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High Prevalence of Drug Resistant *Neisseria gonorrhoeae* in Males with Urethral Discharge at the STD Clinic Mulago Hospital Uganda

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ABSTRACT

Among the treatable sexually transmitted infections (STIs), *Neisseria gonorrhoeae* is considered to be most important because of its ever evolving antibiotic resistance. Currently third generation cephalosporins are recommended as first-line drugs in many countries. Unfortunately, resistance to these cephalosporins (cefixime and ceftriaxone) has been reported elsewhere. The objectives of the study were to determine the prevalence of *Neisseria gonorrhoea* in adult males presenting to Mulago hospital with urethral discharge and to determine the level of resistance of *Neisseria gonorrhea* to the commonly available antimicrobial agents at the clinic focusing on extended-spectrum cephalosporins. In a cross sectional study, we enrolled 112 adult males aged 18-65 yrs with urethral discharge at the STD clinic Mulago Hospital. Prevalence of *gonorrhoea* was determined based on Gram staining and/or *N. gonorrhea* isolation at culture. Antimicrobial susceptibility testing was determined with the Kirby-Bauer disk diffusion method and with the Nitrocefin tests for β -lactamase production. Among 112 adult male patients aged 18-65 years with urethral discharge, the prevalence of *N. gonorrhea* was at 81.3%. Resistance to drugs was identified at the following rates: tetracycline 100%, ciprofloxacin 94.8%, cotrimoxazole 91.4%, penicillin 82.8%, cefixime 20.7%, ceftriaxone 5.2%, spectinomycin 1.7%, and azithromycin 0%. According to this study drug resistance to commonly available antimicrobials for *N. gonorrhea* is very high among isolates from patients attending the STI clinic, Mulago Hospital Kampala, Uganda. Ceftriaxone, spectinomycin and azithromycin appeared to be the most useful drugs in the treatment of *N. gonorrhea* infection at the clinic. This work was completed in November 2017.

Keywords: Antimicrobial resistance, cefixime, ceftriaxone, cephalosporins, *Neisseria gonorrhea*.

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INTRODUCTION

Neisseria gonorrhoea is the bacterium ^[1] that causes gonorrhea a major reproductive health problem. Gonorrhoea follows Chlamydia as the most prevalent sexually transmitted bacterial diseases in humans. Globally the World Health Organization in 2011 estimated that about 88 million new cases of gonococcal infections occurred in 2005. Around 95% of infected males are symptomatic^[2]. An estimated 10%-20% of infected women develop the following complications ^[2] ; Pelvic inflammatory disease, ectopic pregnancy, infertility, and adverse pregnancy conditions. Males develop, testicular, prostate infections and infertility while young ones develop Neonatal ophthalmia and blindness. It is also a co-factor in increasing HIV[by 500 fold] ^[3] and HPV infections; this could be due to viral load increase in the semen or vaginal fluids of those co-infected with gonorrhea and HIV and HPV, and to an increased number of inflammatory cells for HIV and HPV ^[4-6].The gonococci produces only mildly symptomatic or asymptomatic disease in females while around 95% of infected males are symptomatic. Therefore, in the absence of a vaccine, appropriate diagnostics and antimicrobial treatments are the key elements for the reduction and control of gonorrhea and its associated complications. About 30-60 patients with STIs visit the STI clinic daily for treatment in Mulago hospital. A major factor to the continued spread of gonococcal infections is the ability of *N. gonorrhoea* to acquire resistance to antibiotics; over the last two decades , genes which encode for resistance in *N. gonorrhoea* strains to antimicrobial agents like; Spectinomycin, penicillin, tetracycline and, quinolones have been characterized in different countries^[7-9]

The mechanisms for chromosomally mediated resistance to penicillin G and cephalosporin in *N. gonorrhoea* involve the *penA*, *penB*, and *mtrR* mutations. Mutations in *penA* causes insertion of a single amino acid into penicillin-binding protein 2 [PBP 2], and this reduces the level of

binding of penicillin to PBP 2.^[10-11]. The *penB* mutation, which is a mutation that is linked to the porin gene, reduces porin permeability to hydrophilic antibiotics and plays an important role in the development of resistance to penicillin G, ^[12]. *Mtr* gene mutations increase the level of expression of the MtrCDE efflux pump and confers resistance to multiple hydrophobic agents and some hydrophilic antibiotics such as the penicillins ^[13]. The reduced susceptibility of *N. gonorrhoea* strains to broad spectrum cephalosporins such as cefixime and ceftriaxone has been proposed to be associated with polymorphisms in several of these genes and especially with certain *penA* mosaic alleles^[14].These mosaic sequences are thought to have evolved from recombination events involving *penA* gene sequences from several *Neisseria* species, including *N. perflava*, *N. sicca*, and *N. cinerea* ^[14-15].

Before 2010, treatment failures had only been reported in Japan ^[16] however, two cases of clinical failures with cefixime standard treatment were described in 2010 in Norway^[17], and three cases in 2011 in the United Kingdom^[18-19]. In July 2011 the first *Neisseria gonorrhoea* strain with resistance to cefixime was found in Austria^[20]. Furthermore, the first gonococcal strain with high-level clinical resistance to ceftriaxone [the last remaining option for empirical first-line treatment], was recently found in Japan^[21]. In Uganda studies done in Mulago hospital STI clinic indicate that gonorrhea cases are increasing which may be due to; prevalence of resistant strains in communities, and improper treatment guidelines.

There is limited data on the extended spectrum cephalosporin resistance among *Neisseria gonorrhoea* strains in Uganda. This resistance may be chromosomal or plasmid encoded. This study therefore, intended to determine the prevalence of cefixime and ceftriaxone resistant strains and to determine other conventional drug susceptibility patterns among *Nesseria gonorrhoea* strains isolated from adult

males presenting to Mulago hospital with urethral discharge.

Materials and Methods

A cross sectional study to identify *Neisseria gonorrhoea* from urethral discharge samples collected from men was done. Participants aged 18yrs to 65yrs were recruited from sexually transmitted infections [STI] clinic at Mulago Hospital [Uganda] .This hospital admits patients from all corners of the country and its clinic is a national referral centre for patients with STIs. The Clinical Microbiology Laboratory, Molecular Biology Laboratory [Makerere University] and MBN Laboratories did the laboratory work.

Identification of *N. gonorrhea*: 112 samples were collected, Gram stained, cultured on a selective modified Thayer martin agar under 5-7% CO₂ and the following phenotypic characteristics tests; colony morphology, catalase , superoxol , glucose utilization ,colistin and oxidase tests performed. After performing those phenotypic tests , Prevalence was determined using the following procedures ; Gram negative intracellular diplococci and culture negative , Gram negative intracellular diplococci and culture positive , No primary Gram reaction, culture positive with extracellular diplococci on a slide. And negative results were

determined by obtaining no morphotypes suggestive of *neisseria* on gram reaction and culture plate. All cultured samples, which were positive for *N. gonorrhoea*, were tested using the Vitek 2 system for definitive identification according to manufacturer's instructions.

This study used a control strain of *neisseria gonorrhoea* no. ATCC43069

For storage; about 3-5 colonies of each pure isolate was inoculated into a cryovial containing 0.5 - 1.0ml of 20% glycerol in trypticase soy broth and stored at -80 °C

Antimicrobial susceptibility testing; This was performed by the Kirby-Bauer disk diffusion method to obtain the zones of inhibition for the susceptibility of 11 antibiotics as summarized in table 1 below. Briefly, an overnight growth of each isolate was suspended in Muller-Hinton Broth and the suspension adjusted to 0.5 Mc Farland standard .This was used to inoculate plates of GC agar base with Growth factor mixture GFM 80005. Antibiotic disks were then placed onto the inoculated plates which were then incubated at 37°C for 24 hours. Zone diameters were then measured and interpretation made using CLSI break points as seen in the table below.

Table 1 ; Drug susceptibility break points as recommended by CLSI ^[22]

DRUGS	DISC POTENCY	SUSCEPTIBLE	INTERMEDIATE	RESISTANCE
Ceftriaxone	30 µg	39-51mm	----	≤ 39mm
Cefixime	5 µg	≥ 37mm		≤37
Penicillin	10 units	≥47 mm	27 – 46 mm	≤ 26mm
Ciprofloxacin	5 µg	≥41 mm	28 – 40 mm	≤27mm
Tetracycline	30 µg	≥38 mm	31-37 mm	≤ 30 mm
Azithromycin	15µg	≥ 42 mm	-----	≤19mm
Spectinomycin	100µg	≥18 mm	15 – 17 mm	≤ 14 mm
Doxycycline	30µg	ND	ND	N D
Erythromycin	15µg	ND	ND	ND
Septrin	1.25/23.5µg	≥ 16mm	11-15mm	≤ 10mm
Gentamycin	120µg	ND	ND	ND

ND- These ranges have not been determined yet by CLSI

Beta-lactamase production; Nitrocefin strips were used to detect beta-lactamase production and double disc diffusion tests were also performed to detect extended beta –lactamase producing strains using Cefixime and Augmentin discs plated 30 mm apart from the center of each other on GC media .

RESULTS

Prevalence of gonorrhea determined by gram staining or culture .

Of the 112 participants 91 had gram stains and positive cultures suggestive of *Neisseria* giving a prevalence of 81.3%.

Urethral discharge for the ages of 18- 30 years , 31-43yrs and 44-65yrs were 68.7%, 27,6%and 3.5% respectively, a total of 65 [58%] were single and 47 [42%] were married . A total of 47 [42%] admitted to having used an antibiotic while the rest 65[58%] denied history of antibiotic use to treat their urethral condition.

Drug susceptibility profiles

Table 2 below summarizes the drug susceptibility profiles of 11 antibiotics.

Resistances for; ceftriaxone, cefixime, azithromycin ,tetracycline ,ciprofloxacin, septrin , spectinomycin and penicillin was identified at the following rates 5.2% , 20.7% , 0% , 100%, 94.8% , 91.4% , 1.7% and 82.8% respectively.

Gentamycin, doxycycline and erythromycin are commonly used in Uganda however, their standard zones of susceptibility among *N. gonorrhea* strains have not yet been determined by CLSI today and we were not able to put them in any of our susceptibility categories.

However, gentamycin had 36 zones of inhibition ranging from 16mm-30mm, 22 zones of inhibition ranging from 30-60 mm ,

Doxycycline had all its zones below 20mm. and erythromycin had 10 of its zones of inhibition below 22mm

TABLE 2: % DRUG SUSCEPTIBILITY PROFILES FOR FIFTY EIGHT ISOLATES [positive at culture]

DRUGS	%SUSCEPTIBLE	%INTERMEDIATE	RESISTANCE	%RESISTANCE
Ceftriaxone	94.8	----	3	5.2
Cefixime	79.3		12	20.7
Penicillin	12.1	5.1	48	82.8
Ciprofloxacin	0	5.2	55	94.8
Tetracycline		0	58	100
Azithromycin	10 0	-----	0	0
spectinomycin	98.3	0	1	1.7
Doxycycline	ND	ND	N D	
Erythromycin	ND	ND	ND	
Seprtin	3.4	5.2	53	91.4
Gentamycin	ND	ND	ND	

ND- These ranges have not yet been determined by CLSI.

Discussions

The recent emergence and transmission of *Neisseria gonorrhoeae* strains with reduced susceptibility to expanded-spectrum cephalosporins such as cefixime and ceftriaxone

have been reported. Effective control of the disease spread requires ; improved knowledge about the prevalence of the disease , timely and appropriate diagnosis and treatment coupled with increased disease awareness promotion.

The aim of this study was to determine the prevalence of the gonococcus in adult males with urethral discharge and to determine the drug susceptibility profiles of *N. gonorrhoeae* to cefixime and ceftriaxone drugs.

Prevalence of *Neisseria*

112 participants were enrolled for the study. The prevalence of the gonococcus disease in adult males presenting with urethral discharge was found at 81.3%, this is slightly lower than a 95% prevalence found in the UK, by a study done on diagnosis rates for gonorrhoea in 2008 [2]. Another study done in Bangui on aetiology of urethral discharge [23] found a 65% prevalence of the gonococcus in urethral discharges. While a study done on antimicrobial susceptibility survey of *N. gonorrhoea* in a Uganda urban clinic by Kanya et al [1989-1993] reported a prevalence of 73% and one done recently in Uganda on prevalence, socio-demographic factors and antimicrobial susceptibility of *Neisseria gonorrhoea* found a prevalence of gonorrhoea at 55% [24].

These differences could be attributed to the different methods used to collect and to transport urethral samples coupled with the different identification techniques used.

In this study the prevalence is higher than the ever stated one in Uganda, this is true because we recruited male participants presenting with urethral discharge. There usually 95% chances of detecting *Neisseria gonorrhoea* in males with urethral discharge [2]. This prevalence may also be due to the development of resistance to several antimicrobial drugs such as sulfonamides, penicillin, tetracyclines and quinolones by *N. gonorrhoea* and lack of antimicrobial resistance [AMR] surveillance which could help to establish and maintain the efficacy of standard treatment regimens.

Nitrocefin tests were positive reflecting penicillinase production by these strains.

Findings from Urethral Discharge ; Urethral discharge was more among the ages of 18-30 years than among the 31-43yrs and 44-65yrs

which had 68.7%, 27.6% and 3.5% respectively, a total of 65 [58%] were single and 47 [42%] were married, this shows that urethral discharge was high among the unmarried participants. A total of 47 [42%] admitted to having used an antibiotic while the rest 65[58%] denied history of antibiotic use to treat their urethral condition

Antimicrobial susceptibility profiles

Ceftriaxone, cefixime, gentamycin, ciprofloxacin. Spectinomycin, erythromycin. Tetracycline. Doxycycline. penicillin, azithromycin and septrin were antibiotics used to determine the susceptibility of fifty eight culture positive isolates. Ceftriaxone resistance was found at 5.2%, Cefixime at 20.7%, Penicillin at 82.8% while Ciprofloxacin and Tetracycline had the worst resistance rates of 94.8% and 100% respectively. All the gonococcus strains were susceptible to azithromycin and spectinomycin at rates of 100% and 98.3% respectively.

This is the first study to report ceftriaxone and cefixime resistance in Uganda. Before 2010, treatment failures had only been reported in Japan [16], however, two cases of clinical failures with cefixime standard treatment were described in 2010 in Norway [17], and three cases in 2011 in the United Kingdom [18-19]. In July 2011 the first *Neisseria gonorrhoea* strain with resistance to cefixime was found in Austria [20]. Furthermore, the first gonococcal strain with high-level clinical resistance to ceftriaxone [the last remaining option for empirical first-line treatment], was recently found in Japan [21]. This study also revealed that doxycycline a drug which is also used here in Uganda with cefixime to treat Gonorrhoea had very small zones of inhibition below 20 mm and these ranges were similar to those of tetracycline a sister drug which also showed 100% resistance. Since they are both tetracyclines, the organisms may be resistant to both drugs.

Erythromycin which is also used in pregnancy to treat the gonococcus showed very small zones of inhibition [below 22mm] which don't compare with those of Azithromycin [42mm] which showed 100% susceptibility which is a

sister drug and therefore may also be regarded as a drug of resistance.

Conclusions

Gonorrhoea remains a leading cause of urethritis among males accounting for 81.3% of cases of urethral discharge at Mulago . Resistance to 3rd generation cephalosporins exists among Uganda *N. gonorrhoeae* strains at a rate of 20.3% for Cefixime and 5.2% for ceftriaxone among study strains . cefixime and ceftriaxone drugs are now reported as drugs of resistance in Uganda as far as Gonorrhoea treatment is concerned, however, the results cannot be generalized because the study was done in a referral hospital where a small population of patients attend for treatment. The disease was more among adult males below the age of 30years which are the more active years for the youth. Prior use of drugs affected the prevalence of the disease in that many who had the discharge failed to give positive results and the disease was more found in singles other than the married ones.

Recommendations:

Current use of ceftriaxone as a first line therapy for urethritis should continue but treatment should be prolonged, Cefixime use should be withdrawn and a final decision on its future use be made after a broader study while azithromycin and spectinomycin which showed susceptible rates at 100% and 98.3% respectively, should also be included on the treatment guidelines in Uganda. And in order to prevent future resistances, combination therapies with azithromycin and ceftriaxone should be recommended.

Limitations

The small sample size used which was due to a low number of expected cases with urethritis at the STI clinic may have affected the overall prevalence.

Participants who didn't have a clear cut discharge didn't want to give a sample because of the swabbing procedure which is slightly

painful and so loss of these participants may have affected the study.

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