

Behavioural characteristics, prevalence of *Chlamydia trachomatis* and antibiotic susceptibility of *Neisseria gonorrhoeae* in men with urethral discharge in Thyolo, Malawi.

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Summary

A study was carried out in a rural district of Malawi among men presenting with urethral discharge, in order to a) describe their health seeking and sexual behaviour b) determine the prevalence of *Neisseria gonorrhoeae* (*N.gonorrhoeae*) and *Chlamydia trachomatis* (*C.trachomatis*), and c) verify the antibiotic susceptibility of *N.gonorrhoeae*. A total of 114 patients were entered into the study. 61% of study subjects reported having taken some form of medication before coming to the Sexually Transmitted Infections (STI) clinic. The most frequent alternative source of care was the traditional healer. 68 (60%) patients reported sex during the symptomatic period the majority (84%) not using condoms. Using ligase chain reaction on urine, *N.gonorrhoeae* was detected in 91 (80%) and *C.trachomatis* in 2 (2%) of urine specimens. 45 of 47 *N.gonorrhoeae* isolates produced penicillinase, 89% showing multi-anti-microbial resistance. This study emphasises the need to integrate alternative care providers and particularly traditional healers in STI control activities and to encourage their role in promoting safer sexual behaviour. In patients presenting with urethral discharge in our rural setting, *C.trachomatis* was not found to be a major pathogen. Antimicrobial susceptibility surveillance of *N.gonorrhoeae* is essential in order to prevent treatment failures and control the spread of resistant strains.

Key Words: Malawi, urethral discharge, *Neisseria gonorrhoeae*, susceptibility, behaviour

Introduction

Sexually transmitted infections (STIs) are known to facilitate the sexual transmission of HIV¹, and effective STI case management is known to reduce the incidence of HIV². The National HIV prevalence in Malawi is estimated at 15%³, and HIV infection rates among patients with STI's range from 53-83%⁴. As part of its STI and HIV control strategy, Malawi adopted the World Health Organization recommended syndromic approach to STI case management in 1993. Based on clinical efficacy, in-vitro studies and cost considerations, a combination of gentamicin (240-mg single intramuscular dose) and doxycycline (100 mg twice daily for 7 days) was recommended as the treatment of choice for adult men presenting with urethral discharge⁵. Although this regimen is meant to cover for *Neisseria gonorrhoeae* (*N.gonorrhoeae*) and *Chlamydia trachomatis* (*C.trachomatis*) infections⁶, the relative prevalence of these pathogens in rural communities in Malawi is not known.

Regular monitoring of antimicrobial susceptibility of *Neisseria gonorrhoeae* and other STI pathogens is essential as resistance patterns could change rapidly⁷. Due to scarce resources, this is not done in Malawi and there is limited information on the subject.

Control of STIs involves not only providing effective treatment to those that are infected, but should also involve promotion of safer sexual practices during the symptomatic period. The present study was undertaken in a rural district of Malawi among adult men presenting with urethral discharge, in order to a) describe their health seeking and sexual behavior b) determine the relative contribution of *N.gonorrhoeae* and *C. trachomatis* to urethral discharge, and c) verify the antibiotic susceptibility of *N.gonorrhoeae*.

Material and methods

Study population, and data collection.

This study was conducted in Thyolo district, a rural region in southern Malawi. Between October 2000 and May 2001, all adult males presenting with urethral discharge to the district STI clinic were requested to participate in this study. After obtaining informed consent, a semi-structured questionnaire, was used to gather basic socio-demographic data, and information on health seeking and sexual behavior. All patients were managed according to National STI guidelines⁸.

Prevalence of *N.gonorrhoeae* and *C.trachomatis*

Patients were requested to provide a urine specimen at the time of first attendance. Nucleic acid amplification using Ligase Chain Reaction (LCR), was used for determining the presence of *N.gonorrhoeae* and *C.trachomatis* in the urine samples.

Anti-microbial susceptibility testing for *N.gonorrhoeae*

Swabs were also taken from the anterior urethra at the time of first attendance, smeared on a glass slide for gram staining and placed directly in a transport media (Amies media, Oxoid, Basingstoke, UK) containing charcoal. The specimens were transported at the end of each day to the laboratory. Plating was done on modified New York City medium (gonococcal selective) containing Lincomycin, Colistin sulphate, Amphothericin-B and Trimethoprim lactate. Yeast autolysate was used as a growth supplement and incubation was done in a candle jar for 48 hours at 35° with 5-10% CO₂. *N.gonorrhoeae* was identified by colony morphology, gram staining and oxidase testing. Penicillinase producing strains were detected using beta lactam paper strips (Oxoid, Basingstoke, UK), that show b-lactamase activity.

Anti-microbial susceptibility testing was done on Muller Hinton agar (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood using the disk diffusion technique. Inhibition zone sizes for *N.gonorrhoeae* were read according to National Committee for Clinical Laboratory⁹ standards. The E-Test strip (AB-Biodisk, Solna, Sweden) was used for susceptibility testing to gentamicin and minimum inhibitory concentrations for gentamicin were read as susceptible $\leq 16\mu\text{g/ml}$, and resistant $>16\mu\text{g/ml}$ ¹⁰.

Antibiograms were validated with standardized *N.gonorrhoeae* control strains (ATCC NO 49226, American Type Culture Collection, Rockville, MD) on regular basis and the National reference laboratory in Malawi provided monitoring and supervision. Additional technical assistance was provided by the Microbiology laboratory, reference center for infectious disease, Luxembourg.

Data analysis

The Epi Info software (Centre for Disease Control, Atlanta) was used for data analysis.

Results

Socio-demographic characteristics of the study population

A total of 114 adult male subjects with urethral discharge were enrolled in the study. The median age of the study participants was 27 years (range 16-47, SE: 0.6). The majority of patients (79%) came from rural villages, and were married (57%). The mean educational level of participants was 6.5 years in school. Patients included farmers (29%), unskilled employees, (34%), skilled employees (6%), business people (24%) and students (7%).

Health seeking and sexual behavior (Table 1)

The mean reported time with STI symptoms before presenting at the STI clinic was 27 days.

61% of all subjects reported having taken some form of medication before coming to the clinic. The most frequent single source of medication was the traditional healer (43%), (Table 1).

68(60%) patients reported having had sexual encounters during the symptomatic period and the majority (84%), had not used condoms. The main reported reasons for not using condoms during sex in the symptomatic period was partner refusal (37%) and, having sex with a spouse or with someone in a steady relationship (28%) (Table 1)

Table 1: Health seeking and sexual behaviour in adult males with visible urethral discharge (n=114).

Variable	Number	(%)
Previous medication before coming to clinic	70/114	61
Modern (ampicillin, cotrimoxazole etc)	25	36
Traditional (herbs, roots etc)	30	43
Both	15	21
Source of previous medication (n=70)		
Public and private clinics	4	6
Drug vendors/public pharmacy	21	30
Traditional healers	30	43
Several of the above	15	21
Mean duration of urethral symptoms (days)	27	-
Sex during symptomatic period =yes	68/114	60
With same partner	37	54
With different partners	31	46
Condom use during sex in symptomatic period (n=68)		
Always	0	0
Intermittent/sometimes	11	16
No condom use	57	84
Reasons for "no condom use" (n=57)		
Sex with steady partner or spouse	16	28
Refusal by partner	21	37
Condom not available	5	9
Reduces pleasure	3	5
Religious reasons	10	17
Others	2	4

Prevalence of *N.gonorrhoeae* and *C.trachomatis*

Out of 114 patients who submitted urine samples for LCR analysis, data is available for 110 patients; 2 patients having submitted insufficient amounts of urine for analysis and 2 urine containers having leaked during transport.

N.gonorrhoeae was detected in 90 (79%) urine specimens, in 1 (1%) there was *C. trachomatis* and combined infections were found in 1 (1%) urine sample.

Antimicrobial susceptibility of *N.gonorrhoeae*

Out of 47 isolates of *N.gonorrhoeae*, 45 (96%) were found to be beta-lactamase producing (PPNG).

All but one of these strains were resistant to penicillin. 40 (89%) Penicillase-producing *Neisseria gonorrhoea* (PPNG) isolates showed resistance to 2 or more antibiotics. Only 3 PPNG isolates were found to be sensitive to tetracycline. All strains that were resistant to gentamicin were also resistant to tetracycline. The picture is likely to be the same for doxycycline. The susceptibility patterns of isolates to different antibiotics are shown in table 2.

Table 2. Antibiotic susceptibility pattern of *Neisseria gonorrhoeae* isolates (n=47)

Antibiotic	Susceptible		Intermediate		Resistant	
	N	%	N	%	N	%
Gentamicin	40	(85)	-	-	7	(15)
Penicillin	1	(2)	3	(6)	43	(92)
Tetracycline	5	(11)	4	(8)	38	(81)
Erythromycin	21	(45)	3	(6)	23	(49)
Co-trimoxazole	17	(36)	4	(9)	26	(55)
Spectinomycin	40	(85)	2	(4)	5	(11)
Ciprofloxacin	32	(68)	12	(26)	3	(6)

Discussion

In this study, 61% of all participants presenting at the rural district STI clinic with urethral discharge had first sought care at an alternative source. The search for effective treatment is therefore delayed, and meanwhile the men continue to have sex while symptomatic, the large majority (84%) not using condoms. These different alternative sources could be targeted to improve STI control in the district and reduce delays in effective treatment.

The traditional healer was found to be the most important single source of alternative care in our rural setting as was found in a similar study in an urban setting of Malawi⁶. In Malawi, traditional healers are generally reputed as being sympathetic, more confidential, and easily accessible. Considering their importance as an alternative care provider, and the potential role they could play in encouraging safer sexual behaviour, it would be important to integrate them in control activities and ensure condom availability (to clients) at their sites.

The National Tuberculosis control program in Malawi conducts training sessions with traditional healers from around the country, and encourages early referral of tuberculosis suspects. Training on STIs and HIV infection could be linked with such an existing initiative, and might be one way of also encouraging earlier referral (by healers) for antibiotic treatment.

This study is the first in Malawi that has used a highly sensitive and specific nucleic acid amplification technique¹¹ to determine the presence of *C.trachomatis* and *N.gonorrhoeae* in the urine of men presenting with urethral discharge. Infection with *N.gonorrhoeae* was confirmed in 79% of urine specimens whereas *C.trachomatis* was found in only 2% of specimens. In contrast to previous suggestions¹², Chlamydia, was not found to be a major pathogen in men presenting with urethral discharge in our rural setting. A similar low prevalence of *C.trachomatis* was found in Blantyre, an urban town in Malawi, where Chlamydia antigen was found in only 26 (5.2%) of 497 urine specimens tested⁶. We had not screened for other possible organisms associated with urethral discharge such as *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis*.

Only 47 isolates from 90 patients positive for *N.gonorrhoeae* by LCR were available for susceptibility testing. This might be explained by the fact that *N.gonorrhoeae* is a very fastidious organism and despite use of a transport medium, undocumented delays did occur between collection of specimens in the district STI clinic and inoculation at the laboratory in Blantyre. Specimens were transported once a day and could have been subjected to rapid changes in ambient temperature which is characteristic of the region.

We used the E-test for testing the susceptibility of *N.gonorrhoeae* to gentamicin and found it easy and practical to use in our developing country laboratory setting. The disc diffusion method although most widely available and least expensive is not considered reliable for gentamicin susceptibility testing, and agar dilution assays are quite complicated to perform for routine surveillance¹³.

None of the antibiotics tested in our study approached the 95% sensitivity recommended for effective "blind treatment"¹⁴. The clinical cure rate for gentamicin treatment of *N.gonorrhoeae* was 95% in 1993⁶ as compared to 91.8% in 1996¹³. Our study which is the first since 1996 in Malawi, shows that only 85% of *N.gonorrhoeae* isolates are currently susceptible to gentamicin. Although clinical cure rates might differ from in-vitro susceptibility patterns, this finding is of concern since selection of resistant strains may rapidly limit the usefulness of gentamicin for the treatment of *N.gonorrhoeae* in our setting. There is therefore a need to search for alternative antibiotics for the syndromic treatment of urethral discharge caused by *N.gonorrhoeae*.

All isolates tested in 1996¹³ were fully sensitive to ciprofloxacin, ofloxacin and cefixime. We had not tested susceptibility to ofloxacin and cefixime but found 6% resistance and 26% intermediate susceptibility to ciprofloxacin. This could be due to the rising indiscriminate use of this agent which is now readily available (without prescription) at some public pharmacies. Ciprofloxacin is relatively expensive and "cost considerations" often encourage the use of inadequate, or low dosages that will help force the selection of strains exhibiting resistance or reduced susceptibility¹⁵.

The great majority (96%) of *N.gonorrhoeae* isolates in the rural district of study were PPNG strains the majority of which exhibited multi-antimicrobial resistance. Continuing surveillance of antimicrobial susceptibility of *N.gonorrhoeae* is essential in order to detect emerging resistance, prevent treatment failures and control the spread of resistant strains within the population.

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References

1. Clotey C, Dallabetta G . Sexually transmitted diseases and human immunodeficiency virus, epidemiologic synergy? *Infectious Discases Clinics of North America*, 1993, 7:95-102.
2. Grosskurth H, Mosha F, Todd J, Mwijarubi E, Klokke A, Senkoro K, Mayaud P, Changalucha J, Nicoll A, Ka-Gina G. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania. Randomized controlled trial. *Lancet*, 1995, 346:530-536
3. National AIDS Control Programme of Malawi. Sentinel Surveillance Report. 2001, NACP, P.O.Box 30622, Lilongwe 3.
4. Kristensen JK. The prevalence of symptomatic sexually transmitted diseases and human immunodeficiency virus infection in outpatients in Lilongwe, Malawi. *Genitourinary Medicine*, 1990, 66:244-246.
5. Ministry of Health and Population, Essential Drugs Programme, Malawi standard treatment guidelines, 2nd ed. 1993, Ministry of Health, Lilongwe, Malawi.
6. Lule G, Behets F M-T, Hoffman IF, Dallabetta G, Hamilton HA, Moeng S, Liomba NG, Cohen MS. STD/HIV control in Malawi and the search for affordable and effective urethritis therapy: a first field evaluation: *Genitourinary Medicine*, 1994, 70: 384-388
7. Lind I. Epidemiology of antibiotic resistant Neisseria gonorrhoeae in industrialized and developing countries. *Scandinavian Journal of Infectious Diseases*, 1990, 69:77-82.
8. National AIDS Control Program, Ministry of Health and Population/JSI-STAFH, Managing people with sexually transmitted diseases in Malawi 1993, 1st edition.
9. National Committee for Clinical Laboratory Standards. Performance standards for anti-microbial susceptibility testing. Third information supplement. 1998, NCCLS Document M 100-S8.NCCLS: Villanova, PA.
10. Lesmana M, Lebron I, Taslim D, Tjaniadi, Subekti D, Wasfy O, Campbell J, Oyoyo A. In-vitro antibiotic susceptibility of Neisseria gonorrhoeae in Jakarta, Indonesia. *Antimicrobial Agents Chemotherapy*, 2001, 45: 359-362.
11. Strydom A. Chlamydia screening. Which sample for which technique? *Genitourinary Medicine*, 1997, 73:99-102
12. Taylor-Robinson D, Ballard RC, Thomas BJ, Renton A. STD/HIV control in Malawi. *Genitourinary Medicine*, 1995, 70:384-8
13. Daly C, Hoffman I, Hobbs M, Maida M, Zimba D, Davis R, Mughogho G, Cohen M . Development of an antimicrobial susceptibility surveillance system for Neisseria gonorrhoeae in Malawi: comparison of methods. *Journal of Clinical Microbiology*, 1997, 35:2985-2988
14. World Health Organisation, STD treatment strategies. 1989,WHO/VDT/89.447.
15. Handsfield H.H and Whittington W.L . Antibiotic-resistant Neisseria gonorrhoeae: the calm before another storm? *Annals of Internal Medicine*. 1996, 125:507-509

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