TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2002) 96, 232–235

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

R. Zachariah[1], A. D. Harries[2], W. Nkhoma[3], V. Arendt[4], D. Nchingula[5], A. Chantulo[1], F. Chimtulo[6] and P. Kirpach[4] *[1]Médecins sans Frontières–Luxembourg, Thyolo District, Malawi; [2]National TB Control Programme of Malawi/DFID advisor, and [3]National AIDS Control Programme of Malawi, Ministry of Health, Lilongwe, Malawi; [4]Infectious Disease Reference Centre & Laboratory, Central Hospital, Luxembourg; [5]Blantyre Diagnostic Laboratory Services, Blantyre, Malawi; [6]National Reference Laboratory, Ministry of Health and Population, Lilongwe, Malawi*

Abstract

A study was carried out in 2000/2001 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* and *Chlamydia trachomatis*, and (c) verify the antibiotic susceptibility of *N. gonorrhoeae*. A total of 114 patients were entered into the study; 61% reported having taken some form of medication before coming to the sexually transmitted infections clinic. The most frequent alternative source of care was traditional healers. Sixty-eight (60%) patients reported sexual encounters 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%) urine specimens. Forty five of 47 *N. gonorrhoeae* isolates produced penicillinase, 89% showing multi-antimicrobial resistance. This study emphasizes the need to integrate alternative care providers and particularly traditional healers in 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.

Keywords: sexually transmitted diseases, urethral discharge, men, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, prevalence, health-care seeking behaviour, sexual behaviour, antimicrobial susceptibility, Malawi

Introduction

Sexually transmitted infections (STIs) are known to facilitate the sexual transmission of HIV (CLOTTEY & DALLABETTA, 1993), and effective STI case management is known to reduce the incidence of HIV (GROSSKURTH *et al.*, 1995). The HIV national prevalence in Malawi is estimated at 15% (NACP, 2001), and HIV infection rates among patients with STIs range from 53% to 83% (KRISTENSEN, 1990). As part of its STI and HIV control strategy, Malawi adopted the World Health Organization (WHO) 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 men presenting with urethral discharge (MOHP, 1993). Although this regimen is meant to cover for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections (LULE *et al.*, 1994), the relative prevalence of these pathogens in rural communities in Malawi is not known.

Regular monitoring of antimicrobial susceptibility of *N. gonorrhoeae* and other STI pathogens is essential as resistance patterns could change rapidly (LIND, 1990). Because of 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 men presenting with urethral discharge, in order to (a) describe their health-seeking and sexual behaviour, (b) determine the relative contribution of *N. gonorrhoeae* and *C. trachomatis* to urethral discharge, and (c) verify the antibiotic susceptibility of *N. gonorrhoeae*.

Address for correspondence: Dr R. Zachariah, Head of Mission (Mission Malawi), Médecins sans Frontières–Luxembourg, 70 rue de Gasperich, L-1617, Luxembourg; phone +265 644409 (Malawi), fax +265 641 468 (Malawi) +352 335133 (Luxembourg), e-mail zachariah@internet.lu

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 invited 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 behaviour. All patients were managed according to national STI guidelines (NACP, 1993).

Prevalence of N. gonorrhoeae *and* C. trachomatis

Patients were requested to provide a urine specimen at the time of first attendance. Nucleic acid amplification, by ligase chain reaction (LCR), was used to determine the presence of *N. gonorrhoeae* and *C. trachomatis* in the urine samples.

Antimicrobial 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 medium (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, amphotericin-B and trimethoprim lactate. Yeast autolysate was used as a growth supplement and incubation was done in a candle jar for 48 h at 35°C with 5–10% CO_2.

N. gonorrhoeae was identified by colony morphology, Gram staining and oxidase testing. Penicillinase-producing strains (PPNG) were detected using beta-lactam paper strips (Oxoid), which show β-lactamase activity.

Antimicrobial susceptibility was tested on Muller Hinton agar (Oxoid) supplemented with 5% sheep blood, using the disk diffusion technique. Inhibition zone sizes for *N. gonorrhoeae* were read according to National Committee for Clinical Laboratory Standards (NCCLS, 1998). 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 (≤16 μg/mL) or resistant (>16 μg/mL) (LESMANA *et al.*, 2001).

Antibiograms were validated with standardized *N. gonorrhoeae* control strains (ATCC No. 49226, American Type Culture Collection, Rockville, MD, USA) on a regular basis and the National Reference Laboratory in Malawi provided monitoring and supervision. Additional technical assistance was provided by the Microbiology Laboratory, Reference Centre for Infectious Disease, Luxembourg.

Data analysis

The EpiInfo software (Centers for Disease Control, Atlanta, GA, USA) 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). Most 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 behaviour

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 traditional healers (43%) (Table 1). Sixty-eight patients (60%) 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).

Prevalence of N. gonorrhoeae *and* C. trachomatis

From the 114 patients who submitted urine samples for LCR analysis, data are available for 110 patients; 2 patients submitted insufficient urine for analysis and 2 urine containers leaked during transport. *N. gonorrhoeae* was detected in 90 (79%) urine specimens, *C. trachomatis* in 1 (1%) 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 isolates were resistant to penicillin. Forty (89%) PPNG isolates showed resistance to 2 or more antibiotics. Only 3 PPNG isolates were found to be sensitive to tetracycline. All isolates 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.

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 of health care 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 Malawi (LULE *et al.*, 1994). In Malawi, traditional healers are generally reputed to be 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 is important to integrate them in control activities and ensure condom availability (to clients) at their sites.

Table 1. Health-seeking and sexual behaviour in adult males with visible urethral discharge ($n = 114$) (Thyolo, Malawi, 2000/2001)

Variable	Number	(%)
Previous medication before coming to clinic	70/114	61
Modern (ampicillin, co-trimoxazole, 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	18
Others	2	4

Table 2. Antibiotic susceptibility pattern of *Neisseria gonorrhoeae* isolates ($n = 47$) from men with urethral discharge (Thyolo, Malawi, 2000/2001)

	Susceptible		Intermediate		Resistant	
Antibiotic	*n*	(%)	*n*	(%)	*n*	(%)
Gentamicin	40	(85)	–	–	7	(15)
Penicillin	1	(2)	3	(6)	43	(91)
Tetracycline	5	(11)	4	(9)	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)

See Material and Methods for the methodology used.

The National Tuberculosis Control Programme 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 technique (STARY, 1997) 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 80% of urine specimens whereas *C. trachomatis* was found in only 2% of specimens. In contrast to previous suggestions (TAYLOR-ROBINSON *et al.*, 1995), *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 tested (LULE *et al.*, 1994). 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 the rapid changes in ambient temperature that are 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 surveillance (DALY *et al.*, 1997).

None of the antibiotics tested in our study approached the 95% sensitivity recommended for effective 'blind treatment' (WHO, 1989). The clinical cure rate for gentamicin treatment of *N. gonorrhoeae* was 95% in 1993 (LULE *et al.*, 1994) as compared to 92% in 1996 (DALY *et al.*, 1997). 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 1996 (DALY *et al.*, 1997) 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 susceptibility (HANDSFIELD & WHITTINGTON, 1996).

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.

Acknowledgements

This study received ethical approval as part of a package of operational research studies on tuberculosis, STI and HIV by the National Health Sciences Research Council of Malawi. Médecins sans Frontières–Luxembourg financed the laboratory material and reagents required for this study. The National Reference Laboratory of the Ministry of Health, Malawi, ensured quality control. The Laboratory for Infectious Diseases, Luxembourg, provided additional technical assistance and conducted LCR analysis. We thank Dr Romain Poos and Rookie Bowles for their support and in particular Mrs Makombe and Mrs Chipioza for data and specimen collection.

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Received 30 July 2001; revised 31 October 2001; accepted for publication 14 January 2002

Announcements