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Author manuscript

Sex Transm Infect. Author manuscript; available in PMC 2024 July 04.

Published in final edited form as:

Sex Transm Infect. 2021 June ; 97(4): 312–316. doi:10.1136/sextrans-2020-054581.

Implementation of a standardised and quality-assured enhanced gonococcal antimicrobial surveillance programme in accordance with WHO protocols in Kampala, Uganda

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Abstract

Objectives—The emergence of multidrug-resistant *Neisseria gonorrhoeae* (NG) is a major global health threat necessitating response and control measures. NG antimicrobial resistance (AMR) surveillance data from sub-Saharan countries is exceedingly limited. This paper aims to describe the establishment, design and implementation of a standardised and quality-assured gonococcal surveillance programme and to describe the susceptibility patterns of the cultured gonococcal isolates in Kampala, Uganda.

Methods—From March 2018 to September 2019, using the WHO Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) protocol, consecutive males with urethral discharge syndrome were recruited from 10 surveillance sites in Kampala City, Uganda, in

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Contributors FK led the coordination of the surveillance programme and wrote the manuscript with support by BSB, MMH, ML, RK and MU. MW, YCM, ML and RW provided the strategic direction; PM and PK spearheaded the collaborative efforts from Ministry of Health; EM, CL and JMN supported the laboratory data management. All authors reviewed the final manuscript.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The IRB of School of Biomedical Sciences Makerere University College of Health Sciences approved the surveillance protocol and issued a waiver of consent to use the stored surveillance data and isolates (Approval Number: SBS 694).

Provenance and peer review Not commissioned; externally peer reviewed.

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collaboration with the Ministry of Health. Males completed a questionnaire and provided a urethral swab specimen. Culture, identification and antimicrobial susceptibility testing (Etest) were performed.

Results—Of the 1013 males recruited, 73.1% (740/1013) had a positive Gram stain and 51.1% (n=518) were culture-positive for NG. Using Etest (458 isolates), the resistance to ciprofloxacin was 99.6%. Most isolates were susceptible to azithromycin, cefoxitin and gentamicin, that is, 99.8%, 98.5% and 92.4%, respectively, and all isolates were susceptible to ceftriaxone and cefixime.

Conclusions—We established a standardised, quality-assured WHO EGASP. Using Etest, 458 isolates were characterised, with associated epidemiological surveillance data, in 1.5 years, which by far exceed the minimum 100 isolates per year and country requested in the WHO Global GASP, to detect AMR levels with confidence. These isolates with the epidemiological data can be used to develop population level interventions.

INTRODUCTION

Gonorrhoea is a non-ulcerative bacterial STI caused by *Neisseria gonorrhoeae* (NG). Untreated or inappropriately treated gonorrhoea can lead to serious complications and sequelae particularly in women including infertility, chronic pelvic pain, first-trimester abortion and ectopic pregnancy. Furthermore, gonorrhoea increases the risk of HIV transmission.[1–3] In 2016, the WHO estimated 87 million new NG infections among adults globally, with 21.8 million cases occurring in the WHO African Region (an increase of 91% since 2012) and the highest incidence worldwide, with 41 cases per 1000 women and 50 per 1000 men.[4 5] However, estimation of the true burden of gonorrhoea in many sub-Saharan African (SSA) countries, including Uganda, remains extremely challenging due to the use of syndromic patient management algorithms and lack of surveillance and diagnostic capacity.[6–8]

Increasing international reports of NG strains with high levels of antimicrobial resistance (AMR) and multidrug resistance complicate efforts towards effective management and control of gonorrhoea.[4 6 7 9] In the WHO Global Gonococcal Antimicrobial Surveillance Programme, among the countries reporting on susceptibility to ceftriaxone (n=63), azithromycin (n=62) and ciprofloxacin (n=67) in 2015–2016, 23.8%, 80.6% and 100%, respectively, reported isolates showing decreased susceptibility or resistance.[9] Worryingly, only two (4.3%) of the 47 WHO African countries reported data on all these key antimicrobials in the WHO Global GASP in 2015–2016.[9] Increasing levels of decreased susceptibility or resistance to extended-spectrum cephalosporins (ESCs) such as ceftriaxone and cefixime have been reported in most regions globally, including in SSA countries.[7 9–12] In most SSA countries, the high burden of STIs, suboptimal antimicrobial stewardship (AMS) and absence of NG and AMR surveillance programmes contribute to an underestimation of the burden of gonorrhoea and NG AMR.[6 7 9 13] The implementation of the WHO's Enhanced Gonococcal Surveillance Programme (EGASP) protocol,[13] which includes standardised collection of quality-assured AMR and epidemiological data of corresponding participants, remains low internationally.[7 9]

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Calls for concerted efforts to combat AMR are the subject of World Health Assembly resolutions and WHO global action plans.[12 14 15] Objective 2 of the 2015 WHO Global Action Plan to combat AMR aims to strengthen knowledge and evidence bases through surveillance and research.[15] Increased surveillance is also a key strategy in the WHO Global Health Sector Strategy on STI, 2016–2021, and the WHO Global Action Plan to control the spread and impact of NG-AMR.[12 14]

The present paper aims to describe the establishment and design of a standardised and quality-assured EGASP in Kampala, Uganda, which adheres to WHO EGASP protocols,[13] and report the main AMR results from 2018 to 2019.

MATERIAL AND METHODS

Establishment of the AMR surveillance programme

In 2016, an agreement was signed between the National STI Control Program-Ministry of Health (MoH) and the Infectious Diseases Institute (IDI), Makerere University College of Health Sciences, Kampala, Uganda, to commence the implementation of NG AMR surveillance. The selection of surveillance sites was followed by voluntary acceptance of the sites to participate in the surveillance programme and training of health workers at each site. Surveillance teams were then constituted and provided with supplies required to coordinate the surveillance.

Participant identification, sample and data collection

Urethral swab samples were collected, using Amies transport media without charcoal, from consecutive males presenting with urethritis. Samples were transported within a maximum of 8 hours to the laboratory, where isolation, species confirmation and AMR testing of all NG isolates were performed. Participants' demographic, behavioural and clinical information were collected using case report forms.

Laboratory procedures including antimicrobial susceptibility testing

All urethral samples were inoculated on selective modified Thayer-Martin (MTM) and non-selective chocolate agar plates and subsequently incubated at 35°C–37°C in a 5% CO_2-enriched humid atmosphere. Presumptive identification of suspected gonococcal colonies was based on growth of colonies with typical morphology on MTM media, positive oxidase and superoxol tests and observation of Gram-negative oxidase-positive diplococci in stained smears. Determination of the minimum inhibitory concentration (MIC; mg/L) of ceftriaxone, cefixime, cefoxitin, azithromycin, ciprofloxacin and gentamicin was performed using Etest (bioMérieux, Marcy-l'Etoile, France), in accordance with the manufacturer's instructions. The MIC values for all antimicrobials except gentamicin were interpreted using clinical breakpoints stated by the Clinical and Laboratory Standards Institute (www.clsi.org). For gentamicin, previously published resistance breakpoints were applied.[16]

For quality control (QC), *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as Gram-negative and Gram-positive controls, respectively, every week and

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when using new Gram stain kit. NG ATCC 19424 was used for QC in the AMR testing, at a minimum every month and on all new batches of Etest strips or agar media plates.

RESULTS

Demographic, behavioural and clinical characteristics

The frequency of urethritis and NG culture positive cases at the 10 surveillance sites in Kampala, with linked epidemiological data, is summarised in table 1.

A total of 1013 urethral swabs (one swab per male) together with linked epidemiological data were collected from consecutive males presenting with urethritis at 10 surveillance sites in Kampala, Uganda, from March 2018 to September 2019. The highest number of participants presenting with urethritis in this period were attending the Naguru Teenage Health and Information Centre (n=217, 21.4%), followed by the Kisenyi Health Centre IV (n=192, 19.0%) and the Kiruddu General Hospital (n=135, 13.3%). The lowest number was seen at the IDI Clinic (n=2, 0.2%), which was mostly included to recruit males with HIV, followed by the Luzira Upper Prison Health Centre 3 (n=48, 4.7%). The mean age, median age and age range of the 1013 males was 27.2, 25 and from 2 to 79 years, respectively (table 1). Accordingly, also children presenting with a urethral discharge were diagnosed, treated and reported, as well as included in the surveillance programme.

In total, urethral samples from 73.1% (740/1013) of the males demonstrated Gram-negative intracellular diplococci in microscopic smears and 51.1% (517/1013) of the males were NG culture positive. The majority of NG culture positive males were belonging to the Baganda (40.4%) and Bayankole (10.1%) ethnic groups. In regards to the place of residence, the highest numbers of NG culture-positive males were from the following Divisions of Kampala: Nakawa (n=130, 33.2%), Rubaga (n=90, 23.0%), Makindye (n=69, 17.6%) and Kawempe (n=52, 13.3%). The mean (median) age of the NG culture-positive males (n=517) was 26.2 (24) years with a range from 2 to 72 years. The vast majority of the NG culture-positive males (n=517) self-reported inconsistent use or never using condoms (97.5%), with half (47.5%) having >1 current sexual partner. The self-reported prevalence of HIV among the NG culture-positive males was 5%, and 25% of the males reported a previous episode of urethritis in the past 6 months. Nearly one quarter (22.2%) of the NG culture-positive males reported antibiotic use in the past 2 weeks.

Antimicrobial susceptibility and resistance

Using Etests for 458 gonococcal isolates, 99.6% of isolates were resistant to ciprofloxacin. However, the vast majority of isolates were susceptible to azithromycin, cefoxitin and gentamicin, that is, 99.8%, 98.5% and 92.4%, respectively, and all (100%) isolates were susceptible to ceftriaxone and cefixime (table 2).

DISCUSSION

AMR in NG is a global public health concern and enhanced, standardised and quality-assured national and international NG AMR surveillance is crucial for control and treatment efforts. However, despite having the highest gonorrhoea incidence globally,[4–7 17] there

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is limited recent NG AMR surveillance data in the WHO African region.[6–9 16 18] Consequently, in Africa, AMR NG strains may emerge and/or rapidly spread unnoticed. The present paper describes the steps involved and logistics required in the establishment and implementation of a standardised and quality-assured WHO EGASP[13] in Kampala, Uganda, that could be easily instituted in other similar resource-limited countries in SSA. The examined population was diverse and included high-risk and vulnerable populations such as HIV-positive and HIV-negative males who have sex with men, commercial sex workers, people using narcotic drugs, inmates and teenagers.

The establishment of Kampala EGASP demonstrated the feasibility of implementing the WHO EGASP protocol in a resource-limited setting,[13] through collaboration and partnerships between government, WHO and academic partners. National NG AMR surveillance programmes are critical in SSA countries for collecting local data on AMR in order to inform treatment guidelines and contribute to international efforts to combat AMR. As well as local use, Kampala EGASP can add to the international gonorrhoea and NG AMR data, that is, in the WHO African Region but also globally. In Kampala EGASP, the surveillance is systematic and based on WHO standardised laboratory methodologies and surveillance protocols,[12 13] within the largest city, which is a strategic sentinel site for emerging NG AMR given national and international travelling and the frequency and poorly regulated access to empiric antimicrobial treatment. The Kampala EGASP is also informing the national STI management guidelines in Uganda and strengthened AMS.

The Kampala EGASP used Etest for 458 NG isolates and reported associated epidemiological data over 1.5 years. This exceeds the minimum 100 NG isolates per year and country requested in the WHO Global GASP to detect AMR levels with confidence.[7 9 12 14] In contrast, only approximately 50% of the WHO Global GASP countries in 2009–2016 collected the requested number of NG isolates per year.[7 9] Furthermore, the Kampala EGASP being led by the National STI Control Programme at MoH presents an opportunity of a long-term sustainability since the surveillance activities have been integrated into the daily routine patient care at the health facilities. Obtaining epidemiological data provides further opportunities for developing population level interventions including targeting key populations in order to combat the spread of AMR NG strains in Uganda and internationally. Inability to collect epidemiological data has been a major limitation for many of the GASPs worldwide.[7 9 13] The use of the WHONET system for data collection, collation and analysis has also bridged gaps in data management while achieving WHO targets for AMR surveillance.[19]

An extremely high level of gonococcal resistance to ciprofloxacin (99.6%) was identified, which is in line with a previous study conducted in Uganda.[20] Despite the recommended treatment of cefixime 400 mg single oral dose plus doxycycline 100 mg twice daily for 7 days as first-line treatment for urethritis since 2012,[21] complete ciprofloxacin resistance is maintained in NG strains. This suggests that in NG ciprofloxacin resistance does not exert a fitness cost and/or that there is ongoing selection pressure from fluoroquinolone use in the community. However, only 0.7% (n=7) of the 1013 males presenting with urethritis in the present surveillance received the recommended treatment of cefixime and doxycycline. It is imperative to substantially increase the adherence to the recommended treatment in

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the national clinical guidelines. In the present surveillance, all isolates were susceptible to cefixime and ceftriaxone, which is consistent with studies from other African countries such as Kenya,[22] Cote d'Ivoire,[23] Zimbabwe[17] and South Africa in 2016.[9] Nevertheless, decreased susceptibility or resistance to ceftriaxone and cefixime has earlier been identified in, for example, South Africa.[7 11] The susceptibility to azithromycin was also high; however, 0.2% of isolates were non-susceptible to this antimicrobial in this surveillance, and this may represent emerging resistance. Azithromycin non-susceptible/resistant NG strains have earlier been reported in Uganda,[7] as well as in other African countries such as Cote d'Ivoire,[23] Malawi, South Africa and Kenya.[7 9] Kampala EGASP is a vital tool to track changing patterns in MICs of azithromycin and ESCs in particular.

The main limitations of the present surveillance included that only male urethral samples were collected and no samples from other anatomical sites and/or from women, relatively low culture positivity rate of Gram-stained positive samples due to for example long transport times and suboptimal sampling swabs in some time periods, lack of recommended WHO NG reference strains for quality assurance and control (will be included in future surveillance)[7 9 24] and lack of follow-up of participants to determine treatment outcomes.

An EGASP to monitor existing and emerging AMR strains of NG, a WHO priority pathogen for AMR surveillance,[25] has been established in Kampala, Uganda, and contributes to the WHO global action plan to control NG AMR.[7 9 12 13] Through public–private partnerships, we show the feasibility of setting up a structured, standardised and quality-assured EGASP based on a WHO protocols in a resource-limited setting. Continued and strengthened NG AMR surveillance, including test of cure, in Kampala and nationally in Uganda is critical. Whole-genome sequencing of selected NG isolates will be considered to provide further understanding regarding NG AMR determinants and the transmission of NG and NG resistance in different populations and risk groups in Uganda.

Acknowledgements

The authors are grateful to the National STI Control Program, surveillance sites, John Papp, Emily Weston, Mary Kamb (US Centers for Disease Control and Prevention (CDC)) and Teodora Wi (WHO).

Funding

The funding was from Global Health Security Partner Engagement Project 1U2GGH001744-01 through US CDC.

Data availability statement

Data are available on reasonable request.

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Key messages

- Continuous and quality-assured *Neisseria gonorrhoeae* antimicrobial resistance (AMR) surveillance is imperative, with emphasis on extended-spectrum cephalosporins and azithromycin.
- Uganda is one of exceedingly few countries in Africa that has implemented standardised and quality-assured AMR surveillance.
- The current recommended first-line treatment of cefixime plus doxycycline for bacterial urethritis remains appropriate in Uganda.
- Lessons from the establishment and coordination of the Kampala Enhanced Gonococcal Antimicrobial Surveillance Programme could be adopted by other low-income and middle-income countries.

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Table 1

Frequency of urethritis and *Neisseria gonorrhoeae* culture positive cases at the 10 surveillance sites in Kampala with linked epidemiological data

Variable	Males with urethritis (n=1013)	N. gonorrhoeae culture- negative males (n=496)	N. gonorrhoeae culture- positive males (n=517)	Test positivity rate	P value*
Age: mean (SD)	27.2 (8.8)	28.5 (10.0)	26.2 (7.4)		
Age, n (%)					0.000
≤5	1 (0.1)	1 (0.2)	0 (0.0)	0.0	
5–12	0 (0.0)	0 (0.0)	0 (0.0)	0.0	
13–19	124 (12.2)	61 (12.3)	63 (12.2)	6.2	
20–35	707 (69.8)	322 (64.9)	385 (75.2)	38.0	
36–60	146 (14.4)	93 (18.8)	53 (10.3)	5.2	
>60	9 (0.9)	7 (1.4)	2 (0.4)	0.2	
Missing	26 (2.6)	12 (2.4)	14 (2.7)	1.4	
Ethnic group, n (%)					0.004
Baganda	374 (36.9)	165 (33.3)	209 (40.4)	20.6	
Banyankole	126 (12.4)	74 (14.9)	52 (10.1)	5.1	
Basoga	67 (6.6)	38 (7.7)	29 (5.6)	2.9	
Bagishu	40 (3.9)	24 (4.8)	16 (3.1)	1.6	
Batooro	36 (3.6)	13 (2.6)	23 (4.4)	2.3	
others	249 (24.6)	121 (24.4)	128 (24.8)	12.6	
Residence by Kampala subcounty, n (%)	n=758				0.204
Kawempe	104 (13.7)	52 (14.2)	52 (13.3)	5.1	
Makindye	139 (18.3)	70 (19.1)	69 (17.6)	6.8	
Nakawa	279 (36.8)	149 (40.6)	130 (33.2)	12.8	
Rubaga	150 (19.8)	60 (16.3)	90 (23.0)	8.9	
Central	48 (6.3)	22 (6.0)	26 (6.6)	2.6	
Missing	38 (5.0)	14 (3.8)	24 (6.1)	2.4	
Surveillance site, n (%)					0
Infectious Diseases Institute Clinic	2 (0.2)	1 (0.2)	1 (0.2)	0.1	
Kampala Remand Prison Health Centre 3	67 (6.6)	33 (6.7	34 (6.6)	3.4	
Kawaala Health Centre 3	119 (11.7)	51 (10.3)	68 (13.2)	6.7	

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Variable	Males with urethritis (n=1013)	N. gonorrhoeae culture- negative males (n=496)	N. gonorrhoeae culture- positive males (n=517)	Test positivity rate	P value*
Kiruddu General Hospital	135 (13.3)	68 (13.7)	67 (13)	6.6	
Kisenyi Health Centre 4	192 (19.0)	70 (14.1)	122 (23.6)	12.0	
Luzira Prisons Health Centre 4	57 (5.6)	37 (7.5)	20 (3.9)	2.0	
Luzira Upper Prison Health Centre 3	48 (4.7)	42 (8.5)	6 (1.2)	0.6	
Most at Risk Populations Initiative Clinic	106 (10.5)	43 (8.7)	63 (12.2)	6.2	
Murchison Bay General Hospital	70 (6.9)	49 (9.9)	21 (4.1)	2.1	
Naguru Teenage Health and Information Centre	217 (21.4)	102 (20.6)	115 (22.2)	11.4	
HIV status, n (%)					0.129
Negative	746 (73.6)	373 (75.2)	373 (72.1)	36.8	
Positive	63 (6.2)	37 (7.5)	26 (5.0)	2.6	
Unknown	116 (11.5)	50 (10.1)	66 (12.8)	6.5	
Missing	88 (8.7)	36 (7.3)	52 (10.1)	5.1	
No. of sexual partners in the past 6 months, n (%)					0
0	52 (5.1)	44 (8.9)	8 (1.5)	0.8	
1	388 (38.3)	175 (35.3	213 (41.2)	21.0	
2–4	438 (43.2)	204 (41.1)	234 (45.3)	23.1	
>5	43 (4.2)	18 (3.6)	25 (4.8)	2.5	
Missing	92 (9.1)	55 (11.1)	37 (7.2)	3.7	
Condom use in the past 6 months, n (%)					0.11
Always	14 (1.4)	9 (1.8)	5 (1.0)	0.5	
Sometimes	533 (52.6)	244 (49.2)	289 (55.9)	28.5	
Never	442 (43.6)	227 (45.8)	215 (41.6)	21.2	
Missing	24 (2.4)	16 (3.2)	8 (1.5)	0.8	
No. of urethritis episodes in the past 6 months, n (%)					0.665
0	93 (9.2)	44 (8.9)	49 (9.5)	4.8	
1	170 (16.8)	90 (18.1)	80 (15.5)	7.9	
2–4	107 (10.6)	60 (12.1)	47 (9.1)	4.6	
>5	4 (0.4)	2 (0.4)	2 (0.4)	0.2	
Missing	639 (63.1)	300 (60.5)	339 (65.6)	33.5	
Previous antibiotic use in past 14 days, n (%)					0.011

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Variable	Males with urethritis (n=1013)	N. gonorrhoeae culture- negative males (n=496)	N. gonorrhoeae culture- positive males (n=517)	Test positivity rate	P value*
Yes	260 (25.7)	145 (29.2)	115 (22.2)	11.4	
No	753 (74.3)	351 (70.8)	402 (77.8)	39.7	

*χ^2.

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Table 2

Antimicrobial susceptibility testing using Etests of *Neisseria gonorrhoeae* isolates collected from March 2018 to September 2019 in Kampala, Uganda

Antimicrobial	Breakpoints	No. of isolates	S (%)	I (%)	R (%)	NS (%)	NS 95% CI (%)	Geometric mean MIC (mg/L)	MIC range (mg/L)
Ceftriaxone	S≤0.25	457	100	NA	NA	0	0 to 0.8	<0.016	<0.016–0.016
Cefixime	S≤0.25	458	100	NA	NA	0	0 to 0.8	<0.016	<0.016–0.023
Cefoxitin	S≤2, R≥8	458	98.5	1.3	0.2	NA	0.7 to 3.1	1.06	0.25–32
Gentamicin	S≤4, R≥32	458	92.4	7.6	0	NA	5.0 to 9.7	2.463	1–24
Ciprofloxacin	S≤0.064, R≥1	458	0	0.4	99.6	NA	99.2 to 100	2.057	0.5–6
Azithromycin	S≤1	456	99.8	NA	NA	0.2	0.04 to 1.2	0.146	0.023–1.5

I, intermediate; MIC, minimum inhibitory concentration; NA, not applicable; NS, non- susceptible; R, resistance; S, susceptible.

Sex Transm Infect. Author manuscript; available in PMC 2024 July 04.