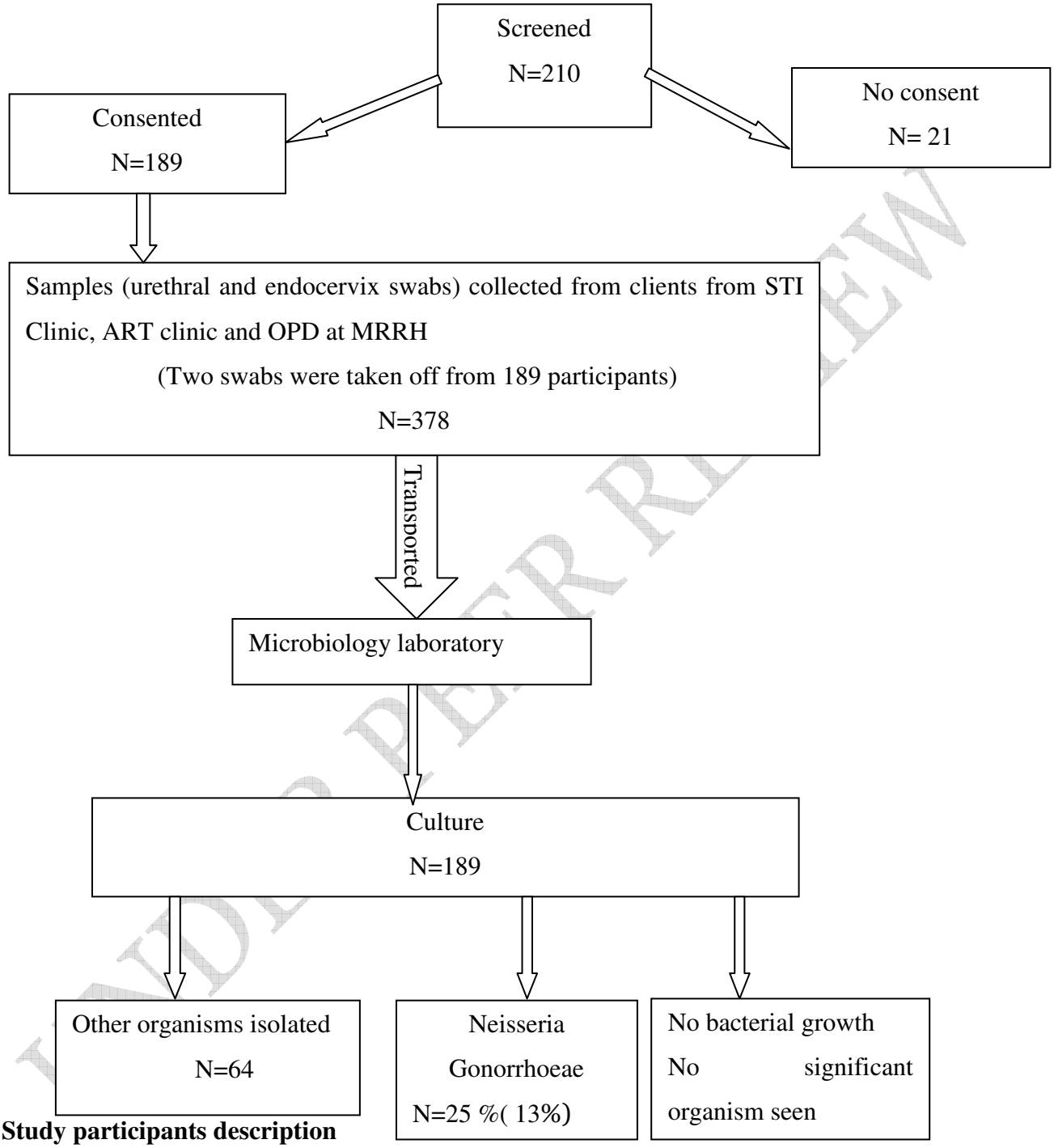
Ethical approval

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.

RESULTS

The total number of participants screened were 210, of which 189 were consented and 21 participants never consented. 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.

Fig 1: Study profile.



Study participants description

Table1: Description of study participants who met the inclusion criteria

Characteristic	Number /Percentage (N=189)
Mean age (S.D)	28.8 (26.5-29.7)
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	13(6.9)
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)

From table one above, 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.

A number of organisms were isolated with the most frequent being *Neisseria gonorrhoeae,* (39%) *and staphylococcus aureus(31%)* respectively followed by Candida spp,(13%), Klebseilla spp （11%) and Streptococcus spp(3%) and the least being E.coli (2%) and candida and Klebseilla (2%).

Prevalence of Neisseria Gonnorrhoea

There were 25(13.1%) clinical isolates of *N.gonorrhoeae* (prevalence of *N.Gonorrhoeae*) and were processed for susceptibility testing during the study period.

Characteristics of participants who had Neisseria Gonnorrhoea

Of 189 samples tested, 25(13%) 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%).

Antimicrobial susceptibility pattern of the N. gonorrhoeae isolates

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%).

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.

Results from this study show that majority of the samples were collected from females giving a female to male ratio of 2.4:1. This ratio is higher than what was observed in a study in adults in Mbarara where a respective ratio was found at 1.2:1 (Melvin et al., 2015).

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 slightly lower than what was observed in a study carried out in a similar setting in 2015 which found out a 32% prevalence rate of gonococcal infection (Melvin et al 2015). In a study that was done in Iran by Afrasiabi*et al.*, 2014, which looked at Neisseria gonorrheae antimicrobial susceptibility patterns in Kashan, Iran, the prevalence of Neisseria gonorrheae 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% (Tukuva et al, 2014). 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 Amito 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 gonorrheae 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 (Afrasiabis *et al.,* 2014). 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 the condition of appropriate antibiotic treatment to mention but a few mat lead to successful disease control and prevention.

CONCLUSION

A high proportion of *N. gonorrhoeae* isolated from genital specimens in adults 18 years and above showed resistance to both the old and new generation antibiotics.

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