The Syndromic Management of Sexually Transmitted Diseases: clinical and microbiological response in relation to aetiology, susceptibility patterns and co-infection with HIV-1

This thesis is submitted by Dr Prashini Moodley in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Medical Microbiology, Nelson R Mandela School Of Medicine, University of Natal, Durban.

Declaration

This thesis in the form of a collection of manuscripts, represents my original work, and has not been submitted previously to this or to any other University. Assistance where received, has been duly acknowledged.

The research described in this thesis was carried out at the Africa Centre for Health and Population studies' field site in the Hlabisa sub-district of KwaZuluNatal, the Prince Cyril Zulu Communicable Diseases Clinic in Durban and the Department of Medical Microbiology, School of Infection, Nelson R Mandela School of Medicine, University of Natal, Durban, South Africa, under the supervision of Professor A. Willem Sturm.

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Prashini Moodley
November 2002

For my darling daughters

Talya and Caitlin

This thesis comprises of a collection of 16 manuscripts:

9 have been published in peer-reviewed journals

2 will be published in peer-reviewed journals in Dec 2002

2 have been accepted for publication in peer-reviewed journals and are ‘in press’ (2003)

3 have been submitted for publication

12 of the manuscripts have been presented at leading international conferences

The author of this thesis contributed in the design of the different sub-studies and supervised the fieldwork at the rural STD clinic at KwaMsane as well as in the STD section of Cyril Zulu Communicable Disease Centre in Durban where she also provided expert consultation on problem cases. She has also supervised the processing of specimens in the classic methodology section of the STD laboratory in the department of Medical Microbiology at the Nelson R Mandela School of Medicine. She was further involved in data analysis and wrote the papers on which she features as first author.

Publications emanating from thesis

1. Prashini Moodley, Patrick Sturm, David Wilkinson, Cathy Connolly, A. Willem Sturm. Male urethritis in South Africa: Impact of HIV-1 coinfection on response to Syndromic Management. Submitted for publication.

2. Prashini Moodley, David Wilkinson, Cathy Connolly, A. Willem Sturm. Influence of HIV-1 Coinfection on Effective Management of Abnormal Vaginal Discharge. Sexually Transmitted Diseases 2002: 30: 1-5

3. Prashini Moodley, David Wilkinson, Cathy Connolly, A. Willem Sturm. Influence of HIV-1 infection on response to treatment of sexually transmitted infections. AIDS 2001; 15: 542-543.

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2. Prashini Moodley, David Wilkinson, Cathy Connolly, A. Willem Sturm. Influence of HIV-1 Coinfection on Effective Management of Abnormal Vaginal Discharge. In Abstracts of the 40th Interscience Conference on Antimicrobial agents and Chemotherapy; Toronto, September 2002; Abstract no: 1194: 447

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PREFACE

HIV-1 is the most prevalent and notorious sexually transmitted pathogen locally, and constantly challenges our foundation of knowledge regarding the classical STIs. The ultimate objective of the syndromic management strategy was to reduce the load of sexually transmitted infections, and hence HIV transmission. This strategy is multifaceted and not only includes the recognition of symptoms by the patient and an effective treatment regime that comprehensively covers the possible aetiological agents for a defined syndrome, but also appropriate health seeking behaviour of infected individuals, recognition of syndromes by the health care worker, partner management (notification and treatment), behavioural counselling and condom promotion. Understanding the complexity of sexual networking and transmission dynamics is part of such a strategy. So, although the rationale and design of syndromic case management appears simplistic, it is by no means easy to implement.

Introduction

Background

Sexually transmitted infections (STIs) are caused by micro-organisms that are fastidious in nature and therefore need intimate contact between individuals for transmission. Since these infections have only one host i.e. man, and no extra-corporal reservoir, they are in principle ideal candidates for elimination. However efforts to this end have been largely neglected. This lack of attention stemmed from the premise that only certain, already marginalised population groups, based on behaviour, race, social status and occupation, were at risk for contracting STIs. As a result of this social stigmatisation, these infections were placed on the lower rungs of the ladder of health priorities. As such, the curable STIs continue to be a large public health problem especially in resource poor settings.

So although STIs deserve effective treatment in their own right, little attention was paid to their management prior to the HIV era. The public health importance of STIs has however been increasingly underscored over the past decade with numerous studies providing epidemiological [1,2,3,4] and biological [5,6,7] evidence that implicate STIs as co-factors in the sexual transmission of HIV.

The relationship between STIs and HIV infection is a complex one with both disease entities having a potential role in the transmission, acquisition and clinical course of the other. Geographically, 86% of the world's burden of STIs occur in the developing world.[8]

It has been reported that the presence of an STI increases the risk of HIV transmission from two to twenty fold.[4] The treatment of these infections has therefore become a priority especially in developing countries where the prevalence of HIV and STIs has reached astronomical proportions. It has been suggested that in resource-poor settings, where access to effective management strategies for HIV infection is unaffordable, successful management of symptomatic STIs (STDs) may reduce transmission of HIV. In such countries, treatment of symptomatic STIs together with the promotion of condom use is seen as an affordable means of controlling the spread of HIV.[4,9,10,11]

The classic approach to STD case management has been fraught with problems. Studies have repeatedly underscored the inaccuracies associated with linking clinical observations with aetiological diagnosis. This is compounded by the fact that patients often present with multiple infections. In addition, in resource poor settings the diagnosis of STDs has been hampered by the lack of trained health care personnel and appropriate laboratory support. Classic laboratory diagnosis requires staff that is properly trained to collect the required specimens. The fastidious nature of the pathogens requires transport under optimal conditions. Detection is labour intensive and slow resulting in delay in obtaining results and treatment. At best, the most sensitive of the classic tests fail to diagnose between 20 to 40 % of infections when compared to the newer PCR based tests (Sturm et al. submitted). Although collection and transport of specimens are less demanding, these tests are expensive and require specialised laboratory facilities and trained personnel.

The soaring HIV epidemic prompted health authorities to look at alternative strategies to improve case management of STDs. For the want of an appropriate point of care test with reasonable sensitivity and specificity to detect the common STI pathogens, the WHO introduced various guidelines for the syndromic treatment of symptomatic patients. [12]

The syndromic approach uses clinical algorithms so designed that primary health care nurses in resource poor settings may

arrive at an appropriate clinical diagnosis based on a patient's symptoms and clinical signs. The clinical diagnosis is then linked with a predefined antimicrobial prescription in which drugs are advised that have shown efficacy against the different STI pathogens in clinical trials.

The main advantage of this approach is that the patient receives effective treatment at the first visit for the common causes of the presenting symptom. The major concern regarding syndromic management is over-treatment and its related development of antimicrobial resistance. In 1995, the Department of Health for KwaZuluNatal adopted and modified the WHO guidelines for the treatment of symptomatic STIs in the region. Non-ulcerative genital disease is treated with an acute dose of 250mg ciprofloxacin and doxycycline 200mg daily for 7 days. This is supplemented with metronidazole 2 g STAT in the non-pregnant female. If pelvic inflammatory disease is suspected, the dose of metronidazole increases to 400mg tds for 5 days. During pregnancy, these drugs are replaced by ceftriaxone, 125 mg intramuscularly STAT and erythromycin 500mg qid for 7 days. Spectinomycin is used in case of β-lactam hypersensitivity. Genital ulcers are treated with 2.4 mu of penicillin intramuscularly and erythromycin 500mg qid for 7days. Although these guidelines have been in place for five years, STD control has been perceived as being far from optimal with the area being ill-famed as having one of the highest HIV and STD prevalence rates in the world.

The WHO recommendation to target symptomatic patients stems from the theory that symptomatic STDs may be more important in facilitating HIV transmission. While it is obvious that symptomatc cases of STIs do play a role in transmisssion of STI pathogens, the role of individuals without a prominent immune response (and therefore few symptoms) in HIV transmission is less clear. The low concentration of traget cells for HIV in such patients might render them less important. The epidemiological impact of targetting symptomatic patients depends on the percentage of symptomatic infected individuals that are cleared of their infection for a period long enough to break the chain of transmission. An obvious limitation is the neglect of the infected, asymptomatic individual. The Piot-Fransen model illustrates that treatment of symptomatic STDs only, fails to impact on the overall burden of disease. This was shown in rural South Africa, where 25 % of 55 974 women in the Hlabisa district aged 15-49 years, were found to be infected on any given day with an STI. [13] Of these, 48% were asymptomatic and 50% were symptomatic but not seeking health care. Less than 2% of these women would seek medical attention and even fewer would be adequately treated. These prevalence figures although alarmingly high, are actually an under-estimation since these are based on classic diagnostic methodology. The problem is compounded by poor treatment seeking behaviour of individuals with STD symptoms. It follows from this that even if targeting symptomatic patients only could lead to the desired effect, its implementation is cumbersome and difficult to achieve since only patients who present with a discharge are treated for an STI.

Historically, STI programmes aimed at preventing infection and treating the disease when present. Reproductive health fell under the domain of family planning programmes. In 1994, the International Conference on Population and Development in Cairo concluded that sexual and reproductive health together with human rights needed to be linked and addressed in a more holistic manner. Research in population studies was identified as being a key factor that would contribute to the success of such initiatives. The rationale behind this was that population studies and reproductive health are inextricably linked. To implement effective health care strategies in a defined geographical area, a pre-requisite is to understand the factors that may influence the dynamics and behaviour of that population. Reproductive health therefore has a wide definition and includes not only the well being of women and men in terms of their

fertility and sexual health, but also safe motherhood, improvement of infant and child survival, growth and development.

In 1995, The Wellcome Trust launched a 5 million pound Population Studies Programme. The Africa Centre for Population Studies and Reproductive Health, the flagship of the Trust's Population Studies Programme, is situated in the Hlabisa district, about 250 km north of Durban in South Africa.

In Hlabisa STIs are highly endemic. Among 321 women attending district antenatal clinics, 52% were found to have at least one STI (gonorrhoea, chlamydial infection, trichomoniasis or syphilis), and 18% had more than one infection. [14] Among 189 women attending the local family planning clinic, 63% had a genital tract infection (any of the STIs plus either candidiasis or bacterial vaginosis); 26% had multiple infections. Our modelling indictates that around 25% of the women of reproductive age who are resident in the district have at least one STI on any given day; about half of these are asymptomatic.

Many of the projects proposed for the Africa Centre have arisen from this background i.e. population studies in an area highly endemic for STIs and HIV. The largest projects within the Africa Centre such as the STD cohort, the pregnancy cohort, the migration study, the adolescent studies and the microbicide trial either focus directly on STIs, or they include STIs as an important component.

As scientists in The STD and HIV Epidemiology, Prevention and Treatment Group of The Africa Centre, we are responsible for providing microbiological and epidemiological data, and advice to our colleagues in the Africa Centre we proposed the formation of a STI Clinic. The Clinic would be nurse-led/ doctor-supervised and provide a high quality STI service at the KwaMsane Primary Care Clinic, adjacent to the Demographic surveillance area of the Africa Centre.

1. It was envisaged that the STD clinic would be used to answer key questions that are fundamental to the correct and safe conduct of many of the projects planned for the Africa Centre, including::
    i. What is the aetiology of the different STD syndromes in the area ?
    ii. What is the drug susceptibility profile of the aetiological agents
    iii. What is the efficacy of the provincial health department's syndromic management guidelines, and hence can they be used to treat people identified with an STD in the projects of the Africa Centre ?
    iv. What is the effect of HIV-1 infection on the symptoms and signs of the different STD syndromes
    v. What is the effect of HIV-1 coinfection on response to syndromic drug therapy

2. To use the clinic to support other research projects that are proposed for the Africa centre including: strategies for improved partner treatment; strategies for improved recognition of and response to symptoms and signs of STDs; determining the prevalence and causes of asymptomatic STD in men and women
3. To use the reference clinic as a resource to provide on-going high quality data which will advise policy makers and local health authorities.

In 1999, Professor A. Willem Sturm and Dr David Wilkinson spearheaded the establishment of a STD clinic within the primary health structure at KwaMsane in the Hlabisa district. The space available was limited and was not conducive to the confidentiality that is required for the interview and examination of patients with STIs. Expansion of these premises was

made possible through a grant from The Welcome Trust. The STD and antenatal arm of this clinic now boasts four fully equipped counselling/examination rooms, a small on-site laboratory, a tearoom and ablution facilities. The clinic is fully integrated with the service component of the KwaMsane Clinic.

The clinic is staffed by four professional nurses, three field workers, two drivers, a laboratory technologist (part-time) and a medical officer. In addition to research activities, they are also responsible for the running of the routine STD and antenatal activities at the clinic.

The STD and HIV Epidemiology, Prevention and Treatment Group also conducts some of its research activities at the The Prince Cyril Zulu Clinic in Durban. This clinic has been established and is run by the Durban City Health Department.

A STI diagnostic laboratory was obviously needed to support the clinical and scientific research programme of the Africa Centre. The laboratory would form the basis for:

vi. the training of technologists and health workers
vii. conducting epidemiological and microbiological surveys
viii. monitoring antimicrobial susceptibilities
ix. validating syndromic management algorithms
x. sentinal surveillance
xi. development of new diagnostic tests
xii. clinical research

The Africa Centre Sexually Transmitted Diseases Laboratory was established within the Department of Medical Microbiology at the Nelson R Mandela School of Medicine, under the leadership and headship of Prof A. Willem Sturm. The facility is equipped for the isolation and detection of all STI pathogens using both classical and DNA based methodologies. In addition, susceptibility testing on isolates is also performed.

The distance between the laboratory and the Africa centre STD and antenatal clinics in KwaMsane posed an obvious problem of timeous and optimal transport of specimens. This was resolved with the acquisition of a bus equipped with a 4^{o}C fridge, -20^{o}C freezer as well as a 35^{o}C compartment, which allows for the specimens to be transported under optimal conditions to the laboratory in Durban.

The STD and HIV Epidemiology, Prevention and Treatment Group of the Africa Centre have been conducting research in KwaMsane and Durban for almost three years. The major thrust of the research thus far has been the evaluation of aspects of syndromic management protocols as recommended by the KwaZuluNatal Provincial Health Department, the impact of HIV on the presentation, course and response to treatment of STIs and antimicrobial susceptibilities of some of the sexually transmitted pathogens.

Outline of Thesis

The spectrum of microbes causing the same disease syndrome and their variable antimicrobial susceptibilities underscore the need for area specific research when addressing syndromic management programmes. For STIs, there are a number of pre-requisites to the implementation of such a programme, most important being an assessment of the prevalence of the causative agents of STIs in the area and the antimicrobial susceptibility patterns of these organisms. These two factors however do not guarantee success. After implementation, success will depend on recognition of symptoms by patient as well as health care worker, condom promotion and treatment of sexual contacts before the patient is re-infected. era of rampant HIV-1 infection and its associated immunosuppresion, the effect of the HIV-1 infection on response to treatment of STIs is unknown.

The impact of HIV-1 infection on the response to treatment of curable STIs has

The public health importance of STIs is primarily its impact on pregnancy outcome and more recently, on HIV transmission. Effective treatment of STIs is likely to decrease the HIV incidence. However, in the been debated and a decreased response to therapy among HIV-1- infected patients has been suggested. Validation of this is important, especially locally where coinfection of classical STIs and HIV-1 is common.

As described in the introduction, local syndromic management guidelines have been in place for more than five years in KwaZuluNatal. However, its efficacy has not been tested.

The main objective of this study was to test the applicability and efficacy of the provincial health department's syndromic management guidelines for ulcerative and non-ulcerative sexually transmitted infections in KwaZuluNatal, against a background of endemic HIV-1 infection.

We therefore set out to:

1.determine the aetiology of the major STD syndromes in KwaZuluNatal
2.assess response to syndromic treatment clinically and microbiologically
3.compare the prevalence and response to treatment of treatable STIs in HIV-1-infected and-uninfected patients
4.determine the susceptibility patterns of the causative organsisms
5.summarise the impact of STIs and adverse pregnancy outcome.

The four papers in **Chapter 1** describe the prevalence of the aetiological agents associated with the 3 major STD syndromes viz: female genital discharge, male urethritis and genital ulcer disease. In addition, the chapter addresses clinical and microbiologic response to syndromic treatment in HIV-1-infected and-uninfected patients.

To advise on antibiotic use in syndromic management protocols, surveillance is necessary. STIs caused by antibiotic resistant organisms are becoming a major therapeutic problem in many parts of the world with *Neisseria gonorrhoeae* being the most extensively studied organism. The first 3 papers in **Chapter 2** address the trends in susceptibility of *Neisseria gonorrhoeae* to various antimicrobials and reports on the emergence of tetracycline resistance and ciprofloxacin resistance. The local STD syndromic management guidelines differ from that of WHO in that the dose of ciprofloxacin used for the treatment of *Neisseria gonorrhoeae* is 250 mg as opposed to 500 mg. The use of the lower dose has been met with international criticism. The 4th paper of this chapter compares the different dosages of ciprofloxacin.

Reinfection of effectively treated patients is the hallmark of STIs that perpetuate and maintain the endemnicity of these infections. To address this aspect, syndromic management incorporates the promotion of condoms as well as partner notification and treatment. In an earlier study**,** (**chapter 1**) we reported on a 20 % failure rate following effective treatment for *N.gonorrhoeae*. Re-infection as a cause of failure of syndromic management is difficult to ascertain since it often relies on subjective history. In **chapter 3** we address this issue by reporting on typing of pre and post treatment strains of *N.gonorrhoeae* from the women in this STD cohort.

The debate on whether bacterial vaginosis (BV) is an STI or not, is rather theoretical since there is a mammoth amount of evidence linking reproductive tract morbidity with this condition. Its recent link in the transmission of HIV leaves no doubt as to the public health significance of this condition. Despite the health risks associated with bacterial vaginosis and its high prevalence in women of childbearing age, this condition continues to be largely ignored by clinicans, particularly in asymptomatic women. It has been argued in STD circles that this condition, because so prevalent, may actually be normal among certain populations living in resource constrained areas. The first paper in **chapter**

4 examines the inter-relationship between the aetiologies of discharge of vaginal origin and HIV-1, while the second paper reports on non-invasive sampling of the genital tract for the diagnosis of BV.

Knowledge of the aetiology of the various STD syndromes is essential for the development of a rational and comprehensive approach to syndromic management. **Chapter 5** addresses questions around unusual aetiologies. In men, the symptoms of urethritis are highly specific of infection. The classic causes of this syndrome are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. As reported by others, in a substantial proportion of the male urethritis cohort (see **Chapter 1**) none of these pathogens were detected. The first paper examines the possible role of other aetiologies in the syndrome of male urethritis.

Pelvic inflammatory disease (PID) has classically been associated with the causes of vaginal discharge viz: *N.gonorrhoeae*, *C.trachomatis* and the anaerobic bacteria associated with bacterial vaginosis. Although *Trichomonas vaginalis* is the commonest discaharge causing STI in developing countries, little attention has been paid to its role as a possible cause of upper genital tract infection. The second paper in this chapter describes a plausible association between *T.vaginalis* in HIV-1 infected women with symptoms and signs of pelvic inflammatory disease.

C.trachomatis biovar LGV is believed to be a rare cause of genital ulcerdisease(GUD).Classic teaching describes this infection as being characterised by inguinal buboes, with an insignificant or absent history of a genital lesion. The third paper describes the prevalence and clinical presentaion of patients with ulcers caused by LGV, as dignosed by a PCR methodology targeting the cysteine-rich outer membrane protein of the organism.

Although the differential diagnosis in women presenting with non-ulcerative genital symptoms and signs is broad, they are often diagnosed and treated as having an STI. This leads to inaccurate data which impacts on policy development and implementation. Using a past history of specific STI symptoms ie: treated genital ulcers, the paper in **chapter 6** attempts to define the different levels of STD risks among rural women attending a STD clinic.

Chapter 7 reviews the major impact of STIs on reproductive health i.e adverse pregnancy outcome

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Chapter One

Impact of HIV-1 infection on Syndromic Management of STIs

Summary

Short term efficacy of syndromic therapy for males with urethritis both clinically and microbiologically is acceptable. The clinical and microbiological response was not influenced by infection with HIV-1.

Infection with all the STI causes of discharge and BV was more frequent among HIV-1 infected women. This was not so in men. In addition, clinical cure was lower among females as compared to males. Cure rates for BV was only 38%. This cure rate is extremely alarming since BV is the commonest cause of vaginal discharge in Africa and has been implicated in facilitating transmission of HIV-1.

For genital ulcer disease, the cure rates following syndromic therapy is adequate and does not appear to be overtly influenced by HIV-1 coinfection.

Male Urethritis in South Africa: Impact of HIV-1 Coinfection on Response to Syndromic Management

Prashini Moodley, David Wilkinson, Cathy Connolly and A.Willem Sturm

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ABSTRACT

Reports on microbiological cure rates following syndromic management (SM) of men with non-ulcerative STIs are limited. The goal of the study was to determine the effectiveness of the drugs used in SM of urethritis in men, and to compare the response among those with and without HIV-1 infection. The study design was a cohort study of men with urethritis who were treated according to local SM protocols. Of 353 men recruited, 198(56%) tested positive for *N.gonorrhoeae* (50%) and/or *C.trachomatis* (9%). HIV prevalence was 44%. Two hundred and seventy two men returned for follow-up, and 254 consented to repeat examination with specimen collection. Clinical cure was independent of HIV-1 serostatus. Microbiological cure ranged from 83% for *C.trachomatis* to 100% for *N.gonorrhoeae* and was also independent of HIV-1 status.Clinical and microbiological cure rates following syndromic management were adequate among these men. HIV-1 co-infection does not appear to reduce cure rates.

Introduction

Although urethritis causes minimal complications in men, the causative organisms are easily transmitted to females, and complications arising from these infections are more frequent and severe in women and neonates [1,2]. The syndrome is also associated with an increase in shedding and potential transmission of HIV in patients co-infected with this virus [3]. It has been suggested that the treatment of sexually transmitted infections (STIs) as a HIV control tool may be one of the most cost-effective health interventions available to resource poor countries [4]. Like with all STIs,

prompt and effective treatment of male urethritis is therefore essential. The syndromic approach for the treatment of symptomatic STIs has been advocated as being the most optimal management strategy in developing countries [5].

The accuracy of this approach in terms of syndrome recognition using currently available algorithms in the management of men with symptomatic urethritis, has been extensively studied and is high [6,7,8]. However, studies on the efficacy of drug treatment in men diagnosed with this syndrome are scanty. The impact of co-infection with HIV on treatment efficacy is largely unknown although a negative impact has been suggested [9]. We recently reported that HIV co-infection had no effect on response to treatment for infections caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* in women [10].

The objective of the study reported here was to assess the clinical and microbiological response of the syndrome of male urethritis to the KwaZuluNatal Provincial Health Department's syndromic management guidelines. In addition, we measured the effect of HIV-1 co- infection on treatment response in an area where both infections are rife.

Methods

The project was done at the Prince Cyril Zulu Communicable Diseases Clinic in Durban, KwaZulu-Natal between September 2000 and December 2000. Patients attending this primary care clinic with symptoms of urethral discharge and/or dysuria were recruited with informed consent. Trained interviewers collected data and a trained HIV counsellor pre- and post-test counselled all patients. All men received a full clinical examination and microbiological work up for the recognised discharge/ dysuria causing pathogens viz. *N.gonorrhoeae* and *C.trachomatis*. According to the local Provincial Health Department's protocols, all were treated on the same day with a single 250 mg dose of ciprofloxacin, and doxycycline 200 mg daily for 7 days.

The men were given an appointment to return 8-10 days later. They were assessed clinically for response to syndromic therapy, by asking about how their symptoms had changed. Repeat specimens were also taken to assess microbial cure if the man consented to further examination. All patients were provided with their baseline microbiological results and offered post-test counselling and their HIV results. Clinical cure was defined as absence of presenting symptoms and microbiological cure as the absence of a STI pathogen at follow-up.

The University of Natal Ethics Committee provided ethical approval for the study.

A Dacron swab was inserted 2-3 cm into the urethra and withdrawn while rotating. The swab was used to make a smear for the diagnosis of chlamydial infection by means of direct immuno-fluorescence (MicroTrak,® Trinity Biotech, Ireland). It was also used to inoculate New York City (NYC) plates (Oxoid Ltd. Basingstoke, Hampshire, England), which were incubated at 37^{o}C in CO_2 for 48 hours, for the isolation *N.gonorrhoeae*. Blood for HIV testing was collected in EDTA tubes. The Determine-HIV® screenings test was used to detect HIV antibodies. Positive tests were confirmed by an ELISA test (Vironostika HIV Uni-Form II *plus* 0 –Organon Teknika). A random sample of 10 % of the negative specimens was also subjected to ELISA confirmation. Urine was tested for the presence of leucocytes and nitrites using the dipstick method. If any of these tested positive, a mid-stream urine for culture on cysteine-lactose-electrolyte-deficient medium was obtained.

Prevalence of infections at baseline and after treatment is reported as the proportion of men with any one of the two organisms. Risk factors associated with urethritis were identified using chi square tests. Data were analysed using SAS statistical software.

Results

Three hundred and fifty three consenting men presented to the clinic within the study period. The median age of these men at presentation was 27 years (range 17-54). The median number of lifetime sexual partners was 12 (range 1-100), while the median number of sexual partners in the last 4 weeks was 1(range 0-15).

At the baseline visit, 198 (56%) were infected with at least one of the STI pathogens. The prevalence of *N.gonorrhoeae* and *C.trachomatis* was 50 % (178/353) and 9% (32/353) respectively. One hundred and fifty six (44%) men in the cohort were infected with HIV. There was no association between HIV and infection with *N.gonorrhoeae* or *C.trachomatis* (table 1)

Estimates of clinical cure were done on the 272 men who returned for follow up 8-10 days later. The baseline prevalence of *N.gonorrhoeae* and *C.trachomatis* in this subset was 50% (135/272) and 9% (24/272) respectively, with 123 (45%) having no detectable pathogen. All men who had a detectable pathogen at baseline reported clinical cure. Of those with no detectable pathogen at baseline, 4 (3%) remained symptomatic.

Table 1: Prevalence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in HIV-1 infected and uninfected men

Pathogen	Total (n=353)	HIV infected(%) n=156	HIV uninfected(%) n=197	p-value	RR (95% CI)
N.gonorrhoeae	178	85(54)	93(47)	0.17	1.15 (0.94-1.42)
C.trachomatis	32	14(9)	18(9)	0.95	0.98 (0.5 – 1.91)

Table 2: Cure rates of sexually transmitted infections among HIV-1 infected and uninfected med with urethritis

	Number (%) cured		
Organism	**HIV infected (n=112)**	**HIV uninfected (n=142)**	**P value**
***N.gonorrhoeae* (n=127)**	**56/56 (100)**	**71/71 (100)**	**NS**
***C.trachomatis* (n=23)**	**9/11 (82)**	**10/12 (83)**	**NS**

Microbial eradication was assessed among the 254 (93%) who consented to repeat specimen collection. The eradication rate for *N.gonorrhoeae* was 100%. Four (17%) men failed to clear their infection with *C.trachomatis*. Of these four, two were infected with HIV-1.

Discussion

Our data show that in this setting, the syndromic approach used for male urethritis leads to equivalent clinical and microbiological cure rates irrespective of HIV status. However, the men in this study were all healthy with no evidence to suggest clinical AIDS. Therefore, the question whether the response to syndromic management is altered by immunodeficiency remains to be answered.

Although this was not associated with HIV infection, clinical and microbiological failure rates for *N.gonorrhoeae* infection were significantly higher in local women [10], than in this cohort of men. Since it is unlikely that these men are less frequently exposed to *N.gonorrhoeae*, the attack rate of this pathogen appears to be higher in women as compared to men. This was not the case for *C.trachomatis*, since failure rates for this infection were similar in this cohort of men as compared to local women previously studied [10].

The syndromic management protocol for male urethritis only caters for the treatment of the classical discharge causing pathogens viz. *N.gonorrhoeae* and *C.trachomatis* [11].

However, in almost half of the men presenting with symptoms suggestive of urethritis, these pathogens were not detected. Since the presence of microbial DNA does not differentiate between viable and killed microbes, we did not use PCR based methodology in our assessment of treatment response, but the less sensitive microscopy and culture. Because it would have increased the prevalence of *C.trachomatis* this may in part explain the discrepancies between clinical presentation and microbial detection. However, the role of alternative aetiologies as a cause of urethritis in our area needs to be investigated.

Interestingly, the clinical response in men without a detectable baseline pathogen was comparable to those with *N.gonorrhoeae* and/or *C.trachomatis* at baseline. This suggests that the undefined and undetected causes of urethritis in these men, were susceptible to the drugs used, or the infection may have been self-limiting.

Trichomonas vaginalis has been isolated previously from the male urethra in our area [12], as well as elsewhere [13], with widely varying prevalence rates. Treatment to eradicate this organism is not part of the syndromic management protocol for the treatment of male urethritis. Locally, this pathogen is the most prevalent STI among female STD clinic attendees [10]. The significance of this organism as a cause of reproductive tract morbidity in women, as well as its role in the transmission of HIV has been reported [3,4,14]. It may therefore be prudent to add metronidazole to the current treatment guidelines for male urethritis, if only to stem transmission from men to women.

While the role of *Mycoplasma genitalium* as a cause of urethritis in men has recently been repeatedly underscored [15,16,17], its clinical role in women remains undefined. As such, the public health significance of the pathology caused by this so-called 'emerging pathogen' is at present not fully understood. Doxycycline, which is part of the syndromic management protocol for the treatment of *C.trachomatis*, has been reported to be effective in the eradication of *M.genitalium*[16]. It is therefore questionable as to whether surveillance testing for this organism is warranted at present.

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Influence of HIV-1 Co-infection on Effective Management of Abnormal Vaginal Discharge

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ABSTRACT

Reports on microbiologic cure rates following syndromic management (SM) of women with non-ulcerative sexually transmitted infections (STIs) are limited. The goal of the study was to determine the effectiveness of the drugs used in SM of non-ulcerative STIs and BV in women, and to compare the response among those with and without HIV-1 coinfection. This was a cohort study of women with non-ulcerative STIs who were treated according to local SM protocols. Of 692 women recruited, 415 (80%) returned 8 to 10 days later, and 290 (70%) consented to a second examination in which specimens were obtained. Clinical cure was reported by 67%, and microbiological cure ranged from 80% to 89% for the three discharge-causing STIs, and was independent of HIV-1 status. Only 38% of those with bacterial vaginosis were cured and HIV-1-infected women were less likely to be cured (28% versus 52%; p<0.001). Clinical and microbiologic response to SM of the nonulcerative STIs was not affected by HIV-1 coinfection, but cure rates for bacterial vaginosis were reduced

Introduction

The epidemiological and biological evidence for sexually transmitted infections (STIs) as important co-factors in the transmission of HIV-1 is accumulating.[1] The incidence and prevalence of STIs in most developing countries is very high, with women bearing the major burden of disease.[2,3] The treatment of bacterial STIs as an HIV control strategy may be one of the most cost-effective health interventions available.[4]

In resource poor settings, the diagnosis and treatment of STIs depend on World Health Organisation-recommended syndromic management protocols.[5] The syndromic approach uses clinical algorithms based on the patient's symptoms and clinical signs to determine antimicrobial therapy aimed to treat the most common causes of a syndrome.[5]

While the diagnostic efficacy of these algorithms [6] has been established, there is limited data to validate their treatment effectiveness. La Ruche et al. and Grosskurth et al. evaluated clinical effectiveness among women presenting with vaginal discharge syndrome and showed the values to be 87% and 96%, respectively.[7,8] These studies did not include microbiologic examination, and as clinical cure may not equate with microbial eradication, studies that include clinical and microbiologic response are important.

It has been suggested that HIV infection may reduce the response of STIs to antibiotic treatment [9] but conclusive evidence is lacking.[10] This is an important issue, as HIV prevalence may be very high among patients with STIs in many developing countries. Failure to respond adequately to treatment could lead to continued transmission of STIs.
The aim of our study was to determine the clinical and microbiologic effectiveness of syndromic management among HIV-infected and -uninfected women who report symptoms indicative of infection in the genital tract.

Methods

Setting

The project was done in the Africa Centre for Population Studies and Reproductive Health primary care clinic in the Hlabisa health district of KwaZuluNatal. Most residents of the area are Zulu speaking rural people living in widely scattered kraals and who are dependent on migrant labour, pension remittances and subsistence farming for money and food. The clinic is part the Primary Health Clinic in KwaMsane, a large formal and informal settlement on a major highway. This nurse-led and doctor-supervised clinic sees approximately 300 patients with STIs each month. All are diagnosed and treated according to the Provincial Health Department's STI syndromic management protocols.

Patients

Women presenting to the clinic between March 1999 and February 2000 with symptoms of vaginal discharge, lower abdominal pain, urinary symptoms or vulvovaginal itching were treated at the clinic. Women were invited to participate in the study after informed consent was obtained. Trained interviewers collected data and a trained HIV counselor counseled all patients before and after testing.

All women underwent a full clinical examination and microbiologic work up for discharge-causing pathogens. According to Provincial Health Department protocols, all were treated on the same day with a single 250-mg dose of ciprofloxacin, a single 2-g dose of metronidazole and doxycycline (200 mg daily for 7 days). Pelvic inflammatory disease was diagnosed if lower abdominal tenderness and cervical excitation tenderness were elicited on examination, and vaginal discharge, dysuria or vulvovaginal itching was reported. For these women, the dose of metronidazole was increased to 400 mg three times a day for 5 days. Women with signs of PID and a fever

(temperature of >38^{0}C) were referred to the local hospital.

Women were given an appointment to return 8 to 10 days later when they were assessed clinically for response to syndromic therapy, by asking about how their symptoms had changed. Specimens were also taken again to assess microbial cure if the woman consented to further examination. All patients were provided with their baseline microbiologic results and offered posttest counseling and their HIV results. Field workers attempted to trace all patients not returning for follow up.

The University of Natal Ethics Committee provided ethical approval for the study.

Specimen collection and transport
After cleaning of the ectocervix, a calcium alginate and a Dacron swab were sequentially inserted 2 to 3 cm under direct vision into the endocervix and withdrawn while being rotated. The calcium alginate swab was put into GC broth (constituents of NYC medium minus agar) and placed in a polystyrene box at room temperature. The Dacron swab was used to make a smear for chlamydia microscopy. A specimen from the lateral wall of the posterior fornix was collected using a Dacron swab. A smear was made for Gram staining, after which the swab was placed in Diamond's media. Blood for HIV testing was collected in EDTA tubes and mid-stream urines were obtained for nitrite-leucocyte esterase dipstick tests and culture. Appropriate storage temperatures were maintained and specimens were transported within 8 hours of collection to the Africa Centre STD Laboratory in Durban for further processing.

Processing of Specimens
New York City (NYC) plates (Oxoid Ltd.™, Basingstoke, Hampshire, England) incubated at 37^{o}C in CO_2 for 48 hours were used for the isolation *N.gonorrhoeae*. *C.trachomatis* infection was diagnosed by means of direct immunofluorescence (MicroTrak, Trinity Biotech ™, Ireland). *T.vaginalis* was grown in home made Diamond media. Cultures were read every day for up to five days or until positive. Nugent Gram stain score was used to quantify abnormal vaginal flora.[11] Bacterial vaginosis was defined by a score of $\geq$ 7. The presence of yeasts on Gram stain was also recorded. The Determine-HIV screenings test was used to detect HIV antibodies. Positive tests were confirmed an ELISA (Vironostika HIV Uni-Form II *plus* 0; Organon™ Teknika). A random sample of 10 % of the negative specimens was also subjected to ELISA confirmation.

Response to treatment
Clinical cure was defined as absence of presenting symptoms, and microbiological cure as the absence of a STI pathogen at follow-up. For bacterial vaginosis cure was defined as a Nugent score < 7 on Gram staining.

Statistical methods
Prevalence of infections at baseline and after treatment are reported as the proportion of women with any one of the three organisms and bacterial vaginosis. We also report the number of women with any infection and with multiple infections. Association with HIV infection was assessed using the chi square test with the Mantel – Haenszel correction. Statistical significance was set as $p<0.05$, and relative risks and 95% confidence intervals are reported.

Results

In all, 692 consenting females, with a mean age of 24 years (range, 15-70 years), presented to the clinic within the study period. The mean age at coitarche was 17 years (range, 11-36 years). A previous history of discharge/dysuria and/or genital ulcers was reported by 314/692 patients (46%) and 116/692 (18%), respectively.

Vaginal discharge was the most common presenting symptom (91%), followed by dysuria (45%), lower abdominal pain (41%),

and vulvovaginal itching (40%). However, only 16 % of patients presented with a single complaint. Discharge was the sole complaint of 15 % of those with this symptom. These symptoms showed no association with any of the discharge etiologies or HIV infection. Of the 311 patients with urinary symptoms, 21 had a positive nitrite and/or leucocyte esterase test. Only 14 (4%) with such symptoms had a positive urine culture; 13 yielded *Escherichia coli* and 1 yielded *Proteus mirabilis*.

At the initial visit, 519 (75%) were infected with any one of the three organisms or had BV (Table 1). Prevalence of HIV-1 infection in the cohort was 56%. Infection with all three organisms and with BV was more frequent among HIV-1-infected women (Table 1). Women with multiple infections were also more likely to be HIV-1-infected.

TABLE 1. Baseline Prevalence of Discharge Etiology in HIV-1-Infected (n = 387) and HIV-Uninfected Women (n = 305)

Infection	Number (%) With Infection			Relative Risk	95% CI	*P* Value
	Total	HIV-Positive	HIV-Negative			
Bacterial vaginosis	479 (69)	302 (78)	177 (58)	1.34	1.21–1.50	<0.001
Trichomonas vaginalis	203 (29)	127 (33)	76 (25)	1.32	1.03–1.68	0.02
Neisseria gonorrhoeae	86 (12)	62 (16)	24 (8)	2.04	1.30–3.18	0.001
Chlamydia trachomatis	73 (11)	58 (15)	15 (5)	3.05	1.76–5.27	<0.001

Follow up

Of those with an infection, 415 (80%) returned for follow-up 8 to10 days later. Estimates of clinical cure were done on all 415. Microbiologic cure was assessed among the 290 (70%) who consented to a second examination (Figure 1).

Clinical cure was reported by 66 % of the 290 women, ranging from 65 to 69% for those infected with each of the three organisms and for BV (Table 2). The clinical response in the 48 women with PID was 60 % and in those without PID it was 70 % (p= 0.4), independent of HIV status.

Microbiologic cure ranged from 80% for *N.gonorrhoeae* infection to 89% for *C.trachomatis* infection (Table 2) but was only 38% for BV. However, when stratified by HIV status (Table 3), HIV-1- infected women with BV were less likely to be microbiologically cured (28% vs 52%; RR, 1.49 [95% CI, 1.19 –1.87]; p<0.001). Microbiologic cure rates for *T.vaginalis*, *N.gonorrhoeae*, and *C.trachomatis* infections were independent of HIV status.

Discussion

The data suggest that in this setting, co-infection with HIV-1 does not have an impact on the response to the syndromic approach used for non-ulcerative symptomatic STIs in women. However, there was an association of HIV-1 infection and lack of microbiologic response of BV to therapy. The latter may have important implications for syndromic management as an HIV control strategy.

Microbiologic cure ranged from 80 to 89% for the three discharge-causing STIs.

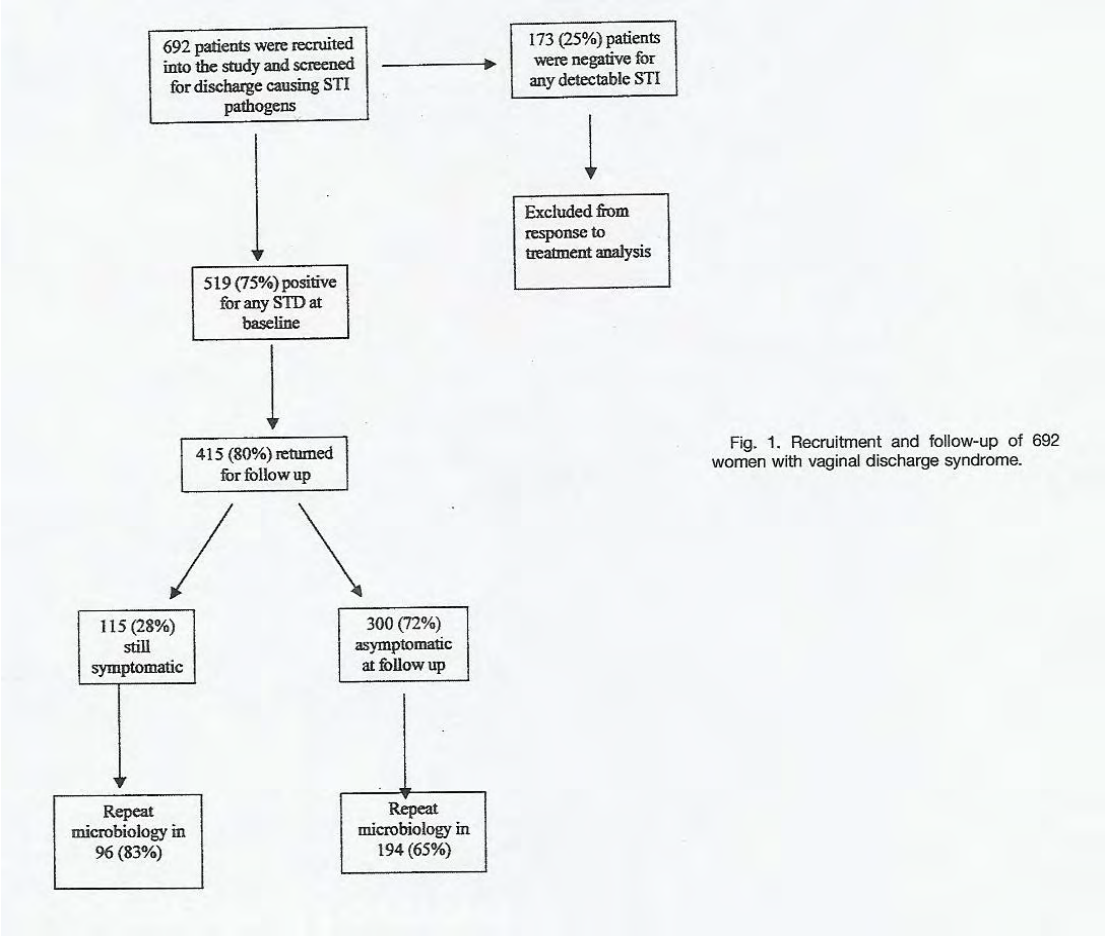

Fig. 1. Recruitment and follow-up of 692 women with vaginal discharge syndrome.

Poor adherence to an otherwise effective therapy may explain these observations. As ciprofloxacin and metronidazole were both given as single doses in the clinic, this seems unlikely. Reinfection by the sexual partner is a likely explanation. This would indicate failure to use the condoms and partner treatment cards that are supplied and promoted and would suggest the health promotion messages provided were not

TABLE 2. Clinical and Microbiological Response to Treatment in 290 Women With Identifiable Discharge Etiology

STD Pathogen/Condition	No. (%) of Women Whose Infections Were Clinically Cured	Microbiologically Eradicated
Bacterial vaginosis (n = 244)	161 (66)	93 (38)
Trichomonas vaginalis (n = 113)	73 (65)	98 (88)
Neisseria gonorrhoeae (n = 51)	33 (65)	41 (80)
Chlamydia trachomatis (n = 44)	30 (69)	39 (89)

TABLE 3. Cure Rates of Sexually Transmitted Infections and Bacterial Vaginosis Among HIV-1-Infected and HIV-Uninfected Women With Vaginal Discharge

Organism/Condition	Number (%) Cured HIV-Positive	HIV-Negative	*P* Value
Bacterial vaginosis	41/144 (28)	52/100 (52)	< 0.001
Trichomonas vaginalis	57/63 (90)	41/50 (82)	0.3
Neisseria gonorrhoeae	26/32 (81)	15/19 (78)	0.9
Chlamydia trachomatis	28/33 (85)	11/11 (100)	0.25

complied with. Failure to eradicate *C.trachomatis* might reflect a compliance problem.

It is also possible that the recommended drugs failed to eradicate infection. On the basis of MIC values available at the time of introduction of syndromic management, as well as on cost considerations, the provincial department of health advocated the use of 250 mg instead of the widely advised 500 mg of ciprofloxacin for treatment of *N.gonorrhoeae* infections. It has been suggested that this lower dose is associated with higher treatment failure. However, the evidence for this is unsubstantiated, and raising the dose of ciprofloxacin from 250 mg to 500 mg to cater for the truly fluoroquinolone- resistant gonococci of the future, would be unlikely to decrease the failure rate. With use of the NCCLS criteria, the MICs of ciprofloxacin against the pretreatment and posttreatment isolates of *N.gonorrhoeae* were within the susceptible range, and opa typing of these strains suggest reinfection by different partners as the most likely cause of failure (accepted in Journal of Clinical Microbiology: Manuscript No: JCM 389-02).

Metronidazole in its varying dosage regimens for the treatment of BV has been shown to provide short-term remission in 50% to 85% of cases.[12,13] Therefore, the high treatment failure rate for BV in this group may in part reflect inadequate treatment of this condition. In addition, the 7-day course of doxycycline could theoretically inhibit recolonisation of the genital tract with lactobacilli-predominant vaginal flora. However, neither of these arguments explains the selective response in HIV- negative women and this association with HIV-1 infection suggest that this virus interferes with normalization of the vaginal ecology.

As with antimicrobial treatment of other infections, the short-term response of infections with *N.gonorrhoeae, C.trachomatis, T.vaginalis,* or the combination of these three was not affected by HIV status. However, the association of BV treatment failure with HIV-1 infection is of concern. BV is the most prevalent cause of vaginal discharge in Africa and has been associated with HIV infection [14, 15] and transmission. [16, 17] Our finding that in a large number of HIV-1-infected women the vaginal flora does not normalise despite syndromic treatment implies that the risk of HIV transmission and acquisition remains particularly high.

Since the presence of microbial DNA does not differentiate between viable and killed microbes, we did not use PCR- based methodology for the diagnosis of *T.vaginalis, N. gonorrhoeae,* and *C. trachomatis* but the less sensitive microscopy and culture. The discrepancies between symptomatology at baseline and microbe detection and in clinical cure and eradication rates of these pathogens may in part be attributed to the insensitivity of the tests used. This is especially so in the case of chlamydial infection. The test used is insensitive, and the follow-up period of 8 to10 days may have been too brief to allow for clearance of inclusions from the original infection.

As with other studies of the diagnostic utility of syndromic management protocols,[6] 25% of the women presenting with symptoms suggestive of an STI had no cause defined microbiologically. The symptoms associated with non-ulcerative genital disease in women have a broad differential diagnosis, including conditions of a non-STI nature. Our data reaffirm the nonspecific nature of the symptoms associated with nonulcerative genital disease in women. Women with this syndrome may present with one or more of the following: discharge, dysuria , vulvovaginal itching, and lower abdominal pain. Since each of these manifestations is rather nonspecific, it is not always easy for the patient or health care worker to decide that there is an abnormal situation. This supports the concern that syndromic management protocols have limited diagnostic utility

among this group. Of the infected women, the majority returned for follow- up as requested. It seems likely that those not returning had been clinically cured. The overall cure rate in the whole cohort is therefore likely to be higher than 67%. However, the problems associated with the utility of symptoms as a trigger for syndromic management also limits the measurement of response to this strategy.

While this study shows that HIV-1 does not affect response to syndromic management of the STIs, about 20% of women still harbor a pathogen 8 to10 days following treatment. This allows for the continued transmission of STIs, including HIV-1. Currently, however, syndromic management remains the only realistic treatment option in this area.

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Impact of HIV-1 Infection on Response to Treatment of Sexually Transmitted Infections

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It has been suggested that infection with HIV-1 might reduce the response to antibiotic treatment of curable sexually transmitted infections [1]. However evidence to support this is lacking. The possibility of reduced response to therapy among HIV-1 infected patients is of critical importance. This is especially so in the developing world where the prevalence of STIs and HIV-1 is very high, co-infection is common, and STIs are likely co-factors for HIV-1 transmission [2].

We studied this issue among women presenting with vaginal discharge to a primary care sexually transmitted disease service in the rural Hlabisa health district in South Africa. Laboratory confirmed infections with *Neisseria gonorrhoea* (GC) (culture on New York medium), *Chlamydia trachomatis* (CT) (tissue culture and direct immunofluoresence), *Trichomonas vaginalis* (TV) (culture on Diamonds medium), bacterial vaginosis [BV] (score > 7 on Nugents criteria from a Gram stain) or a combination of these, were identified in 554 women. All women were treated according to the provincial syndromic management protocols, which consist of ciprofloxacin 250 mg stat, doxycycline 200 mg daily for 7 days, and metronidazole 2 g stat (increased to 400 mg three times a day for 5 days if the patient complained of lower abdominal pain).

Of the 554 women, 412 (74%) returned for follow up 7 - 10 days later. Of these, 129 (31%) declined further specimen collection. Of the remaining 283, 163 (58%) were HIV-1 infected. Cure rates (defined as microbiologically proven eradication) were 30% in the HIV-1 infected and 45% in the HIV-1 uninfected (Table 1). There was no difference in cure rates between HIV-1 infected and uninfected women for TV, CT or GC or for all three organisms combined (Table 1). However, the cure rate for BV was substantially lower among the HIV-1 infected women (28,5% vs 52%, p = 0.001).

These data suggest that HIV-1 infected and uninfected women with a vaginal discharge experience equivalent response to recommended syndromic treatment, with the exception of bacterial vaginosis. Bacterial vaginosis is the most prevalent cause of vaginal discharge in Africa, and has been strongly associated with HIV-1 infection [3-6]. As such, with low cure rates for bacterial vaginosis, the risk of HIV-1 transmission

Table 1. Cure rates of sexually transmitted infections and bacterial vaginosis among HIV-1-infected and uninfected women with vaginal discharge.

	Number (%) cured HIV status		
Organism/condition	HIV positive	HIV negative	*P* value
Any STI (including BV)	49/163 (30%)	54/120 (45%)	0.01
N. gonorrhoeae	26/32 (81%)	15/19 (80%)	0.9
C. trachomatis	28/33 (85%)	10/10 (100%)	0.25
T. vaginalis	56/62 (90%)	41/50 (82%)	0.31
Any of the three	81/101 (80%)	55/70 (79%)	0.8
Bacterial vaginosis	41/144 (28.5%)	52/100 (52%)	0.001

BV, Bacterial vaginosis; STI, sexually transmitted infection..

may be particularly high. Possible reasons for this differential response to therapy require further investigation. However, our data do provide important reassurance that

HIV-1 infection does not reduce the response to antibiotic treatment of the 3 organisms studied, although further studies are warranted to confirm this.

The questions remains, as to why recovery from the only form of vaginal discharge without an identified aetiology, is influenced by HIV-1 infection, whilst those with known aetiologies are not. A general immunological breakdown is an unlikely explanation for this, since the patients we report on did not have AIDS. In addition, the 3 major pathogens showed no significant difference in their eradication rates. It is therefore tempting to speculate that selective immune suppression, directed towards the unknown causes of bacterial vaginosis, is responsible. Therefore, this question may only be answered if we have identified the cause or causes of bacterial vaginosis.

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Association between HIV-1 infection, the etiology of genital ulcer disease and response to syndromic management

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ABSTRACT

Reports on the effect of HIV-1 infection on healing rates of ulcers are conflicting. The goal of the study was to determine the etiology and response to treatment of genital ulcer disease(GUD) in relation to HIV-1 infection. The study was a cohort study of patients with GUD, treated using local syndromic management(SM) protocols. Of the 587 recruited, the prevalence of HSV, *T.pallidum*, *C.trachomatis* (LGV), *H.ducreyi*, *C.granulomatis* and HIV-1 was 48%, 14%, 11%, 10%, 1% and 75% respectively. The prevalence of *T.pallidum* was higher among men (p=0.03) and an association was seen among HIV-1 seronegatives on univariate and multivariate analyses (p<0.001; p=0.01). The prevalence of *C.trachomatis* (LGV) was higher among females (p=0.004), and an association was seen among HIV-1 seropositives on univariate analysis (p=0.04). At follow up, 40/407(10%) showed a decreased healing tendency, not associated with ulcer aetiology or HIV-1 seropositivity. Response to SM of GUD was acceptable and not associated with HIV-1 co-infection.

Introduction

Genital ulcer disease (GUD) has been recognised as an epidemiological and biological risk factor for the transmission of HIV-1.[1,2,3] This relationship underscores the need for effective management of these lesions as part of HIV control strategies.

The clinical diagnosis of genital ulcers has been shown to be imprecise in terms of linking clinical observations with etiological diagnosis.[4,5,6] Classic laboratory based etiological diagnosis is impractical and also lacks sensitivity.[7] The fastidious nature of the pathogens requires transport under optimal conditions. Detection is labour intensive and slow resulting in a delay in obtaining results and instituting treatment. Although the newer PCR based tests make collection and transport of specimens less demanding and more sensitive,[8] these tests are expensive and require specialised laboratory facilities and trained personnel. In an effort to address the above limitations and improve case management of STDs, World Health Organisation introduced the syndromic management approach for the treatment of genital ulcers.[9]

The etiology of GUD is broad and has been described in different areas of the world with varying prevalences.[10-16] The causative agents include herpes simplex virus, *Treponema pallidum, Chlamydia trachomatis* (LGV), *Haemophilus ducreyi* and *Calymatobacterium granulomatis*. The syndromic management of GUD includes drugs active against the four bacteria. Thus far lesions caused by Herpes simplex virus are not treated.

Recent studies suggest a change in the prevalence patterns of the different causative agents of GUD in relation to HIV-1 infection. Some observations also suggest a more severe and prolonged clinical presentation as well as a decreased response to treatment in HIV-1 infected people.[2,17-20]

We determined the etiology of genital ulcer disease in KwaZuluNatal and evaluated the response to syndromic treatment clinically and microbiologically in relation to HIV-1 infection.

Methods

Patients

Consenting patients presenting to the STD clinic at the Prince Cyril Zulu Communicable Diseases Clinic in Durban (formerly the Durban City Health STD Clinic) from October 2000 to March 2001with genital ulcers were enrolled. Patients with a history of antimicrobial use four weeks prior to their current visit were excluded. A trained study nurse administered a standardised questionnaire. Following a general examination, a research medical officer examined the genitalia and lower abdomen of the patients, and recorded the presence and site /absence of lymph nodes, number of ulcerative lesions as well as site of the lesions. In women, a speculum was passed to look for the presence of internal ulcerative lesions. Specimens were collected from the lesions at the initial visit. All patients were treated according to the local syndromic management guidelines for GUD, which comprises penicillin 2.4mU intramuscularly, as well as erythromycin 500mg four times a day for 5 days. Patients were asked to return 8 to 10 days later. The ulcers were evaluated clinically. Repeat specimens were collected from lesions with decreased healing tendency. Treatment was repeated and these patients were asked to return a week later. Venous blood for repeat syphilis serology was also collected from these patients.

This study was approved by the Ethics Committee of the Nelson R Mandela School of Medicine.

Specimen collection and preparation

After cleaning of the ulcer with a sterile dry gauze, impression smears were made onto glass slides for the detection of *C.granulomatis* (CG) by Rapidiff staining.

A specimen was collected by scraping the ulcer base and edges with a sterile disposable plastic loop (Quad loop, Bibby Sterilin, UK). The material was suspended in 1.0 mL PBS, pH 7.6. DNA was extracted from a 0.4 mL aliquot of the PBS suspension using the Qiagen Blood and Tissue Mini Kit (Qiagen, USA). Venous blood was collected for HIV serology as well as syphilis serology.

PCR

PCR for *T.pallidum*, *H.ducreyi*, and Herpes simplex virus 2 (HSV-2) were performed as previously described.[21] PCR for the human □-globin gene was performed on all cases with negative PCR results for all four organisms.[22] Those with a negative □-globin gene were excluded from further analysis. *C.trachomatis* was detected by PCR using previously described primers that target the 60-kDa cysteine-rich outer membrane protein (CrP), followed by LGV-specific digestion with *Acc*I. [23]

HIV and syphilis serology

HIV infection was diagnosed using Determine™ HIV1/2 (Abbott Laboratories, USA). All positive results were confirmed by a second serological test (Capillus™ HIV1/2, Trinity Biotech, USA).

The serological diagnosis of syphilis was based on test results obtained with the Rapid Plasma Reagin test (Becton Dickinson, USA), IgM capture EIA (Meddens diagnostics B.V., The Netherlands), FTAabs (Diagnostic & Technical Services c.c.,South Africa) and TPHA (Omega Diagnostics Limited, United Kingdom).

Definitions

Ulcers were classified as having "decreased healing tendency" if these were still exudative, with no evidence of re-epithelialization on inspection 8 to 10 days following enrolment in the study.
Primary syphilis was defined as a positive PCR result from the ulcer specimen. A negative PCR result in combination with one of the following two serological patterns was also considered to be primary syphilis:
i) FTAabs positive and IgM (Capture syphilis-M EIA) positive, irrespective of TPHA result
ii) FTAabs positive and RPR positive, with a negative TPHA and IgM.

Statistics

Significant risk factors associated with ulcer disease were identified using chi square test for categorical data and Wilcoxon rank sum test for quantitative data. Relative risk and 95% confidence intervals are reported. Factors found significant at the univariate level were included in a logistic model. Data were analysed using SAS statistical software version 6 (1997 SAS Institute, Cary, NC, USA).

Results

Of the 587 patients enrolled in the study, the ratio of male: female was 3:1. Males were significantly older (p=0.04) with a higher number of lifetime sexual partners (p=0.001) as well as partners in the last four weeks (p=0.001). Seventy five percent of this cohort was infected with HIV-1, with the prevalence in women being significantly higher (p=0.001) (Table 1).

The overall prevalence of HSV, *T.pallidum*, *C.trachomatis* (LGV), *H.ducreyi* and *C.granulomatis* was 48%, 14%, 11%, 10% and 1% respectively. No microbiological diagnosis was obtained in 27% (156/587).

The median duration of ulcerative lesions in men and women was 7days (range: 1-365 and 1-90 respectively). When stratified by aetiology, duration of symptoms for 7 days and less was associated with HSV in males (p = 0.006). There was no association in females. This association remained significant after adjusting for possible confounders (HIV, number of lesions, site of lesions and lymphadenopathy) (p = 0.0001). Duration of symptoms for more then 7 days was associated with *C.trachomatis* (LGV) in

Table 1:Demographic data of men and women with genital ulcers attending a STD clinic

	male (n=438)	female (n=149)	p-value
median (range) age in years	26 (16-64)	25(16-47)	0.04
median (range) number of lifetime partners	10 (1-100)	3 (1-30)	0.001
>1 partner in last 4 weeks	158 (36%)	5(3%)	< 0.0001
Median duration (range) of ulcers in days	7 (1-365)	7 (1-90)	-
HIV-1 seropositive	313(71%)	128(86%)	0.0004

males (p = 0.02) and females (p = 0.03). This association remained significant after adjusting for possible confounders (HIV, number of lesions, site of lesions and lymphadenopathy) in both males (p = 0.04) and females (p= 0.03).

The prevalence of *T.pallidum* as a single infection and in a mix was significantly higher in men (p=0.01 and p=0.03 respectively) (Table 2), and among those not infected with HIV-1 (p < 0.001) (Table3). The negative association with HIV remained significant after controlling for possible confounders for its acquisition: number of lifetime partners, number of lesions (p = 0.01). When patients diagnosed on syphilis serology only were excluded, a similar picture was found for association with HIV (p=0.004).

LGV as a single infection was higher in females (p=0.06) (Table2) and was significantly higher among HIV infected (p=0.004) (Table3). When mixed infections were added, the overall prevalence of *C.trachomatis* (LGV) became significantly higher in women (p=0.004) and remained significant among those infected with HIV-1 (p = 0.04). After controlling for confounders for HIV acquisition (number of lifetime partners, number of lesions, duration of lesions), the association was not significant (p= 0.17).

Men were at greater risk for infection with *H.ducreyi* when it occurred as a single infection (p=0.02)(Table 2). This association however did not reach statistical

Table 2: Prevalence (%) of genital ulcer causing pathogens in men and women attending a STD clinic

Pathogens	Total	Male	Female	RR	p-value
	n=587	n=438	n=149	(95%CI)	
HSV	234 (40)	175 (40)	59 (40)	1.0 (0.9-1.1)	0.9
TP	42 (7)	38 (9)	4 (3)	1.2 (1.1-1.4)	0.01
LGV	43 (7)	27 (6)	16 (11)	0.8 (0.7-1.0)	0.06
HD	45 (8)	40 (9)	5 (3)	1.2 (1.1-1.4)	0.02
CG	4 (0.6)	3 (0.6)	1 (0.6)	1.0 (0.6-1.8)	1.0
HSV + TP	28 (5)	23 (5)	5 (3)	1.1 (0.9-1.3)	0.3
HSV +LGV	8 (1)	3 (0.6)	5 (3)	0.5 (0.2-1.2)	0.03
HSV + HD	8 (1)	5 (1)	3 (2)	0.8 (0.5-1.4)	0.4
HSV + CG	3 (0.5)	2 (0.4)	1 (0.6)	0.9 (0.4-2.0)	0.6
LGV + HD	1 (0.1)	-	1 (0.6)	-	0.08
TP + HD	2 (0.3)	2 (0.4)	-	1.3 (1.3-1.4)	0.4
TP + LGV	10 (2)	7 (1.5)	3 (2)	0.9 (0.6-1.4)	0.7
HSV + TP + LGV	1 (0.1)	-	1 (0.6)	-	0.08
HSV + TP + HD	1 (0.1)	1 (0.2)	-	1.3 (1.3-1.4)	0.6
HSV + TP + CG	1 (0.1)	1 (0.2)	-	1.3 (1.3-1.4)	0.6
No aetiology	156 (27)	114 (26)	42 (28)	0.9 (0.7-1.2)	0.6

HSV = herpes simplex virus
LGV = *Chlamydia trachomatis* (LGV)
CG= *Calymatobacterium granulomatis*
TP= *Treponema* pallidum
HD= *Haemophilus ducreyi*

significance when mixed infections were added to single infections (p=0.08).

The prevalence of the other ulcer pathogens was similar in HIV-1 infected and uninfected patients. The prevalence of ulcers with no detectable etiology also did not differ in HIV-1 infected and uninfected patients. (Table 3).

Four hundred and seven (69%) patients returned within two weeks for follow up. The prevalence of ulcer pathogens and HIV-1 in this subset was similar to that of the initial cohort. Three hundred and sixty seven (90%) showed evidence of healing. Repeat specimens were collected from 40 of those whose ulcers showed a decreased healing tendency. The ratio of men:women in this group was 2:1 and the prevalence of HIV-1 was 88%. These changes were not significant when compared to those whose ulcers had healed (p=0.3 and p=0.1 respectively).

Table 3: Prevalence (%) of genital ulcer causing pathogens in HIV seropositive and HIV seronegative patients attending a STD clinic

Pathogens	Total	HIV +	HIV -	RR	p-value
	n= 587	n= 441	n= 146	(95%CI)	
HSV	234 (40)	177 (40)	57 (39)	1.0 (0.9-1.1)	0.8
TP	42 (7)	21 (5)	21 (14)	0.7 (0.5-0.8)	< 0.001
LGV	43 (7)	40 (9)	3 (2)	1.3 (1.2-1.4)	0.004
HD	45 (8)	35 (8)	10 (7)	1.0 (0.9 –1.2)	0.6
CG	4 (0.6)	2 (0.4)	2 (1)	0.7 (0.3-1.8)	0.2
HSV + TP	28 (5)	23 (5)	5 (3)	1.1 (0.9-1.3)	0.4
HSV +LGV	8 (1)	8 (2)	0	1.3 (1.3-1.4)	0.1
HSV + HD	8 (1)	6 (1)	2 (1)	1.0 (0.7-1.5)	1.0
HSV + CG	3 (0.5)	1 (0.2)	2 (1)	0.4 (0.1-2.2)	0.09
LGV + HD	1 (0.1)	1 (0.2)	0	1.3 (1.3-1.4)	0.6
TP + HD	2 (0.3)	1 (0.2)	1 (0.6)	0.7 (0.2-2.7)	0.4
TP + LGV	10 (2)	5 (1)	5 (3)	0.7 (0.4-1.2)	0.06
HSV + TP + LGV	1 (0.1)	0	1 (0.6)	-	0.08
HSV + TP + HD	1 (0.1)	0	1(0.6)	-	0.08
HSV + TP + CG	1 (0.1)	1(0.2)	0	1.3 (1.3-1.4)	0.6
No aetiology	156 (27)	118 (27)	38 (26)	1.0 (0.7-1.4)	0.8

HSV = herpes simplex virus
LGV = *Chlamydia trachomatis* (LGV)
CG= *Calymatobacterium granulomatis*
TP= *Treponema* pallidum
HD= *Haemophilus ducreyi*

The prevalence of the ulcer pathogens in patients returning for follow up stratified by healing tendency is shown in Table 4. Although decreased healing tendency was not associated with ulcer aetiology, patients with HSV as a single infection demonstrated a higher healing tendency [1.07 (1.0-1.1) (p=0.04)]. When mixed HSV infections were added to the single infections, this association was lost (p=0.3).

Eighteen (9%) of the 204 patients with HSV, had ulcers with decreased healing tendency at follow up. HSV was detected in 13/18 follow up specimens, while no pathogen could be detected in 5/17.
Of the 40 patients with *C.trachomatis* (LGV), 6(15%) had ulcers with decreased healing tendency. Three of these were co-infected with HSV and one with *T.pallidum* (Table 4). None of these carried *C.trachomatis* at follow-up: no pathogen could be detected in 3 and HSV was present in the other 3 patients at the subsequent visit.

Table 4: Baseline prevalence (%) of genital ulcer aetiology of patients returning for follow up stratified by healing

	Patients followed up		
Pathogens	Total	Healing ulcers	Delayed healing
	n= 407	n=367	n=40
HSV	172 (42)	161 (44)	11 (27.5)
TP	28 (7)	25 (7)	3 (7.5)
LGV	29 (7)	27 (7)	2 (5)
HD	30 (7)	28 (8)	2 (5)
CG	3 (1)	3 (1)	-
HSV + TP	22 (5)	19 (5)	3 (7.5)
HSV +LGV	5 (1)	2 (0.5)	3 (7.5)
HSV + HD	4 (1)	3 (1)	1 (2.5)
HSV + CG	1 (0.2)	1 (0.2)	-
LGV + HD	2 (0.5)	2 (0.5)	-
TP + HD	1 (0.2)	1 (0.2)	-
TP + LGV	4 (1)	3 (1)	1 (2.5)
Any aetiology	301	275	26
No aetiology	106	92	14

RR 1.05 (0.97 – 1.11)
p =0.17

HSV = herpes simplex virus
LGV = *Chlamydia trachomatis* (LGV)
CG= *Calymatobacterium granulomatis*
TP= *Treponema* pallidum
HD= *Haemophilus ducreyi*

Seven of the 55 patients (13%) with *T.pallidum* showed decreased healing of their ulcers at follow-up. Three of these were co-infected with HSV and 1 with LGV. At follow up, 1 still carried HSV, while in 6 no aetiology could be detected.

Eight percent (3/37) of the patients with *H.ducreyi* at initial visit, had ulcers with decreased healing tendency. One had co-infection with HSV. At follow up: *H.ducreyi* was still detected in 1, HSV in 1 and *T.pallidum* in 1.

Ulcers with a decreased healing tendency were seen in 14 (14%) of the 106 patients with no identifiable aetiology who returned for follow up. Of these, 9 had no detectable pathogen, 2 HSV-2, 1 *H.ducreyi* and 2 *T.pallidum* at the subsequent visit.

The prevalence of the ulcer pathogens stratified by HIV-1 infection in patients with ulcers that showed a decreased healing tendency is shown in Table 5. Co-infection with HIV-1 did not predispose to a decreased tendency for ulcers caused by any

Table 5: The prevalence of the ulcer pathogens stratified by HIV infection in patients whose ulcers showed a decreased healing tendency

Pathogens	Total	HIV +	HIV-
	n=40	n=35	n=5
HSV	11	8	3
TP	3	1	2
LGV	2	2	0
HD	2	2	0
HSV + TP	3	2	1
HSV +LGV	3	3	0
HSV + HD	1	1	0
TP + LGV	1	1	0
No aetiology	14	14	0

HSV = herpes simplex virus
LGV = *Chlamydia trachomatis* (LGV)
CG= *Calymatobacterium granulomatis*
TP= *Treponema* pallidum
HD= *Haemophilus ducreyi*

of the recognised pathogens or those with no detectable aetiology to heal.

On further follow up of patients with non-healing ulcers, 2 showed no evidence of healing in 4 subsequent visits spanning 6 weeks. The initial aetiological diagnosis for GUD in these HIV-1 sero-positive patients was genital herpes. No etiological agent could be detected from ulcer material collected at subsequent visits. Biopsy from these lesions showed non-specific inflammatory response and no HSV inclusion bodies were detected. These ulcers were painful, and displayed evidence of healing at 7 and 8 weeks respectively after the initial visit.

Discussion

The clinical response of genital ulcer disease to syndromic management in this part of the world with a high prevalence of both GUD and HIV-1 infection is acceptable. There have been conflicting reports of HIV-1 infection slowing the healing rates of ulcers despite appropriate antibiotic therapy. Our data do not confirm these observations. Although 75% of this cohort was infected with HIV-1, 90% showed healing of the ulcer within 10 days. This suggests that co-infection with HIV-1 does not impact on response to treatment. However, the level of immunosuppression, which was not measured in this group of patients, could influence response to treatment. This question is currently being investigated.

HSV was the most prevalent cause of GUD among these patients. Although drugs for the treatment of lesions due to HSV are not included in SM protocols, only 9%(18/204) of ulcers caused by HSV showed a decreased healing tendency. The tendency to decreased healing in this group was not influenced by HIV-1 co-infection. It is debatable whether inclusion of drugs active against HSV in these protocols will impact on shortening the course of disease, thereby curbing transmission of this virus as well as HIV-1. In our setting, adaptation of the SM protocols to include antiviral therapy for HSV is likely to be futile in the absence of patient education on early symptom recognition of this disease.

We found that two patients showed very slow healing tendency. Both were HSV-2 infected and HIV-1 sero-positive. The role of HSV specific therapy in such individuals or in those with frequent recurrences is unclear. Both our patients recovered after several weeks without such treatment.

Our definition of primary syphilis excludes single FTAabs positives. In our experience, for primary syphilis, this test is prone to inter-observer variation. We therefore took an FTAabs as indicative for primary syphilis if confirmed by IgM capture ELISA or RPR. Using our definition we found an independent negative association with HIV-1 infection. This could be the result of an incorrect definition of primary syphilis, or a muted antibody response in HIV-1 infected patients. However, adding the single, unconfirmed FTAabs positives or removing the ulcer PCR negatives, seropositive patients from our definition of primary syphilis did not alter this picture. Others, using PCR detection, have also reported primary syphilis in association with the absence of HIV-1 sero-positivity.[24] This observation needs further exploration.

The overall prevalence of ulcers caused by *C.trachomatis* (LGV) was 11 % and of those 40 LGV patients returning for follow up, 15 % showed a decreased healing tendency. However, no *C.trachomatis* DNA was detectable in these ulcers at follow up. Heat shock proteins of *C.trachomatis* could be responsible for a delayed fading of the inflammatory response to this organism.[25]

The prevalence of chancroid was higher in males. This is plausible since *H.ducreyi*, has a high affinity for keratinocytes and prefers a lower temperature .[26] The male genitalia provide a large keratinised surface area at the optimal temperature.

It may be argued that the positive PCR results obtained at follow up could represent dead microbes. However, this is unlikely since these ulcers were all non-healing and the PCR was negative in a considerable number of slowly healing ulcers with the same aetiology at baseline. Organisms that were not detected at baseline were found in five patients with delayed healing and in another two, a different pathogen was found. It is possible that in these patients, an initial aetiology was missed, either due to inadequate collection of material, or a false negative test result. However, the former is unlikely since all patients with a β-globin negative PCR were excluded. If PCR can be used to measure short-term microbial clearance, then these represent new infections. Whether PCR can be used for this purpose warrants further investigations.

In conclusion, we found no association between ulcer etiology and healing tendency for ulcers caused by organisms covered by the antimicrobial regimen. Spontaneous healing of genital herpes was also not impaired. The number of patients who failed treatment is too small to draw firm conclusions on the effect of HIV-1 on response to therapy, but the data presented suggest that such an association, if it exists, is not very strong.

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Chapter 2

Antimicrobial Issues in gonorrhoea

Summary

Neisseria gonorrhoeae has developed resistance to a wide range of antimicrobials. More importantly, from a management perspective, we show the emergence of Tet-M mediated tetracycline resistance as well as increasing MICs to ciprofloxacin and azithromycin.

In addition, the data supports the use of the 250mg dose for the treatment of *Neisseria gonorrhoeae* as opposed to the WHO recommended 500mg dose in this setting.

Evolution in the Trends of Antimicrobial Resistance in *Neiseria gonorrhoeae* isolated in Durban Over a 5 year period: Impact of the introduction of syndromic management

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ABSTRACT

Antimicrobial susceptibility testing was performed on isolates of *Neisseria gonorrhoeae* obtained from patients attending the City Health STD clinic in Durban, KwaZuluNatal, to the following drugs: penicillin, tetracycline, ciprofloxacin, ofloxacin, ceftriaxone, spectinomycin, erythromycin and azithromycin. These isolates were collected over a six year period from 1995 to 2000. Four hundred and fifteen strains were tested: 61 in 1995, 198 in 1997, 98 in 1998/99 and 58 in 1999/2000. A shift to the right is observed in the susceptibilities of *N. gonorrhoeae* to the currently recommended drugs in the syndromic management guidelines viz. penicillin, tetracycline, ceftriaxone, ciprofloxacin, spectinomycin and erythromycin. The prevalence of penicillinase producing *N. gonorrhoeae* (PPNGs) is currently around 30% while that of plasmid mediated tetracycline resistant *N. gonorrhoeae* (TRNGs) is around 50%. There is a definite association between the MICs of strains falling within the penicillin and tetracycline chromosomally resistant group, and strains exhibiting a decreased susceptibility to ciprofloxacin and ceftriaxone. The minimum inhibitory concentrations (MICs) for azithromycin showed a similar distribution of MICs when compared to erythromycin for 1999/2000 isolates. We postulate that the presence of efflux pumps might play a role in the increasing MICs that we observe among structurally unrelated groups of drugs. Furthermore, widespread use of these antimicrobials in the community may offer a selective advantage to the development of resistance. The implications of this are far reaching and the local susceptibility trends of *N. gonorrhoeae* need to be constantly monitored to direct therapy.

Introduction

Decreasing the prevalence of sexually transmitted diseases (STDs) is a priority in developing countries, especially in large parts of Africa where the prevalence of HIV and STDs have reached astronomical proportions.[1,2] In resource poor settings, as is the case for most of KwaZuluNatal, South Africa, the diagnosis and control of STDs has been hampered by the lack of appropriate laboratory support and trained health care personnel. For the want of an appropriate point-of-care test with good sensitivity and reasonable specificity to detect the common STD pathogens, WHO introduced treatment guidelines for the syndromic management of symptomatic patients.[3]

The syndromic approach uses clinical algorithms based on the patient's symptoms and clinical signs to determine antimicrobial therapy. However, the aetiology of the different syndromes as well as antimicrobial susceptibility patterns of the microbes involved may vary significantly in different areas or even within the same area. This highlights the need for area specific research when addressing control programmes. Periodic monitoring of the prevalence of organisms and their susceptibility profiles provides essential clues towards the adjustment of local syndromic management guidelines.

In 1995, the Department of Health for KwaZuluNatal adopted and modified the WHO guidelines for the treatment of STDs in the region. The discharge syndrome is treated with ciprofloxacin 250 mg STAT, with doxycycline 200mg daily for 7 days in male, supplemented with metronidazole 2 g STAT in the non-pregnant female. During pregnancy, these drugs are replaced by ceftriaxone, 125 mg intramuscularly STAT and erythromycin 500mg qid for 7 days. Spectinomycin is used in case of □-lactam hypersensitivity. Although these guidelines have been in place for five years, STD control has been perceived as being far from optimal with the area being ill-famed as having one of the highest HIV and STD prevalence rates in the world.

Up until 1993, *N. gonorrhoeae* strains were susceptible to most antimicrobial agents listed in the current syndromic management protocols[4]. We report on trends in susceptibility to these drugs over the last 5 years and relate these findings to the introduction of syndromic management for STDs.

Methods

Isolates of *N.gonorrhoeae* were collected from patients attending the City Health STD Clinic in Durban, South Africa in 1995, 1997, end of 1998/beginning of 1999 and end of 1999/beginning of 2000. Surveillance studies were not conducted in 1996. Isolates were grown from specimens obtained from patients presenting to the clinic with genital discharge syndrome during surveillance studies. Such studies are performed at regular intervals at this clinic. Data on antibiotic consumption and population size were obtained from the KwaZuluNatal Provincial administration and Department of Tourism respectively.

Urethral and cervical specimens were obtained and New York City plates [GC agar base supplemented with yeast autolysate , lincomycin, colistin, amphotericin-B, trimethoprim (Oxoid Ltd. Basingstoke, Hampshire, England) and lysed horse blood] were inoculated for the isolation of *N.gonorrhoeae*. Plates were incubated at 37^{o}C in 5 % CO_2 for 48 hours. Suspected colonies of *N.gonorrhoeae* were identified by means of gram staining, oxidase test, β -galactosidase, hydroxy-prolylaminopeptidase, γ -glutamylaminopeptidase and acid production from glucose, lactose and maltose.

Minimum inhibitory concentrations (MICs) of penicillin, tetracycline, spectinomycin, ceftriaxone, ciprofloxacin, ofloxacin, erythromycin and azithromycin were determined by means of the agar dilution dilution method. Two fold serial

dilutions of antibiotics were added to molten GC agar base (Oxoid Ltd) supplemented with 1% isovitalex at a temperature of 45^0 C. After solidification, these plates were seeded with 10^4 cfu/spot of bacteria by means of a multipoint inoculator and incubated at 37^0 C in CO_2 for 24 hours. *N.gonorrhoeae* (ATCC 49226), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 10418) were used as controls. Erythromycin and spectinomycin were not tested for isolates obtained in 1995. Plates for the MICs of *N. gonorrhoeae* to erythromycin from the 1997, 1998/99 batches were incubated in CO_2. Plates for MICs to erythromycin and azithromycin in the 1999/00 batch were incubated at 37°C in CO_2 as well as at 37°C in air. The CO_2 incubated plates demonstrated 2 fold higher MICs than those incubated in air. MICs from previous batches for the macrolides were therefore adjusted accordingly. [5,6] The antimicrobial susceptibility was judged using breakpoint criteria defined by the National Committee for Clinical Laboratory Standards [7] for all but erythromycin and azithromycin, for which interpretive criteria for *N. gonorrhoeae* are not available. Susceptibility to penicillin and tetracycline were defined as follows: tetracycline susceptible strains (TET-S): MIC $\leq$ 0.25 mg/L; plasmid mediated tetracycline resistant *N. gonorrhoeae* (TRNG): MIC $\geq$ 16mg/L ; chromosomally mediated tetracycline resistant *N. gonorrhoeae* ($CMRNG^T$): MIC = 1-8 mg/L; penicillin susceptible strains (PEN-S): MIC $\leq$ 0.06 mg/L; decreased penicillin susceptible *N. gonorrhoeae* (DPNG): 0.06-4 mg/L in the absence of β-lactamase. Strains testing positive for β-lactamase production by the chromogenic cephalosporin method are referred to as penicillinase producing *N.gonorrhoeae* (PPNG).

Calculations for the comparison of data to illustrate trends was done using the chi-square test for linear trends. Fisher's exact test was used for comparisons between different years.

Results

A total of 415 *N. gonorrhoeae* strains, obtained from different patients were tested: 61 in 1995, 198 in 1997, 98 in 1998/99 and 58 in 1999/2000.

The prevalence of PPNGs doubled from 1995 to 1997 (p = 0.02) and has remained at around 30% since then (Fig.1). The doubling

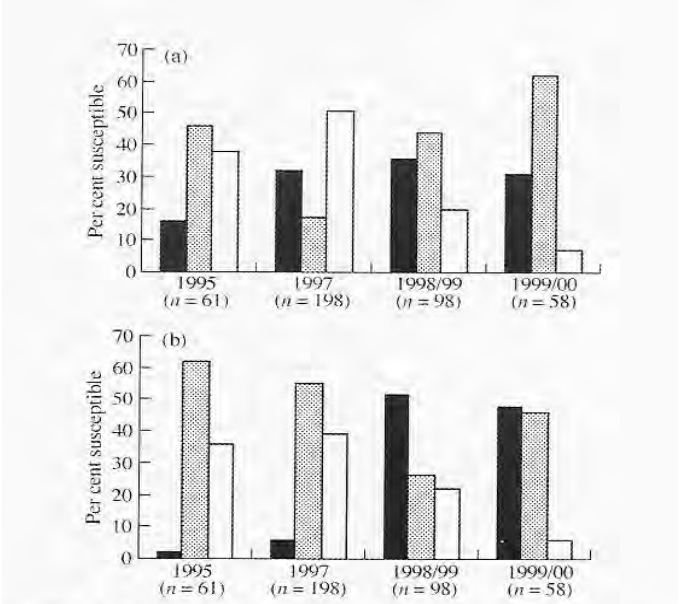

Figure 1. Trends in susceptibility to (a) penicillin (■, PPNG; ▨, DPNG; □, PEN-S) and (b) tetracycline (■, TRNG; ▨, CRNGT; □, TET-S) of *N. gonorrhoeae* isolates over a 5 year period.

of the PPNG prevalence in 1997 was associated with a decrease in the DPNGs (p < 0.001) and an increase in PEN-S in that year. In 1998/99 and 1999/00, a significant increase in the DPNGs ($\chi^2_{\text{trend}} = 49.9$, p < 0.001) was seen and this was accompanied by a decrease in PEN-S strains ($\chi^2_{\text{trend}} = 49.2$, p < 0.001). There was a dramatic increase in the number of strains exhibiting high-level resistance to tetracycline (TRNG) between 1997(3%) and 1998/99(51%) (p < 0.001). This increase was counteracted by a reduction in $CRMNG^T$ in 1998/99. A doubling of the prevalence of $CMRNG^T$ was accompanied by a marked decrease in the

Table 1. Activity of five antimicrobial agents on *Neisseria gonorrhoeae* isolates from 1995 ($n = 61$), 1997 ($n = 198$), 1998/99 ($n = 98$) and 1999/2000 ($n = 58$)

Antimicrobial agent	Year	Percentage of strains with MIC (mg/L)													
		≤0.007	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Ciprofloxacin	1995	100													
	1997	96	4												
	1998/99	84	3	9	4										
	1999/2000	71	19	10											
Ofloxacin	1995	100													
	1997	52	34	11	2		2								
	1998/99	5	47	33	2	2	1	1	1	3	3				
	1999/2000		55	26	19										
Erythromycin	1995	NT													
	1997	32	10	14	12	13	14	6							
	1998/99	15	7	19	21	15	14	7	2						
	1999/2000			10	22	21	26	14	2		5				
Ceftriaxone	1995	100													
	1997	95	3	1	2										
	1998/99	62	26	7	5										
	1999/2000	53	31	10	5										
Spectinomycin	1995	NT													
	1997	9	3	4	4	5	1	2	7	13	10	17	20	8	
	1998/99	3		2				7	2	6	16	10	48	2	2
	1999/2000												22	78	

number of fully susceptible strains to tetracycline in 1999/00 (7%).

Table I shows the trends in susceptibility patterns for the remaining drugs. All 1995 strains had MICs for ciprofloxacin $\leq$ 0.007 mg/L , but only 71% fell in this category in 1999/00. In addition, over 10% of strains demonstrating MICs of $\geq$ 0.03 mg/L were seen in 1998/99 and 1999/00. The same trend was observed with ofloxacin but at higher MIC levels. The susceptibility to erythromycin also changed over this period. The MICs in 1997 ranged from $\leq$ 0.007 to 0.5 mg/L but these values changed to 0.03 to 4 mg/L for the 1999/00 strains. The data for azithromycin (available for 1999/00 only) when compared to erythromycin for the same period, showed a similar distribution of MICs. (Fig.2). No resistance to ceftriaxone was detected. However an increase in MICs was observed with almost 50% of strains in 1999/00. A similar pattern was observed for spectinomycin as evidenced by the increase in MICs with 28% of strains exhibiting MICs $\geq$ 16 mg/L in 1997, 52% in 1998/99 and 100% in 1999/00. Two strains showed a marked decrease in susceptibility to the drug with an MIC of 64 mg/L.

A large proportion of DPNG and CRMNG[T] isolates in 1999/2000 exhibited increased MICs to ciprofloxacin, ceftriaxone, erythromycin,azithromycin and spectinomycin. (Table II)

The amount of drug, prescribed for any indication, of the classes of antimicrobial drugs that are advised for treatment of STDs is given in table III. The consumption of penicillins per potential user of the provincial health care system increased significantly from 1996 to 1999. The use of cephalosporines remained constant.

Table 2. Percentage of DPNG and CRMNG[T] isolates in 1999/2000 with increased MICs of ciprofloxacin, erythromycin, azithromycin, ceftriaxone and spectinomycin

Antimicrobial agent	Number (%) of strains with increased MICs	
	DPNG (n = 37)	CRMNG[T] (n = 26)
Ciprofloxacin	13 (35)	13 (50)
Erythromycin	37 (100)	26 (100)
Azithromycin	37 (100)	25 (96)
Ceftriaxone	24 (65)	16 (62)
Spectinomycin	37 (100)	26 (100)

Table 3. Total consumption of antimicrobial agents used for syndromic management of STDs in the provincial healthcare system of KwaZuluNatal

	1996		1997		1998		1999	
	kg/year	mg/person	kg/year	mg/person	kg/year	mg/person	kg/year	mg/person
Penicillins	2276	260	2845	414	3683	509	9196	1021
Cephalosporins	239	27	216	31	190	26	206	23
Quinolones	46	5	356	52	274	38	296	33
Macrolides	5693	650	5561	808	4996	690	6282	698
Tetracyclines	956	109	805	117	812	112	385	43

Macrolides, mainly erythromycin, were the most frequently prescribed antimicrobial agents. The use of quinolones rose sharply from 1996 to1997 and levelled off thereafter.

Discussion

To establish the aetiological diagnosis of sexually transmitted infections is one of the most challenging issues in human medicine. Most of the 333 million estimated new infections per year [8] occur in developing countries, where clinical and laboratory diagnosis is hampered by the lack of medical personnel and laboratory facilities. However, even in more affluent countries where these are available, aetiological diagnosis proves to be difficult because of the low sensitivity of both the clinical diagnosis as well as even the most sophisticated of tests. Furthermore, a positive test for one organism does not exclude the presence of others, because mixed infections occur frequently.[9] These difficulties in reaching a diagnosis, have resulted in the implementation of syndromic management for STDs.[3] However, the

standardisation of antimicrobial treatment creates a selective advantage for organisms resistant to the drugs used.[10] Hence, sexually transmitted infections caused by antibiotic resistant organisms are becoming a major therapeutic problem in many parts of the world with resistance among strains of *N. gonorrhoeae* posing a particular challenge. [11,12,13,14,15,16,17]

Syndromic management was introduced on a large scale in KwaZuluNatal in 1995. From then to 2000, the distribution of MICs of *N. gonorrhoeae* to all antimicrobials recommended, has shifted to the right. These shifts have resulted in clinically significant resistance to penicillins and tetracyclines. For the other drugs the increase in MICs have had no clinical impact as yet. Others have reported similar trends in resistance[13,14,16,17,18,19]. Mechanisms that cause increase in MICs among unrelated groups of antibiotics are well recognised. Events leading to the expression or upregulation of efflux pumps produce a phenotype with resistance to several structurally unrelated antibiotics [20]. Integrons provide bacteria with a tool to accumulate resistance genes.[21] Although such genetic elements have not been reported in *N.gonorrhoeae*, these are found with increasing frequency in Gram negative bacteria. In addition, a site-specific recombinase has recently been identified in *N.gonorrhoeae.* [22] Such enzymes play a role in the acquisition of resistance gene cassettes.

The gradual increase in MICs as observed with the quinolones, the macrolides/azalides, ceftriaxone and spectinomycin might be the result of a series of independent events. The emergence of plasmid mediated high level resistance to penicillin and tetracycline indicates either the acquisition of genes from other organisms, or the introduction of an already resistant gonococcal strain that takes advantage of the selective environment created by the syndromic management. Both these events may occur simultaneously in gonococci as has been demonstrated in the past. The initial low level chromosomally mediated resistance to penicillin (MIC $\geq$2mg/L)[23] was followed by the emergence of plasmid mediated high level resistance (MIC $\geq$ 8mg/L) through the production of □ -lactamase.[18] The emergence of these penicillinase producing *N.gonorrhoeae* (PPNG) strains in Durban, KwaZuluNatal was in 1977.[24] By 1985, the prevalence of PPNGs among strains from Durban was 29%.[25] In 1988, the use of penicillin as first line therapy for *N.gonorrhoeae* was curtailed and patients were treated with a cephalosporin or spectinomycin. This was changed to ciprofloxacin with the introduction of the syndromic management guidelines in 1995.

We recently reported on quinolone resistance in *N.gonorrhoeae* in South Africa.[26] Molecular characterisation of clinical strains from other areas of the world has shown that resistance occurs in a stepwise fashion, which can be detected by increasing MICs to the drug. The number of organisms with decreased susceptibility to the quinolones in KwaZuluNatal has increased since 1995 (Table 1). This South African province uses a 250 mg STAT dose of ciprofloxacin instead of the WHO and CDC recommended 500 mg. Reports on susceptibility from other parts of South Africa where the 500mg STAT dose is used are lacking. The question as to whether the use of this low dose has fast-tracked this emergence of decreased susceptibility is therefore difficult to answer. Selection of resistance in bacteria is thought to be related to the duration of treatment in individual patients as well as to the total amount of drug used within a population.[10,27] The STAT dose of ciprofloxacin reflects the shortest course possible. Therefore, the vast amounts of antimicrobial agents used in the population is the most likely explanation for our observations. However, if mechanisms conferring resistance to more then one drug are involved, then the selective pressure does not have to come from ciprofloxacin usage. The extremely high consumption of macrolides (Table III) provides a selective advantage for these strains with decreased susceptibility to both erythromycin and

ciprofloxacin (29 % in 1999/2000). Monitoring the emergence of these pre-resistant strains will assist in the prediction of future evolution of quinolone resistance in *N. gonorrhoeae.*

Although tetracycline derivatives are not the treatment of choice for gonorrhoea, these are the recommended drugs for the treatment of *Chlamydia trachomatis*.[3] However, since the differentiation between the gonorrhoea and chlamydial infection is not made when syndromic management is applied, all patients with gonorrhoea are exposed to this group of drugs. Such exposure also occurs when asymptomatic patients or those with unrecognised symptoms [28] are treated with tetracyclines for indications other then STDs. Plasmid mediated high level resistance to tetracycline in *N.gonorrhoeae* (≥ 16mg/L) was first reported in 1983 from the United States of America. Resistance in Africa was first described in Zaire.[29] In 1994, the first high-level resistant strains from South Africa were reported from Bloemfontein.[30] Plasmid mediated high level resistance to tetracycline emerged in KwaZuluNatal in 1998 and spread rapidly. Within a period of 18 – 24 months the prevalence of these highly resistant strains to tetracycline jumped from 3% to over 50%. This resistance is plasmid mediated and has been shown to be due to the presence of the *tetM* gene.[31]

Erythromycin is widely used for the treatment of genital ulcer disease, vaginal discharge in pregnancy as well as for the treatment of respiratory tract infections among children. Mutations leading to resistance to erythromycin may confer resistance to azithromycin.[32,33] Our data support this (Fig 2). Azithromycin has been recommended as an alternative in the treatment of uncomplicated *N gonorrhoeae* and *C.trachomatis* infections. Recent failure of azithromycin therapy in patients with uncomplicated urethritis is alarming, [15,34] but might have been predicted since it is structurally similar to erythromycin. In South Africa, this drug is not recommended for uncomplicated urethritis/cervicitis.

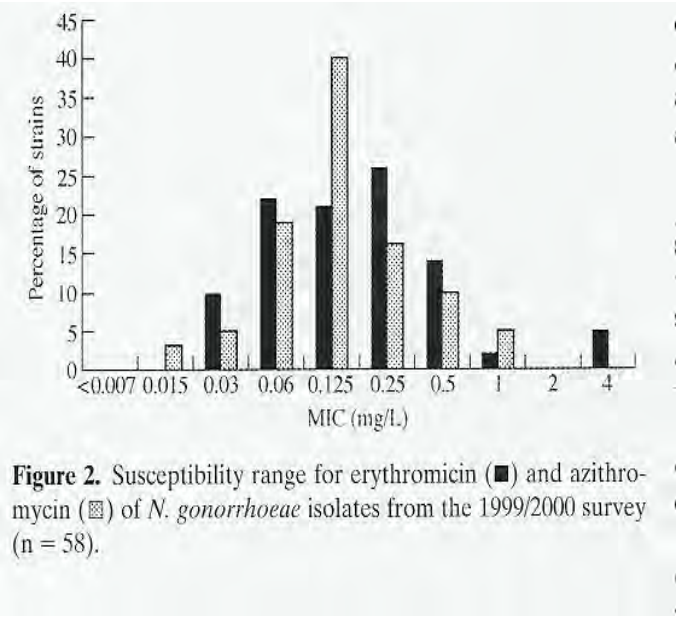

Figure 2. Susceptibility range for erythromicin (■) and azithromycin (▨) of *N. gonorrhoeae* isolates from the 1999/2000 survey (n = 58).

However, it is commonly used in the private sector for the treatment of respiratory tract infections and sexually transmitted infections. The somewhat rapid increase in MICs of *N.gonorrhoeae* in our area to azithromycin should not be taken lightly as this heralds the ineffectiveness of this antibiotic for the treatment of gonorrhoea.

Ceftriaxone is currently the drug of choice for the treatment of *N.gonorrhoeae* in pregnancy.[35] Recently, the use of this drug has increased in both the public and private sectors as first line therapy for bacterial meningitis. In addition the drug is widely used in the private sector for the treatment of other childhood “infections.”

Spectinomycin is a recommended alternative to ciprofloxacin for the treatment of *N.gonorrhoeae* in pregnancy.[35] Our data reveal an increase in the MICs to this drug. This is interesting because the main mechanism of resistance to this drug is a one step chromosomal mutation that results in full resistance.[36] Our observation suggests the presence of another mechanism of which a shared efflux pump is most likely.

The main question is whether we need to respond to the increase in MICs to the different drugs in the absence of documented resistance related clinical

failure. Experience with *Streptococcus pneumoniae*, another community acquired pathogen, suggests that gradual increase in MICs will eventually lead to clinically significant resistance.[37] The high rates of resistance to penicillin, and decrease in susceptibility to azithromycin and erythromycin occurred despite the fact that none of these agents are recommended in the treatment guidelines for genital discharge. In addition, a decrease in susceptibility to ceftriaxone and spectinomycin is seen although these drugs are infrequently used for the treatment of vaginal discharge in pregnancy and are administered as a stat dose. The shift to the right in the distribution of MICs of these antimicrobials may therefore be consistent with the widespread use of these agents in the community for other indications. It is therefore doubtful if changes in syndromic management guidelines at this stage, or a decrease of the total consumption of antibiotics by reverting to aetiology based management of STDs, will make any difference in the absence of a more rational use of the same drugs for other indications.

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Emergence of Tet M mediated tetracycline resistance in rural South Africa

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The Journal of Antimicrobial Chemotherapy 2001; 48: 142-143 (Correspondence)

Sir,

Sexually transmitted infections (STIs) caused by antibiotic resistant *Neisseria gonorrhoeae* are a major therapeutic problem in many parts of the world. Effective therapy against this pathogen has barely stayed ahead of the acquisition of resistance mechanisms and studies continue to document the increase in antimicrobial resistance of *N. gonorrhoeae* to a variety of antibiotics.[1,2]

The high prevalence of mixed infections, compounded by the imprecision of an aetiological diagnosis in developing countries, underscores the feasibilty of the syndromic approach in the management of STIs. In 1995, South Africa implemented a modified version of the WHO's syndromic management protocol for the treatment of STIs. Doxycycline is the recommended drug for the treatment of *Chlamydia trachomatis*. However, since the differentiation between gonorrhoea and chlamydial infection is not made, patients with gonorrhoea are exposed to doxycycline as part of the syndromic management for genital discharge.

In Southern Africa, plasmid mediated high level resistance to tetracycline of *N. gonorrhoeae* (TRNG = MIC $\geq$ 16mg/l) was first described in 1995 among 11 isolates obtained from Namibia and Botswana.[3] These isolates all carried the American type of *tetM* gene. This was followed by a report from Bloemfontein, South Africa where the prevalence was reported to have increased from 2% (3/145) in 1994 to 18.5 % (12/65) in 1995.[4] The resistance patterns of this organism vary geographically and the presence of high-level tetracycline-resistant organisms has not been described in the province of KwaZuluNatal.

The Africa Centre for Population Studies and Reproductive Health is situated in the rural district of Hlabisa in KwaZuluNatal. A direct spin off of this centre has been the establishment of an STD clinic in the area. During STD prevalence and antimicrobial surveillance studies at this clinic in 1999, high-level resistance to tetracycline was observed. This study was therefore carried out to determine whether this was Tet – M mediated and which variant of the gene was involved.

Urethral and cervical specimens were collected from patients presenting with genital discharge to the Kwamsane STD clinic, in the rural Hlabisa district, KwaZulu/Natal , between March 1999 and December 1999. These specimens were inoculated on to New York City agar for the isolation of *N.gonorrhoeae*. MICs of tetracycline were determined using the agar dilution method and breakpoint criteria as defined by the NCCLS.[5]

Selection of potential tetracycline-resistant *N.gonorrhoeae* strains was performed by screening for growth on GC agar (supplemented with 1% yeast autolysate and 5% lysed horse blood) containing 10 mg/L tetracycline.

PCR detection and characterization of the *tet*M gene was performed on strains of *N. gonorrhoeae* that grew on the tetracycline-containing plate, by means of a one step-PCR. [6] PCR products were electrophoresed in a 1% agarose gel containing ethidium bromide and visualised by UV fluorescence.

Two hundred and four strains of *N. gonorrhoeae* were collected during the study period. One hundred and thirty six strains (67%) had MICs of $\geq$ 16mg/l to tetracycline. All these strains grew on the 10mg/l tetracycline screening plate and were found to carry the American variant of the *tet*M gene, as revealed by the production of a 778 base pair PCR product. The Dutch *tet*M gene was not found among these isolates.

In addition to the use of doxycycline as part of syndromic management, tetracycline derivatives being relatively cheap are widely used for the empiric treatment of respiratory and skin and soft tissue infections (P. Moodley *et al*. unpublished results). The emergence of Tet-M mediated tetracycline resistance in this area was sudden and is mediated by a single variant of the *tet*M gene. Whether this is a result of the spread of a single strain or by transfer of the resistance plasmid within the gonococcal population needs further investigation.

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Ciprofloxacin resistance in *Neisseria gonorrhoeae*

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The past 15 years have seen the introduction and establishment of the fluoroquinolones as first line treatment for gonorrhoea across the world. Initially these drugs successfully eradicated *Neisseria gonorrhoeae*, but reports of clinical failure started to emerge around 1992.[1]

In 1995, the syndromic management for sexually transmitted diseases (STDs) was introduced in the province of KwaZulu/Natal, South Africa. Based on minimum inhibitory concentration (MIC) values available at the time, as well as on cost considerations, the provincial department of health advocated the use of 250mg STAT of ciprofloxacin in patients presenting with genital discharge.

Surveillance studies of STDs have been done at two clinics in this province: the urban City Health STD clinic in Durban and the rural STD clinic of Kwamsane. Antimicrobial susceptibility tests to ciprofloxacin were done on isolates obtained from patients attending these clinics during 1999, according to the susceptibility test criteria defined by the National Committee for Clinical Laboratory Standards.

MIC (mg/L)	Cumulative percentage with MIC to ciprofloxacin (%)	
	Urban area (n=204)	Rural area (n=156)
≤0·007	79	88·5
0·015	88	90·5
0·03	98	94·5
0·06	100	96·5
0·125	..	97·0
0·25	..	98·0
0·5	..	98·5
1·0	..	100
2·0	..	..

Susceptibility to ciprofloxacin of *Neisseria gonorrhoeae* isolated in 1999

156 strains were tested from the City Health STD clinic and 204 at the Kwamsane STD clinic. In the urban area, susceptibility of *N. gonorrhoeae* had decreased but the MICs were 0.06 mg/L or lower, which is the cut-off point for susceptibility. In the rural district, more than 3 % of the strains had MICs with higher cut-off values with 3 strains showing resistance and MICs of 1mg/L (table).

Some workers suggest that development and spread of resistance is encouraged by the 250mg dose ciprofloxacin compared with the 500mg acute dose recommended by the CDC and WHO,[2,3] but the time to resistance has been reported as shorter after the higher dose than that which we report.[4] In addition, the rate of reduction in susceptibility of *N.gonorrhoeae* to ciprofloxacin in the two areas that we surveyed varied despite the simultaneous introduction of syndromic management.

Since reports on susceptibility from other parts of South Africa where the 500mg dose is used are lacking, the effect of the lower dose is unclear. The duration of treatment in an individual and amount of drug used in a population might contribute to resistance.[5] However, acute treatment with ciprofloxacin reflects the shortest course possible and the amounts of antimicrobial agents used in this population probably explains our observations.

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Lower dose of Ciprofloxacin is adequate for the treatment of *Neisseria gonorrhoeae* in KwaZulu Natal, South Africa

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ABSTRACT

It has been suggested that 250mg ciprofloxacin for the treatment of gonorrhoea is less effective and encourages the development of resistance as compared to 500 mg. However, evidence to support this is inconclusive. We studied the response of male gonococcal urethritis to a single 250 mg dose of ciprofloxacin vs 500 mg. Both regimens were given in combination with doxycycline in the context of the local syndromic management protocol. There was no significant difference in response between the regimens, inclusive/exclusive of tetracycline susceptible isolates. One patient in the 250 mg arm failed to respond clinically but was microbiologically cured and four patients in the 500 mg arm failed microbiologically but responded clinically. All four isolates had ciprofloxacin MICs $\leq$ 0.007mg/L.

Introduction

In men and non-pregnant women with non-ulcerative sexually transmitted diseases (STDs), a single dose of 500mg of ciprofloxacin for potential infection with *Neisseria gonorrhoeae* was identified for inclusion in syndromic management (SM) treatment regimens [1,2]. In 1995, the department of health for the KwaZuluNatal (KZN) province in South Africa adopted the syndromic management principles and modified the available SM guidelines for the treatment of STDs in the region. Based on minimum inhibitory concentration (MIC) values available at the time, as well as on cost considerations, the department of health advocated the use of a single 250mg dose of ciprofloxacin in men and non-pregnant women presenting with non-ulcerative sexually transmitted diseases. The use of this lower dose has been criticised as being less effective and encouraging the development and spread of quinolone resistance in *N.gonorrhoeae*.

Although there have been several reports on the decreasing in-vitro susceptibility of *N.gonorrhoeae* to quinolones over the last decade, the clinical response to this group of antimicrobials has been excellent [3,4,5,6,7]. Clinical failures of gonococcal infections to fluoroquinolones have been infrequently reported with varying MICs with the single 250 mg dose [8,9] as well as with the single 500mg dose [3,10]. A possible reason for the paucity of treatment failures may be that fact that areas from which in-vitro fluoroquinolone-resistant isolates have been reported, switched to alternative therapeutic regimens.

Two recent studies have attempted to correlate treatment failures with in vitro ciprofloxacin resistance of *N.gonorrhoeae* [11,12]. The conclusions reached in these studies differ. The first study concluded that in-vitro resistance does not always result in clinical failure, and clinical failures are also seen among strains that are not categorised as fully resistant [11]. While reinfection may be responsible for treatment failure at the lower MICs, the clinical response at the higher MICs is not surprising. Interpretive criteria for antimicrobial susceptibilities are based on predictive values of clinical cures. A susceptible result predicts a cure rate of >95%. 'Resistant' breakpoints are set at levels at which < 85% of infections exhibiting that MIC would be expected to be cured [13]. Thus, a large proportion of isolates exhibiting the 'resistant' MIC may respond to fluoroquinolone therapy. However, the proportion of clinical failures would be expected to increase as the in-vitro MICs of isolates increase. The second study, found in contrast a very close correlation and a high predictive value for MIC data with regard to clinical outcome [12].

We recently reported on the emergence of ciprofloxacin resistance in our area [14]. Patients who were found to be infected with resistant strains (MIC = 1 mg/L) were followed up 8-10 days after receiving a single 250mg dose of ciprofloxacin. These patients reported clinical response and repeat microbiology demonstrated organism eradication. Some patients with susceptible strains (< 0.007 mg/L) however did report symptoms at follow up, and repeat microbiology revealed strains with MICs in the susceptible range suggesting reinfection as the most likely cause of treatment failure (unpublished observations).

The Department of Health for KZN has come under pressure to increase the dose of ciprofloxacin from 250mg to 500mg. We therefore prospectively studied patients with urethritis receiving a single dose of either 250mg or 500mg of ciprofloxacin, in an attempt to better define the relationship between MIC and treatment response in our area.

Methods

Male patients presenting to the Prince Cyril Zulu Communicable Diseases Clinic in Durban, KZN, South Africa between April and November 2000, with symptoms of

urethritis (discharge and/or dysuria) were invited to participate in the study.

Urethral specimens were collected and New York City plates (Oxoid Ltd. Basingstoke, Hampshire, England) were inoculated on site for the isolation of *N.gonorrhoeae*. Plates were transported to the laboratory within 3 hours and incubated at 37°C in 5 % CO_2 for 48 hours. Suspected colonies of *N.gonorrhoeae* were identified by means of gram staining, oxidase test, β-galactosidase, hydroxy-prolylaminopeptidase, γ-glutamylaminopeptidase and acid production from glucose, lactose and maltose.

Patients were treated with a single dose of either 250mg or 500mg of ciprofloxacin. The patient allocation of the different dosages followed the dosage schedule of the clinic. Between April and August 2000, the patients attending this clinic were treated with a single dose of 250 mg of ciprofloxacin. Following revision of the guidelines by the Provincial Therapeutics Committee, patients received the higher dose of the drug between August and November 2000. Doxycyline 200mg daily for 7 days was also prescribed for potential infection with *Chlamydia trachomatis*. Patients were asked to return 8-10 days later to assess clinical response to treatment. Repeat specimens were taken at this stage to ascertain microbial eradication.

Minimum inhibitory concentrations (MICs) of ciprofloxacin and tetracycline were determined by means of the agar dilution method. Two fold serial dilutions of antibiotics were added to molten GC agar base (Oxoid Ltd) supplemented with 1% isovitalex at a temperature of 45^0 C. After solidification, these plates were seeded with 10^4 cfu/spot of bacteria by means of a multipoint inoculator and incubated at 37^0 C in CO_2 for 24 hours. *N.gonorrhoeae* (ATCC 49226), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 10418) were used as controls. The antimicrobial susceptibility was judged using breakpoint criteria defined by the National Committee for Clinical Laboratory Standards [15].

Results

Eight hundred and sixty five subjects were recruited, of which 357 were treated with a single dose of 250mg ciprofloxacin while 508 patients received the 500mg dose. There were no significant differences between these groups with regard to age, level of education, marital status, age at first intercourse, lifetime sexual partners and number of partners in the previous four weeks.

N.gonorrhoeae was isolated from 443 subjects: 177 (50 %) in the 250mg arm and 266 (52 %) in the 500mg arm.

Clinical response and microbial eradication rates are summarised in Table I. One patient in the 250mg dose arm reported deterioration of symptoms. This patient had *N.gonorrhoeae* at baseline, but was negative for all detectable sexually transmitted infections at follow up.

All isolates were susceptible to ciprofloxacin with MICs ranging from < 0.007 to 0.015mg/L. Four patients in the 500mg arm were positive for *N.gonorrhoeae* at baseline and remained positive at follow up. The MICs for ciprofloxacin of these isolates were all $\leq$ 0.007mg/L at baseline and follow up.

Sixty four percent of the isolates displayed high level resistance to tetracycline with MICs $\geq$ 16mg/L (Table 1). The distribution of tetracycline resistant and susceptible isolates was similar for both 250mg and 500mg arms. The response-to-treatment parameters did not differ between and within each of the tetracycline phenotype categories (Table 1).

Discussion

Treatment failure of an infection may be the result of lack of compliance, reinfection or failure of the recommended drugs to eradicate the infection. Since single doses are used, compliance is an unlikely issue.

Table 1: Clinical response and microbial eradication in 443 subjects receiving 250mg or 500mg ciprofloxacin in relation to the tetracycline resistance phenotype*

Tetracycline MIC	No. of patients responding to: 250 mg < 1 mg/L (n=16)	250 mg 1-8 mg/L (n=50)	250 mg > 8 mg/L (n=111)	500 mg < 1 mg/L (n=23)	500 mg 1-8 mg/L (n=71)	500 mg > 8 mg/L (n=172)
Clinical response	16	49	111	23	71	172
Microbial eradication	16	50	111	22	71	169

* Tetracycline resistance phenotype: < 1mg/L= susceptible; 1-8 mg/L= chromosomally mediated low level resistance; > 8 mg/L= plasmid mediated high level resistance

The role of reinfection is difficult to ascertain since it relies on subjective history taking. Therefore it would be prudent to exclude these factors before attributing treatment failures to lack of efficacy of the drug.

Although we observed three cases with isolates with MICs of 1 mg/L from a semi-urban/rural area in the Hlabisa District in 1999 [14], the population of gonococcal isolates in this study, from patients attending the largest STD clinic in KZN a year later, were still highly susceptible to ciprofloxacin. Subsequent continued surveillance has not revealed any change thus far. The failure of 4 patients in the 500mg arm to eradicate their infection with *N.gonorrhoeae*, therefore most likely represents reinfection.

Tetracycline derivatives are included in the syndromic management of male urethritis for possible co-infection with *C.trachomatis*. Tetracycline resistance is common in this area [16]. With 64% of the study isolates displaying high level tetracycline resistance and a further 27 %with low level resistance, it is unlikely that this drug would have contributed to a curative effect on gonococcal disease independent of the action of ciprofloxacin. This is supported by our observation that there was no difference in the response rate in either of the study arms when patients with tetracycline susceptible isolates were excluded from the analysis.

Three pharmacodynamic parameters ie. AUC/MIC, C_{max}/MIC and T > MIC (MIC= minimum inhibitory concentration of infecting strain, AUC = area under the concentration curve, C_{max} = peak serum concentration of drug, T = time), which reflect the ratio of serum levels of a drug to the MIC of an organism, are often used in an attempt to relate susceptibility of an organism to a given antimicrobial agent to treatment outcome [17,18]. In such equations, concentrations of a drug at the site of infection should feature as a criterion in defining breakpoints for susceptibility. However, since the concentration curve at the site of the infection is often not known, serum concentrations of drugs are used. It is therefore not surprising that the association between in vitro susceptibility and clinical response is poor with drugs like ciprofloxacin that do not have a linear relationship between serum and tissue concentration. While the binding of

quinolones to serum proteins is generally low, it accumulates in certain tissues and its concentration in macrophages and neutrophils is 2 to 14 fold in excess of that in serum [19].

It has been reported that a therapeutic index (C_{max} /MIC) of 3:1 is required to ensure a high probability that a gonococcal infection will respond to penicillin [20]. In the absence of adequate data on clinical failures, the breakpoints to interpret the susceptibilities of *N.gonorrhoeae* to the fluoroquinolones were adopted based on this study and on published reports of treatment failure [21]. The peak serum level following 250 mg or 500mg of ciprofloxacin is approximately 1.2 μg/ml and 2.5 μg /ml [19] respectively. Since gonococcal infection results in the aggregation of large amounts of neutrophils at the site of infection, the therapeutic index using the peak concentration at the site of infection as the C_{max} should theoretically be at least two times that of the serum concentration (Table 2). This could possibly explain why patients with gonococcal urethritis respond to ciprofloxacin despite having MICs in the resistant range [11,14].

Although clinical isolates of *N.gonorrhoeae* with decreased susceptibilities to fluoroquinolones and treatment failures in gonorrhoea have been reported, our study underscores the lack of evidence to suggest that a 250 mg dose of ciprofloxacin used for the treatment of *N.gonorrhoeae* selects quinolone resistant strains. The duration of treatment in an individual as well as the total amount of drug used within a population is thought to contribute to the selection of resistance in bacteria [23,24]. The former is unlikely since a single dose of ciprofloxacin reflects the shortest course possible, while the total amount of fluoroquinolones used within our population is probably more influenced by the multi-dose regimens used for respiratory and urinary tract infections then by the single dose regimen to treat gonococcal infection.

The development of resistance to fluoroquinolones takes place in a stepwise manner. After initial mutations in the *gyrA* gene that confers low-level resistance, mutations in the *parC* gene result in resistance at a much higher level [25,26]. It has been argued that fluoroquinolone resistance occurs de novo through these stepwise mutations and that the first

Table 2: Therapeutic index for a range of MICs at different levels of ciprofloxacin accumulation at the site of infection

	Therapeutic index at hypothetical concentrations at site of infection		
MIC (mg/L)	[serum] x1	[serum] x2	[serum] x4
0.06	20	40	80
0.12	10	20	40
0.25	4.8	9.6	19.2
0.5	2.4	4.8	9.6
1	1.2	2.4	4.8
2	0.6	1.2	2.4
4	0.3	0.6	1.2

mutational steps increase the chances of subsequent ones occurring in the presence of low concentrations of the drug. In this scenario, the use of a low dose would promote such developments. It is unknown at which dose this will be prevented. It is also unknown what impact the much longer downward slope of the area under the curve obtained with a 500 mg STAT dose, as compared to 250 mg, has on such a selection process. Our data show that 6 years of use of a 250 mg STAT dose for treatment of *N.gonorrhoeae* infections did not have that effect.

Recently, Trees *et al*. provided evidence that this de novo development of resistance may play a minor role while importation of new strains and spread is the most prominent mechanism [27]. Applying the concept of "dose dependent selection" one could argue that in such circumstances a low dose would select for low level resistance and a high dose for high level resistance [23].

In conclusion, for *N.gonorrhoeae* and quinolones, the precise relationship between the $[\text{ciprofloxacin}]_{\text{serum}}$ and $[\text{ciprofloxacin}]_{\text{site of infection}}$ in terms of clinical outcome and selection of resistance remains to be established. Therefore, we feel that there is not enough evidence to warrant a doubling of the ciprofloxacin dosage in our low budget environment. We propose to continue the use of the single 250 mg dose in our area, in conjunction with continued the close monitoring of the trends in MICs of *N.gonorrhoeae* to all relevant antimicrobials [16].

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Chapter Three

Treatment failure of *Neisseria gonorrhoeae* analysed

Summary

Reinfection of effectively treated patients is the hallmark of STIs that perpetuate and maintain the endemnicity of these infections. This study emphasises the problem of rapid reinfection following successful treatment for gonorrhoea. Pre and post treatment isolates of *Neiseria gonorrhoeae* were phenotypically and genotypically typed. The data is highly suggestive of re-infection by a different partner.

Typing of *Neisseria gonorrhoeae* Reveals Rapid Reinfection in Rural South Africa

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ABSTRACT

A recent study afforded us the opportunity to collect pre- and post-treatment isolates of *N.gonorrhoeae* from women who supposedly failed to eradicate the organism when tested 8 to10 days following treatment with a single, directly observed 250-mg dose of ciprofloxacin. In an attempt to differentiate true treatment failure from reinfection, we determined the ciprofloxacin MICs and performed auxotyping, serotyping, and opa typing of the pre- and post-treatment isolates. Paired isolates of *N.gonorrhoeae* were obtained from 7 different women, despite susceptibility of the initial isolates to ciprofloxacin. Six of seven patients were infected with gonococcal isolates that differed significantly from their primary isolate. These most probably represent reinfection with a different strain, which could originate from the same partner infected with multiple strains or reinfected with a new strain or from a different partner. The susceptibility to ciprofloxacin of all isolates makes the possibility of multiple strains in the patient unlikely. The diversity of the isolates within the pairs therefore suggests rapid reinfection within the partnerships.

Introduction

Infections with *Neisseria gonorrhoeae* are of concern to health care providers because they carry an increased risk of morbidity and mortality among women (3) as well as neonates (6). In addition, as with the other causes of sexually transmitted infections (STIs), they represent an elevated risk for infection with human immunodeficiency virus (HIV) (2, 5). Epidemiological surveillance including accurate strain identification is pivotal to the understanding of the transmission dynamics of this organism, which in turn influences public health interventions.

In a recent study, we evaluated the efficacy of the drugs employed in the syndromic management of nonulcerative STIs among women (8). Twenty percent of women treated with ciprofloxacin for culture-proven infection with *N.gonorrhoeae* remained culture positive when tested between 8 and 10 days later. We attributed the subsequent infection to either lack of compliance with antibiotic therapy, failure of the ingested antibiotics to eradicate the infecting organism, or reinfection.

In our setting, ciprofloxacin is the drug of choice for the treatment of cervicitis due to *N.gonorrhoeae* and is administered as a directly observed single dose. Lack of compliance therefore cannot explain the subsequent infection. Although the MICs of ciprofloxacin for *N.gonorrhoeae* have started to increase in this area (7), clinical failures have not as yet been seen. Attempts at partner notification and treatment have been largely ineffectual (16). We suspect that reinfection is common, but this has not been proven.

In an attempt to differentiate true treatment failure from reinfection, we determined the phenotype by using auxotyping, serotyping, and antibiograms of pre- and post-treatment isolates and examined the genetic relatedness by means of opa typing. Our hypothesis was that the isolates within each pair would be susceptible to ciprofloxacin, display identical antibiograms, and have an identical phenotype and genotype. We expected these pairs to be identical based on the assumption that reinfection would be from the same partner, who would harbor a single strain of *N.gonorrhoeae*.

Methods

The Africa Centre for Health and Population Studies' primary care clinic is situated in KwaMsane, a large periurban, combined formal and informal settlement on a major highway in the Hlabisa sub-district of northern KwaZuluNatal.

We tested the efficacy of syndromic drug therapy in women with nonulcerative genital disease who attended this clinic between March 1999 and February 2000 (8). In this study, 692 women were treated for vaginal discharge by using the syndromic approach. Eighty-six (12%) were infected with *N.gonorrhoeae* at baseline. Of the 290 patients returning for follow-up, 51 had positive cultures for *N.gonorrhoeae* at baseline, of whom 10 (20%) had positive cultures at follow up 8 to10 days later. In 3 of these 10 pairs, one or both isolates could not be recovered from the freezer. Therefore, only 7 pairs were available for typing.

Single colonies of *N.gonorrhoeae* isolates were subcultured and stored as suspension in glycerol-peptone at -70^0C. MIC determination and typing were performed on organisms grown from these suspensions.

Typing

Auxotyping was performed using the method described by Copley et al. (1). For serotyping the standard set of monoclonal antibodies and nomenclature described by Knapp et al. was used (4). MIC determinations were performed on all isolates for penicillin, tetracycline, spectinomycin, ceftriaxone, ciprofloxacin, and ofloxacin by using the National

Committee for Clinical Laboratory Standards (NCCLS) defined method (9). In brief, twofold serial dilutions of antibiotics were added to molten GC agar base (Oxoid Ltd.) plus with 1% IsoVitaleX supplement at 45^0 C. After solidification, these plates were seeded with 10^4 CFU of *N.gonorrhoeae* per spot by means of a multipoint inoculator. Plates were incubated at 37^0 C under CO_2 for 24 h. *N.gonorrhoeae* ATCC 49226, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 10418 were used as controls. Isolates were tested for □-lactamase production by the chromogenic cephalosporin method. PCR detection and characterization of the *tet*M gene were performed on isolates of *N. gonorrhoeae* using a one-stepPCR (13). PCR products were electrophoresed in a 1% agarose gel containing ethidium bromide and visualised by UV fluorescence.

Opa typing of the isolates was performed using the method of O'Rourke et al. (10). Briefly, the *opa* genes were amplified from gonococcal chromosomal DNA by PCR using a single pair of primers. The products were digested with frequently cutting restriction enzymes (*Taq*1 and *Hin*P1). The restriction fragments were end labeled with ^{32}P and were separated on polyacrylamide gels, and band patterns were compared.

Results

Paired isolates of *N.gonorrhoeae* were obtained from 7 different women. Demographic data for these patients are shown in Table 1. Their ages ranged from 17 to 30 years, and their risk factor (RF) for acquiring an STI (defined as number of partners divided by present age minus age at coitarche) ranged from 0.3 to 7 (median 0.5).

Table 2 shows the susceptibility of the *N.gonorrhoeae* strains to the antibiotics. Of the 14 isolates, 2 produced □-lactamase and both were isolated from the same patient (patient D). Only 3 isolates did not harbor the *tetM* gene. Two formed the pair isolated from patient E, and the third was the subsequent isolate of patient A. Her initial isolate did not carry this gene. Isolate B2 showed an increase in fluoroquinolones MIC compared to B1, exhibiting reduced susceptibility to ciprofloxacin and resistance to ofloxacin. The MICs of spectinomycin and ceftriaxone for the 14 isolates did not differ.

The results of auxotyping and serotyping are shown in Table 3. Of the 14 isolates, 10 were of the NR auxotype, one (B2) had the ARG auxotype, and 3 (A2, E1, and E2) had the PRO auxotype.

Serotypes differed considerably, one with

TABLE 1. Sexual history of seven women culture positive for *N. gonorrhoeae* 10 days after ciprofloxacin treatment

Patient	Age (yr)	Age at coitarche (yr)	No. of lifetime sexual partners	RF[a]	No. of partners in last month	No. of current partners	Location of current partners(s)	Condom use
A	30	15	4	0.8	1	1	Local, separate	Never
B	23	17	3	0.5	1	1	Local, together	Never
C	21	20	7	7	1	1	Local, separate	Never
D	20	15	2	0.4	2	2	(i) 240 km away; (ii) local, separate	Occasional
E	17	16	2	2	1	1	250 km away	Occasional
F	21	17	3	0.75	1	1	Local, separate	Never
G	22	16	3	0.5	1	1	50 km away	Never

[a] RF = number of lifetime sexual partners (age − age at coitarche).

TABLE 2. Drug MIC, presence of β-lactamase, and *tetM* genes of pre- and post-treatment isolates of *N. gonorrhoeae*

Patient	Isolate no.	MIC (mg/liter) of penicillin/β-lactamase	MIC (mg/liter) of Tetracycline/*tetM*	MIC (mg/liter) of:			
				Spectinomycin	Ceftriaxone	Ciprofloxacin	Ofloxacin
A	A1	0.125/negative	64/positive	16	≤0.007	≤0.007	0.03
	A2	0.25/negative	4/negative	8	≤0.007	≤0.007	0.015
B	B1	1/negative	128/positive	16	≤0.007	≤0.007	0.03
	B2	1/negative	128/positive	16	≤0.007	0.125	2
C	C1	0.5/negative	32/positive	16	≤0.007	≤0.007	≤0.007
	C2	0.5/negative	16/positive	16	≤0.007	≤0.007	0.015
D	D1	8/positive	32/positive	16	≤0.007	≤0.007	≤0.007
	D2	4/positive	32/positive	16	≤0.007	≤0.007	0.015
E	E1	0.5/negative	2/negative	16	≤0.007	≤0.007	0.015
	E2	1/negative	1/negative	16	≤0.007	≤0.007	0.03
F	F1	0.25/negative	32/positive	32	≤0.007	≤0.007	0.015
	F2	0.25/negative	32/positive	16	≤0.007	≤0.007	0.015
G	G1	0.25/negative	16/positive	16	≤0.007	≤0.007	≤0.007
	G2	0.25/negative	32/positive	16	≤0.007	≤0.007	0.015

IB3 being the dominant type (5 isolates). Only 2 pairs showed identical serotypes: D1 and D2 both belonged to the A-NT type, while E1 and E2 belonged to IB3.

The *Taq*1 and *Hin*P1 opa types of the paired isolates are shown in Fig.1. Of the 14 isolates, 8 produced unique opa types with both enzymes. One pair (D1 and D2) was indistinguishable by opa typing with both restriction enzymes. Two other isolates were indistinguishable but belonged to two different individuals (isolates C2 and F1). A further two isolates (B2 and F2) were indistinguishable by *Taq*1 but produced different band patterns by *Hin*P1 and belonged to different individuals.

Of the four typing methods, opa typing was most discriminative, followed by serotyping. Auxotyping and antibiogram typing did not have much discriminative power.

Isolate A1 differed from A2 in its susceptibility to tetracycline. Isolate A1 appeared more resistant than isolate A2 and harboured the *tet*M gene. These isolates belonged to different auxotype/serotype (A/S) classes as well as to different opa types. Isolate B2 was associated with an increase in MIC compared to B1, exhibiting reduced susceptibility to ciprofloxacin and resistance to ofloxacin. Isolates B1 and B2 also belonged to different A/S classes and opa types. The paired isolates from patient D were indistinguishable by antibiogram, A/S typing, and opa typing. Isolates E1 and E2 were indistinguishable by antibiogram and A/S typing but differed by genotyping. The individual isolates of pairs C, F, and G differed by phenotype and genotype.

Discussion

In this study, six of seven patients, who presented 8 to10 days following treatment, were shown to be infected with gonococcal isolates that differed significantly from their primary isolate. This was despite the susceptibility of the

initial isolates to ciprofloxacin that was administered as a directly observed single dose. Hence, the most likely explanation is reinfection. This illustrates the failure of efforts to control the spread of STIs.

TABLE 3. Comparison of pre- and post-treatment *N. gonorrhoeae* isolates from seven women by four typing methods

Patient	Isolate no.	Result of typing system:				Identical isolates
		Antibiogram	Auxotype[a]	Serotype	opa type	
A	A1	Different	NR	IA-6	Different	No
	A2		PRO	IB-2		
B	B1	Different	ARG	IA-6	Different	No
	B2		NR	IB-7		
C	C1	Same	NR	IB-8	Different	No
	C2		NR	IB-3		
D	D1	Same	NR	A-NT	Same	Yes
	D2		NR	A-NT		
E	E1	Same	PRO	IB-3	Different	No
	E2		PRO	IB-3		
F	F1	Same	NR	IB-3	Different	No
	F2		NR	IB-6		
G	G1	Same	NR	IB-3	Different	No
	G2		NR	IA-6		

[a] NR, not requiring; PRO, proline requiring; ARG, arginine requiring.

The phenotypes of the paired isolates from patients A and B were different (Table 3). The isolates in these pairs also differed in their opa type. Theoretically, the subsequent isolate from patient B, could be the result of failure of treatment of a mixed population of gonococci. B1 was susceptible to ciprofloxacin and would therefore have been eradicated by ciprofloxacin, while B2, with its intermediate susceptibility to ciprofloxacin, may have persisted. Isolate A1 differed from A2 both phenotypically and genotypically in its susceptibility to tetracycline. Both these isolates were susceptible to ciprofloxacin. Selection of A2 from a mixed infection is highly unlikely because A2 was susceptible to tetracyclines while A1 was resistant. A2 therefore most likely represents reinfection.

Apart from patient D, the opa types of pairs of isolates from patients with comparable antibiograms (C, D, E, F, and G) were different. This indicates that antibiograms have little value in establishing relatedness between isolates in areas where there is no resistance or, as in our area, widespread acquired resistance to some antibiotics. Both situations lead to similar antibiograms of all isolates.

Like the antibiograms, auxotyping also had little discriminative value within these 14 isolates. This is the result of the large number of nonrequiring isolates. Serotyping, on the other hand, discriminated very well within this group, except for one pair (from patient E), which was identical by serotyping but differed in genotype (Table 3).

The opa types of the initial isolates differed from those of the subsequent isolates in all but pair D. The high degree of unrelatedness of isolates from one individual within a time span of 8 to 10 days suggests rapid reinfection, or infection with multiple isolates at baseline. Reports on the prevalence of coinfection with a mixed population of gonococci have varied from not being present to being common (11,12,14). However, since all but one isolate (B2) was highly susceptible to ciprofloxacin, coinfection is unlikely, since the drug would have eradicated the susceptible mixed population of *N.gonorrhoeae*. Another possible explanation for the discrepancy is short-term genetic instability (14). This is

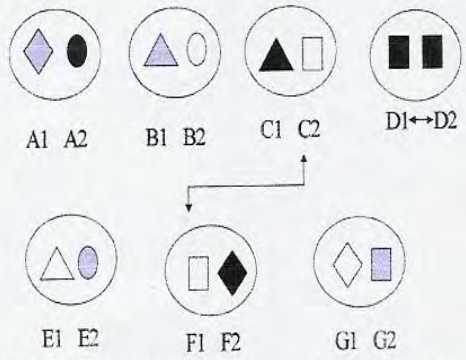

FIG. 1. Opa types of seven pairs (pre- and post-treatment isolates) of *N. gonorrhoeae* isolates. Double-headed arrows indicate identical opa types.

an unlikely explanation for our observations since the interval between sampling was short and opa typing

revealed multiple different bands between the paired isolates. Opa typing has been shown to be highly discriminatory yet is able to identify linked isolates in a transmission chain (15). Therefore, these isolates most likely represent reinfection with a different strain. This strain could originate from the same untreated partner if this partner was coinfected with more than one strain. We have no information on the frequency of infection with multiple strains in our population. This needs further investigation.

The alternative is that this reinfection came from a different partner. This seems to be a likely explanation because the only patient who was reinfected with the same strain (patient D) had, with RF= 0.4, the lowest risk in this small group of women. This was despite the fact that patient D was the only one with multiple current partners. This can be explained by one being a migrant laborer, and the second one a resident in the area. The role of the RF (ie. the number of lifetime partners divided by the number of years of sexual activity) in defining the possibility of acquiring an STI needs further validation in experiments with a larger group. Our conclusion that these women were probably reinfected by different partners is supported by the observation that in our area, as many women as men are HIV-1 positive in discordant couples (M. Lurie, S.S. Abdool Karim, and A.W. Sturm, Abstr. Guide Thirteenth Meet. Int. Soc. Sex. Transm. Dis. Res.,p. 52, 1999). This implies that these women have acquired their HIV infection from a sexual partner other than the stable partner.

Isolates C2 and F1 were indistinguishable by opa typing with both restriction enzymes and by antibiogram and A/S class. The presence of gonococci with indistinguishable opa-types is a good indicator that the individuals from whom they are recovered were sexual partners or part of a short chain of disease transmission (10,15). Therefore, this opa type result from isolates from patients C and F may indicate an undisclosed or unknown sexual linkage between the individuals. The frequency of women having sex with women is unknown in our setting but is believed to be low. Therefore, our data suggest that these women may have a common partner or are part of a common network.

Interestingly, all women, with the exception of patient D, admitted to only one current sexual contact. The typing patterns of their isolates, however, suggest otherwise. The results of this study, in conjunction with our previous report (8), imply that about 20% of female STI clinic attendees become rapidly reinfected following successful drug treatment for *N.gonorrhoeae* infection. Even more alarming is the diversity of the isolates within the pairs, suggesting rapid re-infection within the partnerships.

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Chapter 4

Bacterial vaginosis: A neglected syndrome

Summary

This chapter emphasises the existence of mixed aetiologies in patients presenting with vaginal discharge. The contribution of bacterial vaginosis and *T.vaginalis* to HIV-1 transmission is again underscored.

Self-administered, self-collected vaginal tampons provide a suitable non-invasively collected specimen for the diagnosis of *T.vaginalis, N.gonorrhoeae* and *C.trachomatis* by nucleic acid amplification (NAA) techniques. A diagnosis of BV on these specimens would allow for population-based research investigating the epidemiology of all four major causes of vaginal discharge and their relation to HIV. The second paper in this chapter, reports on the utility of using a tampon for the diagnosis of BV.

Interrelationships amongst Human Immunodeficiency Virus Type 1 Infection, Bacterial Vaginosis, Trichomoniasis and the Presence of Yeasts

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ABSTRACT

Vaginal discharge of mixed aetiology occurs frequently with abnormal vaginal flora being the most common condition. The interrelationships among the disturbance of the vaginal ecology, the presence of yeasts, and infection with *Trichomonas vaginalis* and human immunodeficiency virus type 1(HIV-1) were investigated among women presenting to a sexually transmitted disease service. Analysis was done for 598 women. Although the prevalence of HIV-1 infection increased linearly with increasing Nugent's score (bacterial vaginosis score of Gram stain), the prevalence of *T.vaginalis* increased suddenly, from 12% in patients with a Nugent's score $\leq$ 3 to 33% with a score of 4, and remained at this level at higher scores. Yeast colonization and vulvovaginal candidiasis were inversely related to Nugent's scores. *T.vaginalis* might be responsible for the change in normal vaginal flora and may, therefore, be one of the causes of bacterial vaginosis. This could lead to more effective HIV-1 acquisition.

Introduction

Abnormal genital discharge in women may be differentiated into cervicourethral and vaginal discharge syndromes, on the basis of the site of preference of the causative microorganisms. The usual site of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections is the cervix and/or urethra, whereas discharge caused by *Trichomonas vaginalis* and yeasts as well as that associated with bacterial vaginosis originates from the vagina [1]. Historically, the 3 causes of vaginal discharge have been a lesser public health priority than are cervical infections and have been viewed largely as merely a nuisance and not as a serious threat to the health of women.

Although *T.vaginalis* is considered to be a sexually transmitted pathogen, it only recently has gained prominence as a cause of adverse pregnancy outcome[2,3]. An association of this microbe with increased risk of human immunodeficiency virus type 1(HIV-1) transmission has also been documented [4,5].

Vulvovaginal candidiasis is prevalent among both sexually active and inactive women. Its role in HIV infection is largely undefined [6-9], although a longitudinal cohort study reported a similar prevalence and clinical and microbiological spectrum in HIV-infected and -uninfected women [8].

Controversy has surrounded the inclusion of bacterial vaginosis as a sexually transmitted infection[10], whereas the association of bacterial vaginosis and adverse pregnancy outcome has been a 'hot topic' for ~ 2 decades [11-14]. Therefore, although this condition is highly prevalent in most developing countries [15,16], researchers focussing on sexually transmitted infections, have previously ignored bacterial vaginosis. The link between the recognised sexually transmitted infections and HIV infection[17,18] was made several years before such an association was first reported for bacterial vaginosis [19]. Subsequently, an association between the level of abnormality of the vaginal flora and HIV prevalence has been reported elsewhere [20,21]. Two prospective longitudinal studies show a strong association between abnormal vaginal flora and HIV acquisition [21,22]. It appears that it is not bacterial vaginosis per se that increases the risk of HIV acquisition, but a 'dose related' abnormality of the vaginal flora, with an increase in HIV seropositivity as the flora becomes more abnormal. It is unclear whether the lack of H_2O_2-producing lactobacilli or the increase in the number of anaerobic bacteria facilitates HIV infection.

Methods

Setting

The Hlabisa Health District in northern KwaZuluNatal serves a mainly Zulu speaking population of ~ 210.000 people. Many of the male inhabitants are migrant labourers, most of whom travel between their homesteads and the gold mines in the Johannesburg area or the industrial plants in Richards Bay. There is a high prevalence of sexually transmitted infections in the district, including HIV-1 infection. The district has 1 primary care hospital and 14 clinics. The largest clinic, KwamSane, is situated in the most populated area of the district and serves a considerable proportion of the population. The Africa Centre for Population Studies and Reproductive Health started a specialized primary health care sexually transmitted diseases clinic within the KwamSane clinic premises in January 1999. Study patients were recruited at that clinic.

Clinical investigations

Between March 1999 and February 2000, consecutive consenting women with symptoms of female discharge disease (vaginal discharge, dysuria, vulvovaginal itch) who attended this sexually transmitted disease service during office hours were invited to participate in the study. Trained interviewers collected demographic data including age, sexual history, and presenting symptoms. An HIV counsellor performed pretest counseling. The patients received a full clinical examination and

microbiological work up for the discharge-causing pathogens. At the time of this visit, patients were treated according to the provincial syndromic management guidelines for female discharge disease. The treatment comprises an acute dose of 250mg ciprofloxacin and 200mg doxycycline for 7 days and an acute dose of 2g metronidazole. A diagnosis of vulvovaginal candidiasis was made if patients presented with a clinical picture of thrush in combination with yeasts on Gram stain. Although the syndromic management protocol provides for the treatment of vulvovaginal candidiasis, topical imidazoles are not freely available and the treatment of this condition is largely neglected. Patients who wanted to know their HIV test result received posttest counseling.

Specimen collection and transport
A Dacron swab was used to collect vaginal specimens. A smear was made onto a glass slide for Gram staining, after which the swab was placed in Diamond's medium at room temperature until arrival in the laboratory. Blood for HIV testing was collected in EDTA-containing tubes. All specimens were transported within 10 h of collection to the Africa Centre Sexually Transmitted Diseases Laboratory in Durban for processing or appropriate storage. The type culture strain of *T.vaginalis* (ATCC 50138) is recoverable at a concentration of 10 cells/tube of Diamond's medium when stored and transported under these circumstances (data not shown).

Microbiology
The Gram stain of the vaginal smear was scored for bacterial vaginosis by use of Nugent's criteria [23]. Each slide was read by two independent researchers and evaluated by another when discrepancies arose. The Gram stain also was evaluated quantitatively (number of yeast cells/ oil immersion field) for the presence of yeast cells as well as pseudohyphae. If the patient did not have vulvovaginal candidiasis clinically, then the presence of yeast on Gram stain was interpreted as colonization of the genital tract. The inoculated Diamond's medium was incubated at 37 ^{o}C for 2 days. A wet mount was then prepared for the visualization of motile trichomonads. Cultures yielding negative results were re-incubated and read every day for up to five days or until results became positive.

An HIV screening test (Determine Test ;Abbott Diagnostics) was used on the plasma specimens. Those testing positive were confirmed by two different ELISAs (HIV 1.2.0 [Abbott/Murieux]and Vironosticka HIV uniform 2+0 [Omnimed]). A random sample of 10 % of the screening test-negative specimens was also subjected to ELISA confirmation.

Statistical methods
Significant risk factors associated with infection and associations among infections were determined by χ^2 tests and relative risks with 95% confidence intervals reported for categorical data and *t*-test with Spearman's correlation for continuous numerical data. A logistic model was used to test whether STIs were independently associated with HIV. Relative risks were compared using Mantel-Haenszel χ^2 test of homogeneity. Data were analysed using SAS statistical software (version 6 ; SAS Institute).

Results

A total of 722 women were enrolled in the study; the median age 24 years (17-70 years). For logistical reasons, there were no results available for bacterial vaginosis for 20 patients, for *T.vaginalis* for 35 patients, for yeasts for 112 patients, and for HIV for 11 patients. One or more of these results were missing for 124 patients. Therefore, 598 women are included in this analysis.

When Nugent's Gram stain score was applied to the smear of the vaginal discharge of the 598 patients, 106 (18%) patients scored from 0 to 3, 73 (12%) patients scored from 4 to 6 and 419 (70 %) scored from 7 to 10. There was little difference between the percentages of patients with low and intermediate scores, but there was a

significantly higher prevalence of patients with a high score (p < .001). *T.vaginalis* was present in samples from 177 (30%) patients, yeasts were detected in samples from 158 (26%) patients and HIV-1 antibodies were found in samples from 333 (56%) patients. Six percent of those with yeasts on Gram stain, were clinically diagnosed as having vulvovaginal candidiasis.

Table 1 shows the distribution of *T.vaginalis* infection, the presence of yeast on Gram stain, and HIV-1 seropositivity over the different categories of bacterial vaginosis score of Gram stain. There was a statistically significant difference in prevalence of *T.vaginalis* in patients with low (0-3 [12%]) and intermediate scores (4-6 [33%]; p<.001) (p<0.001). However, the prevalence of *T.vaginalis* infection in women with intermediate and high ($\geq$ 7) scores was similar (~ 33%). Detection of yeast on Gram stain was associated with a decreasing bacterial vaginosis score (r = -0.6; p=.06). The prevalence of HIV-1 infection showed a positive association with increasing bacterial vaginosis score (r = 0.6; p = .05). However, this did not reach statistical significance.

Table 1. Distribution of *Trichomonas vaginalis* infection, yeast colonization, and human immunodeficiency virus type 1 (HIV-1) among women, according to Gram staining categories for bacterial vaginosis.

Nugent's score	No. of women	Infected with *T. vaginalis*	Colonized by yeasts	HIV-1 positive
0–3	106	12	42	33
4–6	73	33	26	52
7–10	419	33	23	62

NOTE. Data are percentage of women, except where noted otherwise.

Table 2 shows the relative risks of being HIV-1 infected when having a form of abnormal vaginal discharge and the relative risk of having each of these conditions when being HIV-1 infected. The relative risk of being HIV-1 infected was 1.5 (95% confidence interval [CI], 1.3-1.8; p=.001) with a bacterial vaginosis score $\geq$ 7, whereas the risk of having such

Table 2. Association between causative agents of vaginal discharge and human immunodeficiency virus type 1 (HIV-1) seropositivity.

Comparison, result indicating vaginal discharge pathogen	No. (%) positive	Relative risk (95% CI)	*P*
HIV-1 positive if pathogen positive			
Culture positive for *Trichomonas vaginalis* ($n = 177$)	106 (60)	1.1 (0.9–1.3)	.2
Bacterial vaginosis score of Gram stain of ≥ 7 ($n = 419$)	260 (62)	1.5 (1.3–1.8)	.001
Yeast on Gram stain ($n = 158$)	84 (53)	0.9 (0.8–1.1)	.5
Pathogen positive if HIV-1 positive			
Culture positive for *T. vaginalis*	106 (32)	1.2 (0.9–1.5)	.2
Bacterial vaginosis score of Gram stain of ≥ 7	260 (78)	1.3 (1.1–1.4)	.001
Yeast on Gram stain	84 (25)	0.9 (0.7–1.2)	.5

NOTE. CI, confidence interval.

a level of imbalance in the vaginal ecosystem for HIV-1 infected women was 1.3 times higher (95% CI, 1.1-1.4; p=.001) than that for non- HIV-1 -infected women. Bacterial vaginosis remained independently associated with HIV-1 after adjusting for the presence of yeast on Gram stain, as well as *T. vaginalis* (odds ratio, 2.3; 95% CI, 1.6 – 3.3).

Although the risk of being infected with *T.vaginalis* for HIV-1-positive women was 1.2 times that of HIV-1- negative women, this did not reach statistical significance (95% CI, 0.9 - 1.5; p=.2). There was no increased risk of being HIV-1-infected when infected with *T.vaginalis* or colonized with yeasts, and HIV-1 seropositivity did not increase the risk of being colonized with yeasts (table 2).

Patients with trichomoniasis had recurrent episodes of discharge in the 6 months before enrollment significantly less frequently than did those with bacterial vaginosis (p=.03) or yeast colonization (p=.048). Patients with yeast colonization had vaginal itching more frequently than did those with trichomoniasis (p=.025), and the prevalence of this symptom in patients with bacterial vaginosis or with yeast colonization was similar(p>.05). No differences in clinical characteristics were observed between patients with trichomoniasis or bacterial vaginosis.

Discussion

Bacterial vaginosis is recognized as a syndrome in which there is an imbalance of the vaginal ecosystem [24]. Hydrogen peroxide-producing lactobacilli have been identified as key components in maintaining a normal vaginal milieu [25]. The pathogenetic mechanism for bacterial vaginosis is unknown, but it may vary from the use of topically applied substances with antimicrobial effect to a localized immunologic defect or it may result from infection with an organism that competes or interferes with the lactobacilli. Such an infection may be sexually transmissible or of endogenous origin.

Trichomoniasis was strongly associated with abnormal vaginal ecology. This association has been described by other researchers [21,26]. However, we showed that this association is not linear. The prevalence of *T.vaginalis* in women with a normal Nugent's score was low, but this rose sharply from a score of 4 and remained the same for all levels of vaginal imbalance. This can be explained in 2 different ways: a normal vaginal ecology (score 0-3) may inhibit infection with *T.vaginalis*, or trichomoniasis may change the vaginal ecology, causing it to resembles bacterial vaginosis. If *T.vaginalis* is inhibited by normal vaginal flora, one would expect the prevalence of this organism to rise gradually, with a decrease in the normal bacterial flora. The sudden increase at a score of 4, and constant prevalence of *T.vaginalis* infection in patients with all levels of abnormal vaginal flora (bacterial vaginosis score of Gram stain $\geq$ 4) suggest that this microbe might contribute to the change in vaginal flora. If this is indeed the case, then *T.vaginalis* may be considered an important factor in the development of bacterial vaginosis. This needs to be studied further by means of a longitudinal approach.

The presence of yeasts on microscopy was inversely related to the level of ecologic disturbance. This supports the view that the bacterial vaginosis environment is not conducive to yeast multiplication, and yeast vaginitis therefore does not occur in the presence of bacterial vaginosis. This is supported by the very low prevalence of vulvovaginal candidiasis (6%) in our patients with an extremely high prevalence of bacterial vaginosis.

The significance of positive microscopy or culture for yeasts of routine vaginal discharge specimens is controversial [7]. Our observation that there is no difference in discharge characteristics between patients with yeasts on Gram stain and those with another microbiologic etiology is in keeping with the low prevalence of clinically diagnosed yeast infection among our patients. We considered the possibility that this infection was underdiagnosed clinically. However, topical imidazoles are not freely available locally, and only 2 women received treatment for candidiasis, whilst cure rates were similar in women with and without yeasts on microscopy (data not shown). This indicates that attempts to diagnose yeast infection by means of routine laboratory tests in our setting, where the prevalence of bacterial vaginosis and *T.vaginalis* are high, is futile. These tests should be exclusively applied for confirmation in cases of clinical suspected yeast infection.

Whether HIV-induced vaginal immune impairment plays a role in the development of bacterial vaginosis cannot be concluded from our data. Like other researchers [20-22], we found a strong association between the prevalence of HIV infection and the level of abnormal vaginal ecology. These observations may reflect more efficient HIV transmission with changing vaginal ecology, as well as an HIV-driven change in the vaginal flora. We found that the risk of being HIV- positive was 1.5 times higher in patients with a bacterial vaginosis score $\geq$ 7, whereas the risk of having such a bacterial vaginosis score when HIV-positive was only 1.3 times higher than for those who were HIV-negative ($p = .15$; table2). Other researchers have reported that bacterial

vaginosis favours the acquisition of HIV-1[21,22].

A recent in vitro model which studied the effects of bacterial vaginosis -associated anaerobic bacteria on HIV-1 expression in monocytoid cells and T cells supports a mechanism by which disturbances in vaginal flora could lead to a higher rate of shedding of HIV. *Gardnerella vaginalis*, *Peptostreptococcus asaccharolyticus* and *Prevotella bivia* were shown to stimulate HIV-1 expression in monocytoid cells, and *P. asaccharolyticus* also enhanced HIV-1 expression in T-cells[27].

In conclusion, we showed a positive linear association between HIV-1 infection and increasing bacterial vaginosis score of Gram stain. The presence of yeasts on Gram stain showed an inverse relationship while there was a sudden increase of *T.vaginalis* infections at a score of 4, which remained constant to a score of 10. We propose that *T.vaginalis* has a role in the pathogenesis of abnormal vaginal ecology, which, in turn, results in more effective HIV-1 acquisition. A prospective longitudinal study is needed to show such a temporal relationship.

In contrast to *T.vaginalis* infections that showed a non-linear association with increasing vaginal flora abnormality, HIV-1 showed a positive linear association (table 1). This difference explains why we did not find an independent association between HIV-1 infection and trichomoniasis. If there is any association between trichomoniasis and abnormal vaginal flora, then *T.vaginalis* infection promotes the acquisition of HIV through alteration of the vaginal flora. If this hypothesis holds true, then control strategies that focus on decreasing *T.vaginalis* prevalence may be of crucial importance in containing the HIV-1 epidemic in sub-Saharan Africa where *T.vaginalis* prevalence is extremely high. Although men act mostly as asymptomatic transmitters, the inclusion of metronidazole in the treatment of those who seek medical attention for any sexually transmitted disease has the potential to decrease the prevalence of this infection in women.

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Diagnosis of Bacterial Vaginosis on Vaginal Tampon Specimens

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ABSTRACT

Background Bacterial vaginosis, like sexually transmitted infections, causes severe complications and is associated with increased transmission of HIV. Population studies are necessary to gain insight in the epidemiology of bacterial vaginosis, sexually transmitted infections, and HIV. A self-administered vaginal tampon was evaluated for the diagnosis of BV in pregnant women.

Methods Gram stain results from unconcentrated tampon fluid, cytospin preparations, and vaginal smears collected during speculum examination were evaluated by two observers using the Nugent score. The presence of the Amsel criteria was assessed.

Findings Using the Amsel criteria, 21% of the 84 women enrolled were diagnosed with bacterial vaginosis, and using the Nugent score on vaginal smears 29% and 31% by observer 1 and 2, respectively. The Nugent score results of unconcentrated tampon fluid and vaginal smears showed excellent agreement for both observers (Pearson >0.87). The interobserver agreement for a Nugent score >6 on these preparations was similar as well as the sensitivity and specificity using the Amsel criteria as reference standard.

Interpretation A simple drop of expressed fluid from a self-administered vaginal tampon can be used for the aetiological diagnosis of vaginal discharge including bacterial vaginosis. Because of the non-invasive nature of the specimen, it can be used in population based studies.

Introduction

Bacterial vaginosis (BV) is one of the most common causes of vaginal discharge. The pathogenesis of this condition is still unclear and it is unclear whether BV is sexually transmitted. However, there is a clear association of BV with other STIs, sharing identical risk factors, symptomatology, and complications including the association with HIV [1,2]. Bacterial vaginosis is a clinical syndrome and the gold standard for a diagnosis of BV is based on the rather subjective Amsel criteria. Microbiologically, bacterial vaginosis is characterised by a disbalance of the vaginal flora with decreased numbers of lactobacilli and increased numbers of anaerobic bacteria resulting in a distinct microscopic picture. Therefore, the diagnosis of BV is increasingly based on a gram stain evaluation of a vaginal smear using the Nugent score [3]. This method has been shown to be highly sensitive compared to the Amsel criteria [4]. The relative low specificity can be explained by the subjective nature of the Amsel criteria resulting in false negative results. In addition, although the gram stain features in these cases are identical as seen in BV patients, the full BV syndrome fulfilling the Amsel criteria may not have developed as yet.

Self-administered, self-collected vaginal tampons provide a suitable non-invasively collected specimen for the diagnosis of GC, CT, and TV by nucleic acid amplification (NAA) techniques [5-7]. A diagnosis of BV on these specimens would allow for population based research investigating the epidemiology of all four major causes of vaginal discharge and their relation to HIV.

We have shown previously the possibility to diagnose BV on a smear made from vaginal tampons [8]. However, this would increase the risk of contamination if this is done before processing for NAA tests. For the previously described molecular diagnosis of the STIs the tampons are collected in a transport buffer and the expressed tampon fluid is subsequently used for analysis. Thus, to be able to use the vaginal tampon for the combined diagnosis of the 4 major causes of vaginal discharge, it is necessary to diagnose BV on a Gram stain made from the expressed tampon fluid rather than on a smear of the tampon. Since the Nugent score is based on the semi-quantification of different morphological types of bacteria, the dilution of the vaginal secretions in the transport buffer may affect the results.

In this study, we evaluated the use of vaginal tampon fluid for the microscopic diagnosis of BV in a cohort of pregnant women. We compared the Gram stain score on vaginal smears collected during speculum examination with the Gram stain made directly from the tampon fluid and from tampon fluid concentrated by means of cytospin centrifugation.

Materials and Methods

Study participants were randomly recruited among new admissions to the obstetric wards of King Edward VIII hospital, Durban, South Africa. Women with contraindications for speculum examination and/or specimen collection during this procedure were excluded. Contra-indications were apparent labour, cervical insufficiency, per vaginal bleeding, and placenta praevia.

The vaginal tampon was inserted by the women and left in place for 15-30 minutes. The tampon was removed by the study doctor to assess the degree of insertion and collected in 10 ml phosphate buffered saline, pH 7.6. Subsequently, speculum examination was carried out. Endocervical swabs were taken after cleaning of the cervical os for the culture of *N. gonorrhoeae* on New York City media and the detection of *C. trachomatis* by immunofluorescent staining (MicroTrak,® Trinity Biotech, Ireland). One vaginal swab was taken for *T. vaginalis* culture in Diamond's media and a

second swab was used to make a smear for gram staining.

The Amsel criteria were assessed in all women. There was always sufficient discharge in the posterior fornix to perform the tests. The release of amines from vaginal secretions was noted after the addition of 10% KOH. The pH of the secretions was measured using a narrow range pH strip (Merck), range 0 to 6 with 0.5 intervals. The presence of clue cells was evaluated by light microscopic evaluation (X40) of a wet mount preparation.

Vaginal tampon specimens reached the laboratory within 1 hour and were processed on arrival. Tampons were expressed thoroughly with a sterile tongue depressor. A drop of the expressed fluid was air dried on a slide. Subsequently cytospins were made from 25 and 50 µl with 10 minutes centrifugation at 2000 rpm.

All gram stains of the vaginal smears and tampon fluid preparations were coded and evaluated independently by 2 observers (AWS, PM) using the Nugent scoring system.

Test parameters of the Nugent score for the diagnosis of BV were calculated after exclusion of the patients with *T. vaginalis* infection.

Results

A total of 84 women were enrolled in a 3-month period. The mean age of the cohort was 28 years (SD $\pm$ 6.98), mean gravidity 2.63 (SD $\pm$ 1.51), mean parity 1.48 (SD $\pm$ 1.52), and mean gestational age 32 weeks (SD $\pm$ 5.41). STIs were diagnosed in 30% of the women, 20(24%) were infected with *T. vaginalis* of which 3 were co-infected with *C. trachomatis* and 1 with *N. gonorrhoea*. Another 5 women were diagnosed with *C. trachomatis*. The presence of infection with GC and CT could not be assessed in 17 women because the cervix was not visualised. BV was diagnosed in 18 women (21%) using the Amsel criteria and in 24 (29%) and 26 (31%) women using Nugent score on vaginal smears by observer 1 and 2, respectively.

For both observers the intraobserver agreement of a Nugent score >6 was similar comparing the different tampon fluid preparations with the vaginal smear . The correlation of the Nugent scores on the different tampon fluid preparations and the vaginal smear was excellent for both observers (Table 1). The interobserver agreement for a Nugent score >6 was similar on all specimen preparations with an excellent correlation of the Nugent scores (Table 2).

There was no significant difference of the test parameters of the Nugent score when performed on vaginal smears or on the different tampon fluid preparations using the Amsel criteria as reference standard (Table 3).

Tampons were completely inserted in 51% of the women, and at least half of the tampon was inserted in 75%. Incomplete insertion was not related to disagreement between results from vaginal smears and tampon fluid (Table 4).

A gradual increase of the presence of any of the Amsel criteria, excluding homogeneous discharge, with increasing Nugent score was observed in women without BV defined by the Amsel criteria and without *T. vaginalis* infection. Any of the Amsel criteria were present in 20% of the women with a Nugent score <4, 50% with a Nugent score 4-6, and 55% with a Nugent score >6 . A homogeneous discharge was excluded from above calculations since its presence was equally distributed over women with and without BV (67% and 60%, respectively) as opposed to the other criteria; in women with BV and without BV the amine test was positive in 94% vs 0%, clue cells were present in 89% vs 16%, and pH >4.5 in 89% vs 16% .

Of the 20 women with *T. vaginalis* infection, 9 were Amsel criteria positive. The Nugent scores on the vaginal smears were >6 by both observers in 6 cases, >6 by 1 observer but 4-6 by the other in 2 cases, and 4-6 by both observers in 1 case. The remaining 11 women with *T. vaginalis*

infection had Nugent scores of >6 by both observers in 9 cases, 4-6 by both observers in 1 case, and <4 by both observers in 1 case. The women with C. trachomatis infection only were Amsel criteria negative and had Nugent scores <4 (4 cases) and 4-6 (1 case) by both observers.

Discussion

Vaginal tampon specimens were previously shown to be useful for the molecular diagnosis of TV, GC, and CT, and more sensitive for the diagnosis of TV and GC as compared to first catch urine [7]. This study shows that a simple drop of the expressed tampon fluid can be used for the diagnosis of BV using the Nugent score.

The sensitivity and specificity of the Nugent score for the diagnosis of BV on a drop of tampon fluid and the vaginal smear was similar and there was excellent correlation of the Nugent score results from both specimen preparations. The sensitivities and the relative low specificities reported in this study for the diagnosis of BV on gram stain with the Amsel criteria as the reference standard are in keeping with a previous study that showed a sensitivity ranging from 78 to 91% and a specificity of 67 to 94% [4]. The cytospin preparations did not increase the correlation with the vaginal smear, nor did they increase the diagnostic yield on the tampon fluid. The interobserver agreement for a Nugent score of >6 was similar to the 89% reported by Nugent et al [3].

Incomplete insertion of the tampons was not related to disagreement between results of vaginal smears and tampon fluids. Even if only the tip of the tampon was inserted, results from vaginal smears and unconcentrated tampon fluids categorised patients identically in 88%.

As previously discussed, the low specificity of the Nugent score for the diagnosis of BV as defined by the Amsel criteria probably represents the ability of the Nugent score to detect changes in the vaginal flora at a different, may be earlier,

Table 1. Intraobserver agreement for a Nugent score >6 and the correlation of the Nugent scores (0–10) between the tampon fluid preparations and the vaginal smear for both observers O1 and O2 (n=84)

	Agreement (%)		Spearman r	
Specimen	O1	O2	O1	O2
Unconcentrated	86	90	0.86	0.90
25 μL cytospin	83	93	0.80	0.86
50 μL cytospin	87	90	0.82	0.89

Table 2. Interobserver agreement for a Nugent score >6 and the correlation of the Nugent scores (0–10) (n=84)

Specimen	Agreement (%)	Spearman r
Vaginal smear	93	0.89
Unconcentrated	86	0.90
25 μL cytospin	93	0.87
50 μL cytospin	82	0.87

Table 3. Test parameters of the Nugent score on vaginal smear and tampon fluid preparations for a diagnosis of bacterial vaginosis defined by the Amsel criteria (n=64, women with T. vaginalis infection excluded)

	O1	O2
Vaginal smear		
Sensitivity (%)	83	94
Specificity (%)	80	80
Unconcentrated		
Sensitivity (%)	94	89
Specificity (%)	78	80
25 μL cytospin		
Sensitivity (%)	100	89
Specificity (%)	74	80
50 μL cytospin		
Sensitivity (%)	94	89
Specificity (%)	80	80

Table 4. Percentage agreement of Nugent score categorization (0–3, 4–6, 7–10) on vaginal smear and unconcentrated tampon fluid related to degree of tampon insertion

		Agreement (%)	
Tampon insertion	n=84	O1	O2
Complete 1.00	38	76	79
0.75	5	100	100
0.67	7	86	86
0.5	6	83	83
0.33	11	91	73
0.25	4	50	100
Tip	8	88	88
Unknown	5	80	100

stage of disease. The low sensitivity of the Amsel criteria due to their subjective nature can also be responsible for this observation. Our finding that more than 50% of the women with a Nugent score >6 and no BV according to the Amsel criteria have 1 or more of the Amsel criteria positive supports both these possibilities.

The clinical significance of an intermediate Nugent score has not been well established. There is evidence that the intermediate score is a transitional phase between normal flora and BV and that the vaginal flora can develop in either direction [9-11]. Increased risk of postoperative infection after surgical termination of pregnancy and increased prevalence of HIV infection has been reported in women with an intermediate BV score [1,2,12]. The high prevalence of abnormal findings, i.e. Amsel criteria, in cases with an intermediate Nugent score further supports the possible significance of this condition. Therefore, it maybe be more important to identify women with a Nugent score 4-10 rather than only those women with BV. This could also done reliably on a drop of tampon fluid with a percentage agreement of 94% with the vaginal smear.

Interestingly, a third of the women who were Amsel criteria positive and half of the women who were Amsel criteria negative but with a Nugent score >6 had a *T. vaginalis* infection, and 85% of the women with a *T. vaginalis* infection had a Nugent score >6. This confirms our observations in a cohort of STD clinic attendees [13].

BV is associated with adverse pregnancy outcome and PID. Furthermore there is evidence that BV like STIs facilitate the transmission of HIV. The high prevalence of BV and STIs among pregnant women predominantly admitted for pregnancy related pathology illustrates the extent of the problem of BV and STIs in South Africa. A self-administered self-collected vaginal tampon provides a suitable specimen for the combined diagnosis of the 4 major causes of vaginal discharge facilitating self-initiated testing and population based studies to investigate the epidemiology of these diseases and their relation to HIV.

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Chapter 5

Aetiology of STIs readdressed

Summary

Knowledge of the aetiology of the various STI syndromes is essential for the development of a rational and comprehensive approach to syndromic management.The study described in paper 1 suggest a a role for *M.genitalium* as a cause of urethritis. In addition, a history of urethritis in HIV-1infected males supports the hypothesis that non-ulcerative STDs are co-factors in HIV transmission.
The data in paper 2 suggest that T.vaginalis is a risk factor for pelvic inflammatory disease, but only in HIV-infected women.
LGV is found to be the second most common cause of genital ulceration locally. Paper 3 looks at the PCR based diagnosis and presentation of these ulcers. This needs to be taken into account in the development of new syndromic management protocols.

Etiology of male urethritis in patients recruited from a population with a high HIV prevalence

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ABSTRACT

The etiology of urethritis, the significance of potential pathogens, and the relation of urethritis to HIV infection were determined in 335 men (cases) with and 100 men (controls) without urethral symptoms. Urethral swab specimens were tested for the different organisms by PCR or culture (*N.gonorrhoeae*). The prevalence of *N.gonorrhoeae* and *C.trachomatis* was 52% and 16%, respectively. The potential pathogens *M.genitalium, U.urealyticum, T.vaginalis,* and Herpes Simplex virus, were present in 5%, 36%, 6%, and 6% of the cases, respectively. *M.genitalium* was the only potential pathogen associated with microscopic urethritis. After exclusion of gonococcal infections, *U.urealyticum* was more frequent in symptomatic patients, while the prevalence of *T.vaginalis* was similar among cases and controls. These results strongly suggest an etiological role for *M.genitalium* in male urethritis, a possible role for *U.urealyticum*, but not for *T.vaginalis*. The control group, with 97% genital ulcer disease patients, was unsuitable to investigate the role of HSV. The sero-prevalence of HIV was 45%. Current infections were not associated with HIV. However, a history of previous urethral discharge remained associated with HIV in a multivariate analysis and supports the hypothesis that non-ulcerative sexually transmitted diseases facilitate HIV transmission.

Introduction

Non-ulcerative sexually transmitted diseases (STDs) play a role in the transmission of HIV, and there is evidence that effective STD control programmes in developing countries may decrease the incidence of HIV (4,5). Knowledge of the etiology is essential for the development of a rational and comprehensive approach for such programmes. The established causes of male urethritis are *N. gonorrhoeae* and *C. trachomatis*. However, in a significant proportion of symptomatic men these pathogens are not detected (7,14,18, 22).

Currently, *T.vaginalis*, *U.urealyticum*, and *M.genitalium* are thought to be potential causes of non-chlamydial non-gonococcal urethritis (NCNGU) in men but their role remains controversial. The prevalence of the different organisms varies between studies, but the number of studies is limited and most of these were performed in developed countries with only two studies from the African continent (14,18). The latter indicated that the prevalence of *M.genitalium* in the African setting is similar to that in other parts of the world, and that the prevalence of *T.vaginalis* varies significantly between countries. Another potential cause of symptomatology compatible with urethritis is herpes simplex virus-2 (HSV-2) (12, 27). It is currently the most prevalent cause of genital ulcer disease in Africa and is considered one of the most important STDs facilitating HIV transmission (15).

We investigated the prevalence of established as well as potential pathogens of male urethritis in an area with a high HIV prevalence. We compared this with STD clinic attendees from the same population without symptoms or signs of urethritis.

Materials and Methods

Patients

Men attending the City Health STD clinic in Durban, KwaZulu Natal, South Africa between 16 October and 29 November 2000 who reported symptoms of urethral discharge or dysuria were recruited. Men who presented to this clinic with other symptoms (predominantly genital ulcer disease (GUD)) were recruited as controls. Those on antimicrobial therapy in the two weeks prior to the clinic visit were excluded. Men who gave consent to urethral specimen collection and HIV testing were enrolled. Informed consent was obtained from all participants. Pre-test counseling was offered to all and post-test counseling to those that wanted to know their HIV results. The ethics committee of the Nelson R Mandela School of Medicine, University of Natal, approved the study.

Patient's history was obtained by a trained nurse using a standardized questionnaire and physical examination with specimen collection was performed by the research medical officer.

Specimens

To minimize blood contamination, urethral specimens for molecular diagnosis were collected first: a swab provided with the BDProbeTec^{TM} ET assay (Becton Dickinson Microbiology systems, USA) for detection of *C.trachomatis* and a dacron swab in 1 ml of phosphate buffered saline, pH 7.6 (PBS), for *T.vaginalis*, *U.urealyticum*, and *M.genitalium*. Following this a calcium alginate swab was taken for Gram stain and *N.gonorrhoeae* culture. Specimens for molecular diagnosis were stored at the collection site at 4^0C, transported to the laboratory on ice within 4 hours from collection and stored again at 4^0C till DNA extraction on the same day.

Microscopic urethritis

Microscopic urethritis was defined by the presence of $\geq$ 5 polymorphonuclear cells (PMNs)/high power field (HPF) on the Gram stain.

Diagnosis of *N. gonorrhoeae* infection

N.gonorrhoeae was cultured on New York City media (Oxoid, UK). Media were

inoculated at the bedside, placed in a candle jar, and transported to the laboratory at room temperature within 4 hours. On arrival in the laboratory, the media were incubated for 48 hours at 37^0C in 7% CO_2. Suspected colonies were identified by means of gram staining, catalase, oxidase, □-galactosidase, hydroxy-prolylaminopeptidase, □-glutamylaminopeptidase and acid production from glucose, lactose, maltose and sucrose.

Performance of culture was assessed by performing strand displacement amplification (SDA) (BDProbeTec™ ET assay, Becton Dickinson Microbiology systems, USA) for *N.gonorrhoeae* on a random selection of 88 culture negative specimens.

Cases with gram-negative cocci suspect for *N.gonorrhoeae* on Gram-stain in the absence of a positive culture were considered positive if the microscopic observations were confirmed by additional SDA.

Diagnosis of *C. trachomatis* infection

C. trachomatis infection was diagnosed by means of SDA (BDProbeTec™ ET assay, Becton Dickinson Microbiology systems, USA) using the manufacturer's protocol.

Diagnosis of *T.vaginalis*, *U.urealyticum*, *M.genitalium* and HSV infection

Specimens for PCR detection of *T.vaginalis*, *U.urealyticum*, *M.genitalium* and HSV-2 were stored and transported at 4^0C. On arrival at the laboratory, 0.5 ml of the PBS suspension was spun at 13000 g for 15 minutes. The pellet was incubated with 100 □l of a lysis buffer (50mM TrisCl pH 8.5, 1 mM EDTA, 0.2% Tween-20, and 100 □g/ml proteinase K). After vortexing, the specimens were incubated at 56 ^{0}C overnight followed by a 10 minute incubation at 96 ^{0}C. The specimens were stored at -20 ^{0}C until further use. PCRs for each of the organisms were performed as previously described (11,16) and recently developed (P.D.J. Sturm, S. Naidoo, S. Ebrahim, and A.W. Sturm, submitted for publication), using 10 □l of the proteinase K digest.

PCR inhibitors

All specimens with negative PCR results for all organisms were assessed for inhibition of the PCR by performing a PCR for the human □-globin gene (19).

HIV serology

HIV infection was diagnosed using Determine™ HIV1/2 (Abbott Laboratories, USA). All positive results were confirmed by a second serological test (Capillus™ HIV1/2, Trinity Biotech, USA).

Diagnostic categories

GONOCOCCAL URETHRITIS (GU): patients with urethral symptoms and positive for *N.gonorrhoeae* by culture or with gram-negative cocci on urethral smear confirmed by SDA, with or without other organisms or microscopic urethritis.

CHLAMYDIAL NON-GONOCOCCAL URETHRITIS (CNGU): patients with urethral symptoms and positive for *C.trachomatis* by SDA in the absence of *N.gonorrhoeae* with or without other organisms or microscopic urethritis.

NON-CHLAMYDIAL NON-GONOCOCCAL URETHRITIS (NCNGU): patients with urethral symptoms and microscopic urethritis in the absence of *N.gonorrhoeae* or *C.trachomatis*.

CLINICAL URETHRITIS: patients with urethral symptoms but without microscopic urethritis.

CONTROLS: patients without urethral symptoms and without microscopic urethritis.

Statistical Methods

Diagnostic categories were compared using Wilcoxon nonparametric tests for numeric data and chi-square or Fisher's exact tests for categorical data. Factors found significantly associated with HIV at the univariate level as well as known HIV confounders were included in a logistic model.

Results

Of the 341 symptomatic men enrolled, 6 were excluded from analysis because of incomplete laboratory results. Hundred and nine men were enrolled as controls. Results of four of these were excluded because of incomplete results and five because of the presence of microscopic urethritis. Therefore, the results of 335 cases and 100 controls are presented.

Cases and controls were similar with respect to sexual behavior, i.e. sexual experience as defined by age minus age at first intercourse (median, range) and total number of lifetime partners (median, range), 10 years (0-43) versus 11 years (2-34) (p=0.13) and 10 (1-100) versus 10 (1-100) (p=0.23), respectively. A history of a previous episode of discharge was more common among the cases (76% versus 61%, p 0.002), while a history of a previous episode of genital ulcer disease was more common among the controls (57% versus 36%, p 0.001). The prevalence of HIV was also higher in the control group (69% versus 45%, p 0.00005) that included almost exclusively GUD patients (97%). Sexual behavior, history of previous STDs, and HIV infection were similar in the different diagnostic categories of urethritis (data not shown).

Table 1 shows the etiologies in the cases, the different diagnostic categories of urethritis, and in the controls. Among the 335 cases, the prevalence of the established pathogens *N.gonorrhoeae* and *C.trachomatis* was 52% and 16%, respectively. *U.urealyticum* was the most common of the potential pathogens (36%), followed by HSV (6%), *T.vaginalis* (6%), and *M.genitalium* (5%). In 54 (16%) patients none of the organisms were detected, and in 11 (3%) patients there was evidence of PCR inhibition.

N.gonorrhoeae was absent in the controls but *C.trachomatis* and all 4 potential pathogens were present. The frequency of *C.trachomatis* was higher in the cases compared to the controls (p=0.04). There was no difference in the prevalence of *M.genitalium* (p=0.59), and *U.urealyticum* (p=0.26) in cases and controls, but both *T.vaginalis* and HSV had a significantly higher prevalence in controls as compared to cases (p=0.03 and p<<0.0001, respectively).

GU was diagnosed in 174 and CNGU in 29 patients. Of the remaining 127 men, 16 had microscopic urethritis and were diagnosed with NCNGU and 116 with clinical urethritis only. After categorization of the cases, the prevalence of *M.genitalium* was significantly higher in patients with NCNGU as compared to patients in the other diagnostic categories of urethritis (p<0.01) and the controls (p=0.0002). *M.genitalium* was the only potential pathogen associated with the presence of $\geq$ 5 PMN/HPF. When GU patients were excluded, the prevalence of *T.vaginalis* in the other urethritis categories was similar compared to the controls (p>0.11), but the prevalence of *U.urealyticum* was higher (p<0.05). HSV prevalence was similar among the different categories of urethritis (p>0.59).

Eighty-eight (26%) of the 335 patients presented with dysuria only. Of these, 65 (74%) had no microscopic urethritis, which was 56% of all patients categorized as clinical urethritis. No microscopic urethritis was present in 7 patients with GU (4%) and 17 patients with CNGU (59%).

None of the current infections were associated with HIV, and HIV prevalence was similar in the different categories of urethritis (data not shown). Sexual experience (age minus age at first intercourse), total number of lifetime partners, history of previous urethral discharge or GUD, but not circumcision were associated with HIV infection in univariate analysis (Table 2). After adjustment for each of the characteristics above, only a history of previous discharge or GUD remained associated with HIV infection.

Table 1. Prevalence of the organisms in the different diagnostic categories.

	Total		β-globin negative		*N.gonorrhoae*		*C.trachomatis*		*M.genitalium*		*T.vaginalis*		*U.urealyticum*		HSV	
Diagnostic category	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
All Cases	335	100	11	3	174	52	55	16	17	5	19	6	121	36	19	6
Gonococcal Urethritis	174	51	6	3	174	100	26	15	2	1	7	4	45	26	9	5
Chlamydial Non-Gonococcal Urethritis	29	9	0	0			29	100	2	7	2	7	16	55	2	7
Non-Chlamydial Non-Gonococcal Urethritis	16	5	1	6					6	38	3	19	10	63	0	0
Symptomatics without *N.gonorrhoeae* or C.trachomatis, and <5PMNs/HPF	116	35	4	3					7	6	7	6	50	43	8	7
Controls	100	100		2		0		8		3		12		30		31

Table 2. Factors associated with HIV.

Characteristic	No	No with HIV	%	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Sexual experience							
<10 yrs (median)	163	60	37				
≥10 yrs (median)	168	90	54	1.5 (1.1-1.9)	0.003	1.5 (0.9-2.5)	0.1
No of life time partners							
<10 (median)	125	44	35				
≥10 (median)	208	106	51	1.4 (1.1-1.9)	0.007	1.4 (0.8-2.4)	0.2
History of previous urethral discharge							
Yes	255	130	51	1.8 (1.3-2.7)	0.001	2.0 (1.1-3.8)	0.03
No	80	22	28				
History of previous GUD							
Yes	121	71	59	1.6 (1.2-2.0)	0.001	2.3 (1.4-3.7)	0.001
No	214	81	38				
Circumcision							
Yes	42	15	36	0.8 (0.5-1.2)	0.2	0.6 (0.3-1.2)	0.1
No	283	134	47				

Sexual experience, age minus age at first intercourse; OR, odds ratio; CI, confidence interval

Discussion

The prevalence of established and potential pathogens of urethritis was determined in men presenting to an STD clinic with symptoms of discharge and/or dysuria, irrespective of the presence of a visible discharge on examination or a microscopic diagnosis of urethritis. To assess the significance of the potential pathogens, prevalence rates in symptomatic men and asymptomatic STD clinic attendees were compared.

Data from Africa on the prevalence of potential pathogens in male urethritis are scanty (14,18,22). Prevalence rates were shown to be different in different parts of

Africa (18). The prevalence rates reported in the current study were in keeping with those from some other parts of Africa. However, higher rates for *N.gonorrhoeae*, *T.vaginalis*, and *M.genitalium* have been
found using similar methodology. An important difference between two of the previous studies from Africa and this study were the inclusion criteria, which makes comparisons between the studies invalid. In these previous studies patients with complaints of urethral discharge were included, while in the current study all patients with complaints of urethral discharge as well as patients with dysuria only were included. Differences in inclusion criteria will affect prevalence rates, e.g. *N.gonorrhoeae* infection is strongly associated with urethral discharge, and exclusion of patients with dysuria only will affect the relative prevalence rates. The third study used the same inclusion criteria as in this study but reports only on *N.gonorrhoeae*, *C.trachomatis* and *M.genitalium* (22).

The inclusion of patients with dysuria only resulted in a large proportion of patients without microscopic urethritis. However, a dilutional effect on the prevalence rates by inclusion of patients with diseases other than STDs in our study is unlikely and similar rates of patients without microscopic urethritis in consecutive patients with any urethral symptom have been reported (7,17). Besides STDs, cystitis and chronic prostatitis can cause urethral symptoms. No attempt was made to exclude these because the age distribution of the men presenting to our clinic is not compatible with these diseases. This was demonstrated in a study in which cystitis in male STD clinic attendees was diagnosed in less than 1% (7). *Schistosoma haematobium* is endemic in our area and this can also cause dysuria. The high prevalence of men in this cohort with dysuria only and without microscopic urethritis prompted us to investigate in an additional study the prevalence of schistosomiasis in such patients attending our clinic. *S.haematobium* ova

were found in only 10 (2.5%) of 400 consecutive men (data not shown). Moreover, seven of these were also infected with *N.gonorrhoeae*. Taking this into account together with the similar sexual behavior, history with respect to STDs, and HIV prevalence among the different categories of urethritis suggests a homogeneous population of male STD clinic attendees.

Our diagnostic methods were similar compared to most recent studies using previously described sensitive nucleic acid amplification tests for all organisms except *N.gonorrhoeae*. We investigated the presence of PCR inhibition in the PCR negative specimens and evidence of PCR inhibition was only found in 3% of all specimens. Randomly selected specimens negative for *N.gonorrhoeae* culture, were also negative using SDA suggesting a sensitive culture method.

Pathogenic properties of all potential pathogens of urethritis have been established, but what proportion of male urethritis can be attributed to them remains so far unclear. To investigate the causal role of the potential pathogens, the prevalence rates in symptomatic and asymptomatic individuals were compared. STD clinic attendees without urethral symptoms were selected as controls because some of the (potential) pathogens may be colonizers that are transmitted during sexual intercourse and therefore could be present with higher frequency in people at high risk for STDs without causing such infection (23). *N.gonorrhoeae* was not detected in the control group, but, although significantly lower compared to the cases, 8% were infected with *C.trachomatis.* The role of *M.genitalium* as a cause of male urethritis has been studied most extensively and the evidence of a causal relationship is substantial (21). This is supported by our findings. Although the prevalence of *M.genitalium* was similar in cases and controls, 5 and 3%, respectively, this organism had a significantly higher prevalence in NCNGU patients as compared to the controls and the other categories of urethritis. The prevalence of 38% in patients with NCNGU is twice as high as in a recent South African study (22) but within the range reported from other parts of the world (21). *M.genitalium* was the only potential pathogen associated with microscopic urethritis.

The causative relationship between male urethritis and *U.urealyticum* is controversial. It has been associated with non-gonococcal urethritis, but others could not confirm this. It has been proposed that the number of organisms present in the urethra is important in *U.urealyticum* infection. This cannot be established by qualitative PCR. But findings using quantitative cultures also do not concur (8,20). In two recent studies from Africa, *U.urealyticum* prevalence was similar among symptomatic patients and patients with complaints unrelated to the genitourinary tract (14,18). We also did not find a difference in prevalence between cases and controls. However, when gonococcal infections were excluded, *U.urealyticum* was associated with the other categories of urethritis.

The reported prevalence rates of *T.vaginalis* in male urethritis vary enormously (1,10). Surprisingly, in addition to the low prevalence of *T.vaginalis* in the symptomatic patients, *T.vaginalis* was more prevalent in the control group. The prevalence in cases and controls was similar after exclusion of patients with gonorrhoeae who carried less *T.vaginalis* as compared to patients in the other diagnostic categories. These findings suggest that this organism is not an important cause of urethritis in our population. This came as a surprise because of the high prevalence of trichomoniasis in antenatal clinic attendees in our area (P.D.J. Sturm, S. Naidoo, S. Ebrahim, and A.W. Sturm, submitted for publication) and the high rates reported in males from most parts of Africa (6,14,18,24). However, our findings are consistent with a recent population based study from Tanzania where a high *T.vaginalis* prevalence was found (11%). Only 50% of these patients were symptomatic with in most cases dysuria as the single complaint, and the majority had no microscopic urethritis (78%) (24). Two other studies from Africa reported a significantly higher rate of *T.vaginalis* infection among patients with urethritis compared to a control group consisting of men presenting to a clinic without genitourinary related complaints. Although controlled for a number of differences between the two groups, the difference in sexual behavior between cases and controls may have attributed to the association between *T.vaginalis* and urethritis in these studies (6,18).

Urethral HSV infection was uncommon in our study population. It was evenly distributed over the different diagnostic groups, but was more frequent in the control group. However, the control group was not suitable with respect to HSV since many patients had GUD (97%). Since HSV is now the most common cause of GUD in our area, the detection of HSV from the urethra likely represents urethral shedding or contamination of the urethral specimen with ulcer material. HSV as a cause of male urethritis has been described. Besides a case report, two studies reported the presence of HSV in urethral specimens from 12% and 10% of patients with NGU (2,12). A third study that did include a control group could not demonstrate a significant role for HSV in NGU (26). In one of the studies, HSV-2 antibody tests in serum were negative in 65% of the positive patients, which suggested primary infection (12).

The frequency of clinical urethritis in the absence of microscopic urethritis was 35%. This percentage is similar to other studies that included consecutive men with any urethral symptom (7,17). As in our study, most of these men had symptoms of dysuria only (56%). However, one such study from Africa reported only 11% of men with dysuria as the single complaint and less than 10% without microscopic urethritis (3). Since at least 23% of the patients in the latter study had received treatment elsewhere, the clinic at which their patients were recruited may have functioned partly as a referral clinic rather than a primary clinic and this may have influenced the pattern of presentation. Patients in our study were recruited at a primary STD clinic and only 2% reported antimicrobial treatment for their current disease within the last four weeks while only 12 patients were excluded from the study because of treatment in the two weeks prior to their visit.

Clinical urethritis in the absence of microscopic urethritis was also found in 24 patients with established pathogens but these were categorized according to the presence of the organism: 17 with CNGU and 7 patients with GU. These 17 *C.trachomatis* infections represented 12% of all patients without microscopic urethritis and 59% of all *C.trachomatis* infections in the absence of gonococci. This absence of microscopic urethritis in the presence of *N.gonorrhoeae* or *C.trachomatis* in symptomatic patients has been reported previously and therefore the accuracy of a cut-off of $\geq$5 PMNs/HPF to determine the presence of urethritis is questionable (7,14).

Apparently there is a group of patients with symptoms compatible with urethritis that has a poorly defined clinical picture of unknown etiology. As discussed above, the presence of $\geq$5PMNs/HPF on urethral smear may not be an adequate definition of urethritis. The role of anaerobes in male urethritis is unclear and has had little attention so far, but bacteria associated with bacterial vaginosis may be one of these unknown etiologies (9,20,25,26), and this should be considered in the face of the high prevalence of bacterial vaginosis in Africa (13).

Our data suggest an etiological role for *M.genitalium* in male urethritis, and possibly for *U.urealyticum*, but not for *T.vaginalis*. The role of HSV in urethritis could not be established because the control group was inappropriate to evaluate this. However, the data presented should be interpreted with caution because of the low number of patients with positive findings for some of the organisms. Although in many cases the etiology of the urethritis remains uncertain, current syndromic management of urethritis with ciprofloxacin and doxycycline but not metronidazole is effective. Recent evaluation of our syndromic management protocol showed no clinical response in only 1.4% of 272 patients (P. Moodley, P.D.J. Sturm, D. Wilkinson, C. Connolly, and A.W. Sturm, submitted for publication). A self-limiting course of disease rather than effective therapy cannot be excluded.

Urethritis increases the number of HIV viral copies in genital secretions in HIV infected patients thereby increasing their infectiousness (4). At least in women, there is some evidence that non-ulcerative STDs make HIV negatives more susceptible to HIV infection by recruitment of HIV target cells to the site of infection (4). The association of HIV with a history of urethritis independent of other risk factors for acquiring HIV including duration of sexual activity, number of lifetime sexual partners, and a history of previous genital ulcer disease, suggests that non-ulcerative diseases do play a role in HIV transmission also in the direction of female to male. Alternatively, HIV infected patients are more susceptible to infection with urethritis pathogens.

The control group included almost exclusively GUD patients which explain the high HIV prevalence rate in these patients.

In conclusion, like in other parts of Africa *N.gonorrhoeae* and *C.trachomatis* are common causes of male urethritis in our area with a preponderance of *N.gonorrhoeae* infections. This differs from most developed countries. Although the results indicate a role for *M.genitalium* and *U.urealyticum*, the significance of these potential pathogens remains uncertain. It is likely that all can cause urethritis, but, as in other studies, a substantial proportion of men harboring each of these organisms were asymptomatic. Possibly, infection with these organisms results in only mild self-limiting disease that without treatment results in persistent carriage. The association of a history of urethritis with HIV supports the hypothesis that non-ulcerative STDs are a cofactor in transmitting HIV in the female to male direction.

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Trichomonas vaginalis is Associated with Pelvic Inflammatory Disease in Women Infected with Human Immunodeficiency Virus Women

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ABSTRACT

We assessed the association between the causative agents of vaginal discharge and pelvic inflammatory disease (PID) among women attending a rural sexually transmitted disease clinic in South Africa; the role played by coinfection with human immunodeficiency virus type 1 (HIV-1) was studied. Vaginal and cervical specimens were obtained to detect *Neisseria gonorrhoeae, Chlamydia trachomatis , Trichomonas vaginalis* and bacterial vaginosis . HIV-1 infection was established by use of serum antibody tests. A total of 696 women with vaginal discharge were recruited,119 of whom had clinical PID. Patients with trichomoniasis had a significantly higher risk of PID than did women without trichomoniasis (p = .03). PID was not associated with any of the other pathogens. When patients were stratified according to HIV-1 status, the risk of PID in HIV-1- infected patients with *T.vaginalis* increased significantly (p= .002); no association was found in patients without HIV-1. *T.vaginalis* infection of the lower genital tract is associated with a clinical diagnosis of PID in HIV-1- infected women.

Introduction

Pelvic inflammatory disease (PID) is a condition in which there is infection of the reproductive tract of women above the internal os of the cervix. This has classically been associated with an ascending cervical infection caused by the vaginal discharge pathogens of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and by the anaerobic bacteria associated with bacterial vaginosis (BV). Although *Trichomonas vaginalis* is a common cause of vaginal discharge that infects ~ 120 million women per year [1], less attention has been paid to its role as a possible cause of upper genital tract infection. This organism has previously been isolated from peritoneal fluid samples [2], and it has been implicated in potentiating bacterial upper genital tract infection [3-5].

Paisarntantiwong et al. [6] reported an association between vaginal trichomoniasis and PID among women colonised with *C.trachomatis*. In addition, trichomoniasis has been independently associated with adverse pregnancy outcomes, such as premature rupture of membranes, preterm delivery, and low birth weight [7-9].
The evidence linking the discharge-causing pathogens, including *T.vaginalis*, with HIV-1 transmission and acquisition is substantial and clear [10,11]. However, literature addressing the influence of the interaction of HIV-1 infection and PID in terms of clinical presentation, microbiologic etiology and response to therapy is inconclusive [12-16]. We hypothesized that *T.vaginalis* causes PID in HIV-1- infected women independent of the other established discharge-causing pathogens and BV. Therefore, we compared the prevalence of these pathogens in patients with and without HIV-1 who presented with vaginal discharge with or without clinical PID.

Methods

Setting and Patients
Women who attended the Africa Centre for Population Studies and Reproductive Health Sexually Transmitted Diseases Clinic in Kwamsane between March 1999 through November 2000 and who had symptoms of vaginal discharge only or vaginal discharge with lower abdominal pain were invited to participate in the study after informed consent was obtained. A diagnosis of PID was made if, in addition to the presenting symptoms of genital discharge and lower abdominal pain, lower abdominal tenderness and cervical motion tenderness were elicited on examination [17]. The clinician who determined the subject's PID status was blinded to all results.

Specimen collection and processing
A calcium alginate swab and a Dacron swab were sequentially inserted 2-3 cm into the endocervix under direct vision; they were withdrawn while rotating. The swabs were used to inoculate New York City plates (Oxoid) for the detection of *N.gonorrhoeae* and to make a smear for chlamydia direct immunofluorescence testing (MicroTrak; Trinity Biotech) respectively.

A Dacron swab was used to collect vaginal specimens. A smear was made onto a glass slide, which was then stained with Gram stain and scored for BV using by use of Nugent's criteria [18]. The swab was placed into Diamond's media for the isolation of *T. vaginalis*.

Blood samples were collected in EDTA tubes and screened with the Determine-HIV test for HIV testing. Those samples that

tested positive had their results confirmed by 2 different ELISA tests (Murex HIV-1.2.0, manufactured by Abbott; Vironosticka HIV UNI-FORM *plus* 0, manufactured by Organon Teknika). A random sample of 10 % of the negative specimens was also subjected to ELISA confirmation.

Statistical analysis

χ^2 Tests were used to identify significant factors at baseline for PID. Relative risks and 95% CIs are reported. Breslow-Day tests were used to test for an interaction effect. Significant risk factors were examined in a logistic regression model to identify independent factors affecting PID.

Results

A total of 577 subjects with vaginal discharge only and 119 subjects with clinical PID were recruited into the study after informed consent was obtained. The prevalence of HIV-1 infection in all women studied was 56 %. There was no association between HIV-1 infection and a clinical diagnosis of PID: 76 (64 %) of 119 women with PID were infected with HIV-1, as compared with 312 (54 %) of 577 women with discharge and no signs of PID (p =.6).

The overall prevalence of sexually transmitted infections in this population was high; 525 (75%) of 696 subjects had at least 1of the following: *N.gonorrhoeae* infection, *C.trachomatis* infection, *T.vaginalis* infection or BV. BV was the most frequently diagnosed cause of vaginal discharge (in 481 subjects [69%]), followed by *T.vaginalis* (in 205 [29%]), *N.gonorrhoeae* (in 86 [12%]), and *C.trachomatis* (in 74 [11%]; table 1).

There was no difference in the overall prevalence of identifiable sexually transmitted infection aetiology in women with PID (80%), as compared with women who had a discharge only (76%; p = .8). A significant association was seen between *T.vaginalis* and PID (45 of 119 subjects were coinfected), as opposed to those with discharge only (160 of 577; p = .03). A similar trend was seen with *N.gonorrhoeae,* although this did not reach statistical significance (p=.06; table 1).

When the prevalence of the various sexually transmitted infections and of BV was stratified according to the patient's HIV-1 serostatus, the associated risk of PID (as opposed to discharge only) in those infected with *T.vaginalis* disappeared among HIV-1-seronegative patients (RR, 0.8; 95% CI, 0.4 – 1.5; p = .4). However, this association with PID increased significantly

Table 1. Prevalence of sexually transmitted infections and bacterial vaginosis among women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

Infectious agent or condition	No. (%) of patients: All (*n* = 696)	Had discharge only (*n* = 577)	Had discharge with PID (*n* = 119)	*P*	RR of PID (95% CI)
Bacterial vaginosis	481 (69)	397 (69)	84 (71)	.7	1.1 (0.7–1.5)
Trichomonas vaginalis	205 (29)	160 (28)	45 (38)	.03	1.5 (1.1–2.1)
Neisseria gonorrhoeae	86 (12)	64 (11)	22 (18)	.06	1.5 (1.0–2.3)
Chlamydia trachomatis	74 (11)	61 (10)	13 (11)	.8	1.0 (0.6–1.8)

among HIV-1-infected women who had *T. vaginalis* (RR, 1.9; 95% CI, 1.3 – 2.8; p= .002). No such association was seen with any of the other infections (tables 2 and 3).

The association of PID and *T. vaginalis* in HIV-1- infected women remained, regardless trichomoniasis occurred as a single infection (RR, 3.4; 95% CI, 1.1-11.1) or in combination with ≥ 1 of the other 3 conditions (RR, 2.1; 95% CI, 1.2 –3.6). HIV-1- infected women who had *N.gonorrhoeae* alone (OR, 2.2; 95% CI, 0.5 –9.1; p=.3), BV alone (OR, 0.8; 95% CI, 0.4 –2.6; p=.5), or acombination of the 2 (OR, 0.8; 95% CI, 0.2-2.7; p=.7) were not associated with PID. The number of HIV-1-infected women infected with *Chlamydia* species was too small to model.

Table 3. Prevalence of sexually transmitted infections and bacterial vaginosis among HIV type 1–uninfected women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

	Percentage of patients				
Infectious agent or condition	All (n = 308)	Had discharge only (n = 265)	Had discharge with PID (n = 43)	P	RR of PID (95% CI)
Bacterial vaginosis	58	59	56	.7	0.9 (0.52–1.6)
Trichomonas vaginalis	25	26	21	.4	0.8 (0.4–1.5)
Neisseria gonorrhoeae	8	7	12	.3	1.5 (0.7–3.6)
Chlamydia trachomatis	5	6	0	.1	0.2 (0.01–3.2)

Discussion

PID is associated with significant morbidity and mortality. Its management consists of the immediate initiation of antimicrobial therapy to cover the possible causes of infection. *T.vaginalis* is the only vaginal-discharge-causing pathogen that is not part of the microbial differential diagnosis of PID. However, if present, this organism is inadvertently treated with metronidazole, which is used for possible infection with anaerobes. In this study, we show that, in HIV-1- infected women, lower genital tract infection with this organism is associated with an increased risk of PID (p=.002).

The association we describe does not necessarily imply a causal relationship. However, infection with *T.vaginalis* is increasingly recognised to be associated with reproductive tract complications including sepsis that occurs after abortion and after cesarean section [19,20], as well as adverse pregnancy outcome[7-9]. This association has always been reported in combination with an altered state of the reproductive tract (e.g., pregnancy), the puerperium [13,21], and coinfection with other sexually transmitted infections [6]. Our data suggest that coinfection with HIV-1 may also alter the host-microbe relationship, resulting in an apparent increased risk of PID in the presence of *T.vaginalis.* There are, however, no data suggesting a mechanism for such an

Table 3. Prevalence of sexually transmitted infections and bacterial vaginosis among HIV type 1–uninfected women with vaginal discharge only and among those with vaginal discharge and clinical pelvic inflammatory disease (PID).

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association. The pathogenesis of infection with this protozoon is poorly understood. Lower genital tract infection results in an aggressive inflammatory response with punctate hemorrhages, thus allowing effective transmission and acquisition of HIV-1 infection [21,22]. Theoretically, the proteinases produced by *T.vaginalis* have the potential to break down the protective cervical mucus plug, facilitating access of microbes from the lower to the upper genital tract [23]. These microbes may include not only the vaginal anaerobes, which are already implicated as causes of PID, but also *T.vaginalis* itself.

Although our data also suggest an increased risk of PID among patients infected with *N.gonorrhoeae*, irrespective of HIV-1 serostatus, this did not reach statistical significance. A lack of association is also noted for *C.trachomatis* and PID. A possible explanation for this is that the comparison group is women with vaginal discharge and not “healthy” women. Therefore, a large proportion of both the PID and non-PID groups are infected with *N.gonorrhoeae* and *C.trachomatis*.

This study has obvious limitations. The clinical diagnostic criteria used are subjective, but a diagnosis based on sonography or surgery is not always possible or feasible, especially in resource-poor areas, such as ours. In addition, *T. vaginalis* is associated with punctate hemorrhages and inflammation of the cervix (strawberry cervix), which may mimic the cervical motion tenderness associated with PID. If the latter is true, then PID may have been over diagnosed among our patients, in which case, our results imply a more severe presentation of lower genital tract trichomoniaisis among HIV-1- infected women.

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Diagnosis of lymphogranuloma venereum by PCR: Primary genital ulcer lesion not in keeping with text book description

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ABSTRACT

Background *Chlamydia trachomatis* serotypes L1-3, the causative organism of lymphogranuloma venereum (LGV), is believed to be a rare cause of genital ulcer disease (GUD). Typically, primary lesions have been described as transient and insignificant. This may partly be due to inadequate diagnostic methods available to specifically diagnose the LGV biovar of *C.trachomatis*.

Methods Consecutive GUD patients attending a public health STD clinic were investigated for the different aetiologies by PCR on genital ulcer specimens. PCR for *C.trachomatis* was followed by restriction fragment length polymorphism to distinguish between the LGV and trachoma biovar.

Findings In 715 patients, the prevalence of *C.trachomatis* biovar LGV was 12%. Prevalence of Herpes Simplex virus, *Haemophilus ducreyi*, and *Treponema pallidum* was 54%, 9%, and 9%, respectively. Clinically, patients with primary lesions of LGV were indistinguishable from patients with non-LGV ulcers.

Interpretation

LGV was the second commonest cause of GUD in Durban, South Africa. In contrast to the classic description, genital lesions were not transient. They were painful and of significant size causing the patient to seek medical help. The discrepancy of the classic description of ulcers caused by *C.trachomatis* biovar LGV and our findings is likely the result of a lack of sensitivity of previous diagnostic methods.

Introduction

Chlamydia trachomatis serotypes L1, L2, and L3, comprise the biovar of this organism that causes lymphogranuloma venereum (LGV). LGV is believed to be a rare cause of genital ulcer disease (GUD). Classically, infection with *C.trachomatis* biovar LGV is characterised by inguinal buboes preceded by a recent, often insignificant, and at time of presentation already resolved genital lesion, or without any history of genital lesions at all. Prevalence rates among GUD patients vary from 1-25% [1-12]. Other studies do not report on LGV [13-15] possibly because it is believed to be a rare cause indeed or because the diagnostic methods to identify *C.trachomatis* LGV biovar are either insensitive and non-specific, or difficult to perform [16]. Diagnostic methods used are clinical presentation, serology, and demonstration of *C.trachomatis* in the ulcer by immunofluorescent staining, culture, or nucleic acid amplification methods.

A PCR targeting the cysteine-rich outer membrane protein of *C.trachomatis* has been described recently [17]. Restriction digestion patterns of the product obtained can distinguish between the different biovars of *C.trachomatis*. This PCR was applied to genital ulcer specimens to determine the prevalence of LGV and its clinical presentation in STD clinic attendees in Durban, South Africa.

Methods

Patients

All patients presenting to the STD clinic at the Prince Cyril Zulu Communicable Diseases Clinic in Durban between 16 October 2000 and 22 August 2001 with complaints of a genital ulcer and an ulcer on examination were enrolled. Patients with evidence of antimicrobial use four weeks prior to their current visit were excluded. A standardised questionnaire was administered by a trained study nurse and examination of the genitalia and lower abdomen was performed by a research medical officer. The ulcers from the last 182 consecutive patients enrolled were photographed. All patients were treated according to the local syndromic management guidelines for GUD. Informed consent was obtained from all participants prior to enrolment. The study was approved by the Ethics Committee of the Nelson R Mandela School of Medicine.

Specimen collection and preparation

After cleaning of the ulcer with a sterile dry gauze, a specimen was collected by scraping the ulcer base and edges with a sterile disposable plastic loop (Quad loop, Bibby Sterilin, UK). The harvest was suspended in 1.0 mL PBS, pH 7.6. DNA was extracted from a 0.4 mL aliquot of the PBS suspension using the Qiagen Blood and Tissue Mini Kit (Qiagen, USA). Venous blood was sampled for HIV serology.

PCR

C.trachomatis was detected by PCR using previously described primers that target the 60-kDa cysteine-rich outer membrane protein (CrP) [17]. The reactions were performed in 50 □l containing 1 □M of each primer PCR-D1 and PCR-D2, 300 □M of each dNTP, 1.75 mM $MgCl_2$, 1.25 U Taq polymerase, 5 □l of 10x PCR buffer (Perkin Elmer, USA), and 10 □l of DNA template. PCR was performed for 35 cycles. After an initial denaturation step at 94 ^{0}C for 3 minutes, the cycling conditions were as follows: annealing at 55 ^{0}C (60 sec), denaturation at 94 ^{0}C (20 sec), and extension at 72 ^{0}C (90 sec). A final extension was performed at 72 ^{0}C for 10 minutes. A positive (*C.trachomatis* L2) and a negative control (water) were included with each amplification. The sensitivity of the PCR was estimated to be less than 10 copies when performed on serial diluted organisms.

PCR for *T.pallidum*, *H.ducreyi*, and Herpes simplex virus 2 (HSV-2) were performed as

previously described [18]. PCR for the human □-globin gene was performed on all cases with negative PCR results for all four organisms [19].

Restriction digestion and gel electrophoresis
PCR products obtained with primers PCR-D1/D2 were digested with the restriction endonuclease AccI. The restriction reaction contained 13 □l PCR product and 0.5 U of enzyme in a total volume of 30 □l. Digested products were analysed by electrophoresis using ethidium-bromide containing 2% agarose gels. LGV and trachoma biovar were identified based on the restriction patterns [17].

Sequence of PCR products
To confirm the restriction sites for endonuclease AccI, the sequence was determined on six randomly selected PCR products with LGV biovar and one with trachoma biovar restriction patterns. PCR products were purified using QIA quick PCR Purification kit (Qiagen, USA) following the manufacturer's protocol. Sequence reactions were done using the BigDye™ Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, South Africa) according to the manufacturer's manual. The sequencing reaction products were analysed on an ABI 3100.

HIV serology
HIV infection was diagnosed using Determine™ HIV1/2 (Abbott Laboratories, USA). All positive results were confirmed by a second serological test (Capillus™ HIV1/2, Trinity Biotech, USA).

Statistical methods
Groups were compared using Wilcoxon nonparametric tests for numeric data and chi-square or Fisher's exact tests for categorical data. Factors found significant at the univariate level were included in a logistic model.

Results

Patients
Of the 788 patients enrolled, 73 (9.2%) were excluded from analysis because of negative results with any of the diagnostic PCRs and with the PCR for the human □-globin gene.

Prevalence (Table 1)
The prevalence of *C.trachomatis* biovar LGV was 12 %, the second commonest cause of genital ulcer disease in our patients. The prevalence of HSV, *H.ducreyi*, and *T.pallidum* was 54 %, 9 %, and 9 %, respectively. No microbiological diagnosis was obtained in 23 %.

C.trachomatis DNA was initially detected in 88 patients. In four cases the signal obtained with the initial PCR was repeatedly feint and the restriction pattern was considered unreliable. Hence these four were not considered confirmed LGV cases. After restriction of the remaining 84, 83 were identified as LGV biovar and one as trachoma biovar. The one trachoma biovar was found in the ulcers of a woman also complaining of vaginal discharge, urinary symptoms and abdominal pain. None of the other organisms were detected. On examination, two ulcers were present, one on the right labia minora and one on the clitoris. There was no evidence of cervicitis on speculum examination. No cervical or urethral specimens were collected.

In 68 (82%) of the 83 LGV cases, *C.trachomatis* was the single etiological agent found. Twelve cases (15%) were concurrently infected with HSV-2, one with *H.ducreyi* and one with *T.pallidum*. One patient had a triple infection with *C.trachomatis* (LGV), HSV-2 and *H.ducreyi*.

Clinical presentation (Table 2)
LGV was more common in women than in men, 19% versus 10% (p=0.001) (Table 1). Median age and history with respect to sexual behaviour, i.e. age minus age at first intercourse, total number of lifetime partners, and number of partners in the last 4

Table 1. Aetiology of genital ulcers in 715 patients (%).

Aetiology	All	Male	Female	p value[1]
C.trachomatis LGV biovar	12	10	19	0.001
T.pallidum	9	10	3	0.02
Herpes simplex virus	54	54	54	
H.ducreyi	9	10	5	0.05
None	23	22	27	

weeks, was similar among patients with and without LGV (p>0.1). A previous ulcer was more often reported in women with a non-LGV ulcer (p=0.02). This difference disappeared if cases with HSV-2, which are known to have recurrent disease, were excluded (p=0.23). Previous discharge was reported with equal frequency among cases with and without LGV.

The median duration of symptoms was longer in patients with LGV compared to those without (p=0.0006). This was the case for both, men and women, 12 versus 7 days (p=0.01), and 9 versus 7 days (p=0.02), respectively. Symptoms of concurrent discharge were not associated with LGV in either sex (p>0.15); neither were complaints of inguinal pain nor enlarged lymph nodes (p>0.22).

More than 90% of the ulcers were painful irrespective of the aetiology. In men, 65% of the LGV cases had single lesions compared to 38% in the non-LGV cases, (p=0.0001). This difference remained after exclusion of HSV infections (p=0.008). In women there was no association between single lesions and LGV. In both men and women, LGV ulcers were more commonly found on the perineum (p<0.05). This was the most frequent location of the LGV ulcer in women (41%).

Enlarged inguinal lymph nodes were more frequently observed among LGV cases as compared to patients with other aetiology in men (p=0.0003), but not in women. The lymph nodes had no particular features when present in LGV patients (data not shown). HIV infection was associated with LGV in men (p=0.05) but not in women. This association remained (p=0.02) after controlling for other risk factors for HIV (age minus age at first intercourse, total number of life time partners, history of previous discharge and GUD).

Ulcers were photographed during the enrolment of the last 182 patients. Among these, 15 of the 16 LGV ulcers were photographed: three in women and 12 in men. The ulcer of one male was a small lesion in keeping with the classical description of LGV. In the remaining ulcers the maximum diameter was estimated to be more than 1 cm. Eight showed a chancroid like appearance with deep ulcers and irregular undermined edges. Two resembled herpes lesions with multiple round excoriated lesions. However, these lesions were larger and more separated from each other as typically found with herpes lesions. In the remaining four patients the ulcers were raised and resembled primary syphilis.

Confirmation of restriction pattern

The sequence of PCR products obtained with six ulcer specimens with the LGV restriction pattern and with one urethral specimen with the trachoma biovar restriction pattern demonstrated the expected number and location of restriction sites for endonuclease AccI [17].

Discussion

As in most African countries HSV-2 is now the most common cause of genital ulcer disease in South Africa. With the application of PCR, *C.trachomatis* LGV biovar was the second most prevalent cause of this disease in our patient population. To our knowledge, this is the first large series of GUD patients in which an accurate diagnosis of *C.trachomatis* infection was made by identifying *C.trachomatis* biovar LGV from the genital lesion. The diagnosis was made with a previously described PCR and RFLP method [19]. In our hands, the analytical sensitivity of the PCR was less than ten copies, similar as reported previously. LGV was distinguished from the trachoma biovar by demonstration of a specific restriction digestion pattern of the PCR products. This restriction pattern was confirmed in selected cases by sequence analysis.

Most previously reported prevalence rates of LGV ulcers were based on an indirect diagnosis by clinical features and/or serology [3-6,8,10,12]. An aetiological diagnosis of GUD based on clinical presentation is known to be inaccurate [12,20,21]. Currently, serology by means of micro-immunofluorescence (MIF) is the diagnostic method of choice [16]. However, this test is not widely available, and as with all serology, no distinction can be made between past and current infection.

Some studies report on LGV diagnosed by direct detection of the organism in the genital ulcer by culture or DIF [6,8,12]. In general, culture of *C.trachomatis* is cumbersome and was often not followed by serotyping which is necessary to exclude potential 'contamination' of the ulcer with *C.trachomatis* trachoma biovar from the urethra or cervix. Direct immunofluorescence (DIF) is also labour intensive, results need to be evaluated by experienced staff, and again serotypes cannot be distinguished. Furthermore, although the performance of culture and DIF has not been evaluated in the diagnosis of *C.trachomatis* from genital ulcers, it is likely that the relative low sensitivity of these tests on urethral and cervical specimens also applies to genital ulcer specimens [22].

Nucleic acid amplification tests are being used successfully for the diagnosis of GUD pathogens but have hardly been applied for the diagnosis of LGV. One study used a PCR method [20] targeting the cryptic plasmid of *C.trachomatis*. However, these plasmids are present in all serotypes.

Therefore, in previous studies where the organism was detected from the ulcer, it was impossible to distinguish between the LGV and trachoma biovar, and thus 'contamination' of the ulcer with material from the urethra or cervix could not be excluded. However, we only detected one trachoma biovar from an ulcer lesion suggesting that this 'contamination' is a rare event. This suggests that detection of this organism in a genital ulcer specimen by means of DIF or non-type specific nucleic acid amplification is highly specific for LGV.

Classically, LGV can be divided into three stages [16,23]. The primary stage is described as a papule or a small herpetiform genital lesion that does not produce significant symptoms. The secondary stage is characterised as lymphadenitis mostly without a genital lesion. The tertiary or anorectal/elephantiasis stage results from destructed inguinal lymphoid tissue.

Due to the selection criteria, all our patients were in the primary stage. However, the ulcers that we found did not follow the classic picture of a small painless, non-significant lesion of short duration. All patients attended the clinic because of the presence of the ulcer. Over 90% of the LGV ulcers were painful and the median duration of the lesion was 11 days as opposed to 7 days in case of non-LGV ulcers. Because of the recognised non-specific appearance of genital ulcers, the ulcers were not described systematically [12,20,21]. However, photographs that were available from 15 consecutive LGV ulcers showed an estimated size of the lesions larger than 1 cm in all but one case. Most of the ulcers

resembled chancroid, but herpetiform lesions and syphilis-like ulcers were also described. However, these descriptions were done using photographs and a prospective study is needed to detail the clinical presentation of LGV. The variation in clinical picture confirms the non-specific nature of genital lesions. LGV ulcers in male were more often single lesions as compared to other ulcers. This was not observed in female, which may be because complete examination of the genitalia, i.e. speculum examination, may be painful and therefore in case of other aetiologies additional ulcers if present could have been missed. The only characteristic that made patients with LGV ulcers different was an association with inguinal lymphadenitis in males. We also diagnosed LGV ulcers twice as often in women as compared to men. The absence of an association with lymphadenitis in women, and the previously reported low prevalence of the secondary stage of LGV in women may be a reflection of the different lymphatic drainage. In women, the genitalia drain to the retro peritoneal lymph nodes in addition to the inguinal nodes, giving rise to the anorectal syndrome. Therefore, women with the non-ulcerative stage of LGV may present to clinics other than STD clinics and remain undiagnosed or are not reported [23]. Why the prevalence of LGV ulcers in our study was higher in females remains unclear. The reason for the association of LGV ulcers with HIV infection in men is also unclear. While current infection is unlikely to be responsible for HIV seroconversion, other risk factors for HIV were similar between LGV and non-LGV cases. This association was not present in women, but the number of HIV negative women was small.

A likely explanation for the discrepancy between our findings and the textbook description is that patients with larger ulcers were treated in the past, without an aetiological diagnosis, with drugs like sulphonamides or macrolides, while nowadays, macrolides are invariably included in syndromic management protocols. Therefore, these cases never evolve into the next stages. We show here that the number of infections with *C.trachomatis*, LGV biovar, that do result in significant lesions causing the patient to seek medical treatment is substantial.

In conclusion, our findings suggest that difficulty in detecting *C.trachomatis* in genital ulcers has lead to the typical description of the natural course of LGV with an initial, insignificant, self-healing primary lesion and progression to severe lymphadenitis. This may be incorrect and merely represent a small proportion of infections that go unnoticed and untreated, resulting in these complications. In contrast, symptomatic patients with undiagnosed aetiology, receive adequate treatment, aborting further progression of disease. Further work is needed to characterise the clinical syndrome of LGV.

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Chapter 6

Differentiation between STD and non- STD vaginal discharge

Summary

This study emphasises the non-specific presentation of vaginal discharge in women, as well as the broad array of causes including non-STIs. Women attending STD clinics do not form a homogenous group of STD population regardless of current presence vulvovaginal symptoms. A past history of vulvovaginal symptoms, however selects for a group of women at higher risk for HIV seropositivity and for current STI.

Identification of women at high STD risk among STD clinic attendees: implications for STD programmes

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ABSTRACT:
We showed an association between current infection with a recognised STI pathogen and HIV infection in women but not in men with non-ulcerative genital disease. While the accuracy of recognition of male urethritis and genital ulcer syndromes is high, this is significantly less for non-ulcerative STIs in women. The symptoms associated with the latter have a broad differential diagnosis including conditions of a non-STI nature. Local STD clinic attendees often comprise of patients with and without STIs. We hypothesised that this maybe responsible for the association of current STI pathogens and HIV in women. To identify a group of women that would be representative of a true STD clinic population we looked at those with a past history of treated genital ulcers. When we analysed in this subset the association of current STI pathogen and HIV infection, a pattern emerged that was comparable with that in men.

Introduction

The success of syndromic management of symptomatic sexually transmitted infections (STIs) largely hinges on the accuracy of syndrome recognition. Several studies that address this aspect have been published[1]. While the accuracy of recognition of male urethritis and genital ulcer syndromes is high, this is significantly less for non-ulcerative STIs in the female. Symptoms and signs of the latter can be divided into discharge from vagina and/or cervix, dysuria, vulvo-vaginal itch and lower abdominal pain. Women with this syndrome may present with one or more of these non-specific symptoms and signs. It is therefore not always easy for the patient, or health care worker, to decide that there is an abnormal situation [2-4].

Women presenting with non-ulcerative genital symptoms to our local primary health care clinics, are automatically routed to the sexually transmitted diseases (STD) section of these facilities. The differential diagnosis for these symptoms is however broad and includes sexually transmitted infections, urinary tract infections, non-sexually transmitted infections eg.yeast vaginitis, malignancies, or physiological discharge. Therefore, whilst patients with genital ulcer disease and men with urethritis, who present to a STD facility, form a fairly homogenous group with STIs, women with non-ulcerative genital disease, represent a heterogenous group, and only a subset of these will have a STI.

A recent study at the local STD clinic afforded us the opportunity to examine the response to syndromic management in HIV infected and uninfected patients. We found an association between current infection with a recognised STI pathogen or BV and HIV-1 infection in female patients [5], but not in male patients. We explored the hypothesis that the association between HIV infection and current STI or BV in women results from the fact that symptoms of non-ulcerative STIs are similar to those of other non-ulcerative, non-STI genital pathology.

Methods

Setting

The project was carried out in the Africa Centre for Health and Population Studies' STD clinic in the Hlabisa health sub-district in KwaZuluNatal. This clinic is part of a primary health clinic, serving a rural/semi-urban population.

Patients

Patients presenting to this service between March 1999 and February 2000 with symptoms of non-ulcerative genital disease viz. vaginal discharge, lower abdominal pain, dysuria or vulvo-vaginal itch in the female, and urethral discharge or dysuria in the male were invited to participate in the study with informed consent. Trained interviewers collected data and a trained HIV counsellor pre-test counselled all patients.

All recruits received a full clinical examination and microbiological work up. Syndromic treatment was given to all patients.

Ethical approval was obtained from the Ethics Committee of the Nelson R Mandela School of Medicine.

Specimen collection and transport

Female patients: After cleaning of the ecto-cervix, a calcium alginate and a Dacron swab were sequentially inserted 2-3 cm under direct vision into the endocervix, and withdrawn while rotating. The calcium alginate swab was put into GC broth (New York City (NYC) plates minus agar). The Dacron swab was used to make a smear on an immunofluorescence slide. A specimen from the lateral wall of the posterior fornix was collected using a Dacron swab. A smear was made for Gram staining after which the swab was placed in Diamond's media.

Male patients: A Dacron swab was inserted 2 cm into the urethra and withdrawn while rotating. This swab was used to make a smear on an immunofluorescence slide and then placed into GC broth.

All Patients: Blood for HIV-1 testing was collected in EDTA tubes and mid-stream urines were obtained for nitrite-leucocyte esterase dipstick tests and culture. While maintaining the appropriate storage temperatures, specimens were transported within 8 hours of collection to the Africa Centre STD Laboratory in Durban for further processing.

Processing of Specimens
NYC plates (Oxoid Ltd. Basingstoke, Hampshire, England) incubated at 37^{o}C in CO_2 for 48 hours, were used for the isolation *N.gonorrhoeae*. *C.trachomatis* infection was diagnosed by means of direct immuno-fluorescence testing (MicroTrak,® Trinity Biotech, Ireland). *T.vaginalis* was grown in home made Diamond's media. Cultures were read every day for up to five days or until positive. Nugent's Gram stain score was used to quantify abnormal vaginal flora [6]. BV was defined as a score of $\geq$ 7. The Determine-HIV® screenings test was used to detect HIV-1 antibodies. Positive tests were confirmed by an ELISA test (Vironosticka HIV UNI-FORM II *plus* 0 – Organon Teknika). A random sample of 10 % of the negative specimens was also subjected to ELISA confirmation.

Statistical methods
Prevalence of infections are reported as the proportion of men and women with any one of the STIs or BV. Association with HIV-1 infection was assessed using the chi square test with the Mantel – Haenszel correction. A logistic model was used to test whether STIs were independently associated with HIV-1. Data were analysed using SAS statistical software version 6 (1997 SAS Institute, Cary, NC, USA).

Results

Nine hundred and sixty six patients were recruited. Complete data for analysis were available for 222 male and 692 female subjects. The prevalence of HIV-1 infection was 56% among females and 48% among males (p=0.04).

Table 1a shows gender related age differences. The median age at presentation with the current STI, as well as the median age of those with HIV-1 infection was significantly lower among females (p=0.001and p=< 0.0001 respectively). There was no difference between male and female in the HIV uninfected cohorts. Men had a higher number of lifetime sexual partners as well as partners in the last 4 weeks (p=0.001 and p=0.001 respectively) (Table 1b). Regular condom usage was significantly higher among women than men (p < 0.001).

The presence of STI pathogens as well as bacterial vaginosis (BV) at the current visit was associated with HIV-1 infection in women. No association was found between HIV infection and STIs in men (Table 2).

Following on from our hypothesis that the association between HIV-1 infection and current STIs or BV in women results from the fact that symptoms of non-ulcerative STIs are similar to those of other non-ulcerative genital pathology, we attempted to identify a subset of women that like the men would have previously been exposed to a STI, and could thus represent a 'true' STD population.

Table 3 summarises history and treatment of previous symptomatic STIs. Women reported more recent episodes of symptomatic STIs than men (p=0.002). The proportion of men and women with a history of discharge/dysuria was similar. Men reported a previous history of an ulcer more frequently than women. Although as compared to women, a significantly higher proportion of men were previously treated for discharge/dysuria (p=0.04), a similar proportion of both sexes reported previous treatment for an ulcer.

In men, a history of ulcers or discharge/dysuria was significantly associated with HIV-1 [OR 2.76 95% CI (1.42-5.40); p=0.001 and OR 3.21 95%CI (1.78-5.81); p<0.0001 respectively] (Table 4). This association remained significant after controlling for possible confounders

Table 1a: Age differences between male (n=222) and female (n= 692) patients with non-ulcerative genital disease

	Median (range) age in years		
	Male	Female	p-value
at current visit	25.5 (16 –71)	24 (15 – 70)	0.001
coitarche	17 (10 – 28)	17 (11 – 36)	0.2
HIV-1 infected	27 (18 – 62)	24 (16 – 51)	< 0.0001
HIV-1 uninfected	23 (16 – 71)	24 (15 – 70)	0.2

Table 1b: Differences in numbers of sexual partners and condom usage between male (n=222) and female (n= 692) patients with non-ulcerative genital disease

	Male	Female	p-value
Sexual partners:			
Lifetime sexual partners median(range)	6 (1-100)	2 (1-25)	0.001
Partners in last 4 weeks median(range)	2 (1-11)	1 (1-5)	0.001
Condom usage:			
Regular	1%	9%	< 0.001
Occasional	58%	27%	< 0.001
Never	41%	64%	< 0.001

Significant p-value $\leq$ 0.05

Table 2: Prevalence of non-ulcerative STIs and BV in HIV-1 infected and uninfected men and women

	HIV-1 Infected	HIV-1 uninfected	p-value
Males	n =107	n =115	
N.gonorrhoeae (n = 132)	62%	57%	0.5
C.trachomatis (n = 15)	6%	8%	0.5
Females	n = 387	n = 305	
N.gonorrhoeae (n = 86)	16%	8%	0.001
C.trachomatis (n = 73)	15%	5%	0.001
T.vaginalis (n = 203)	33%	25%	0.03
BV (n = 479)	78%	58%	0.001

Significant p-value ≤ 0.05

Table 3: History and treatment of previous STDs in men and women with non-ulcerative genital disease

	Male	Female	p-value
of any STD:			
	122/222 (55%)	374/692 (54%)	0.7
weeks	29/222 (13%)	201/692 (29%)	0.002
ge/dysuria:			
s history	95/222 (43%)	314/692 (46%)	0.4
nt	88/95 (93%)	265/314 (84%)	0.04
s history	60/222 (27%)	116/692 (18%)	0.004
nt	51/60 (85%)	85/116 (73%)	0.08

Significant p-value ≤ 0.05

Table 4: Association of previous history of a symptomatic STI in HIV-1 infected men (n=107) and women (n=387) with non-ulcerative genital disease

	Yes	No	OR (95%CI) p-value
Male (n=107)			
any previous STD	72/122 (59%)	34/100 (34%)	2.80 (1.56-5.03) p=0.0002
previous discharge/dysuria	61/95 (64%)	46/127 (36%)	3.21 (1.78-5.81) p<0.0001
previous ulcer	40/60 (67%)	68/162 (42%)	2.76 (1.42-5.40) p=0.001
Female (n= 387)			
any previous STD	213/374 (57%)	172/318 (54%)	1.12 (0.82-1.53) p=0.4
previous discharge/dysuria	179/314 (57%)	212/378 (56%)	1.04 (0.76-1.42) p=0.8
previous ulcer	77/116 (66%)	311/576 (54%)	1.68 (1.09-2.61) p=0.01

like age, condom use, number of lifetime sexual partners, age at first intercourse and number of partners in last 4 weeks [OR 2.3 95%CI (1.3 – 4.3); p = 0.008]. In women, there was no association between a previous history of discharge/dysuria and HIV-1 infection, while a previous history of ulcer was associated with HIV-1 infection [OR 1.68 95% CI (1.09-2.61); p=0.01](Table 4).

A history of treatment for genital ulcers implies verification of a fairly specific STI symptom by a health care worker. The proportion of men and women with this history was similar (Table 3). When we analysed in the latter subset of women the association of current infection with a STI or BV and HIV-1 infection, a pattern emerged that was comparable with that in men i.e. no association was found (Table 5a).

We analysed the association of HIV-1 infection with current STI or BV in the subset of women with a previous history of treatment for discharge/dysuria (Table 5b). The association of current *C.trachomatis* infection and BV with HIV-1 infection remained significant (p=0.02 and p= 0.001 respectively), while the relationship between current infection with *N.gonorrhoeae* and *T.vaginalis* with HIV-1 infection disappeared.

Discussion

Globally, the preponderance of the social and health burden of STIs and BV, as well as HIV infection, is borne by women [7,8]. Our data further emphasise this by showing that women are more easily infected. Although the coitarche was similar in both genders, women presented at a younger age with their current disease than men. If we assume that both sexes experience a similar level of exposure to STIs, then this implies that the period of exposure needed to acquire an STI is shorter in women than in men. This is further illustrated by two other observations: the median age of HIV-1 infected women was significantly lower than that of the men (Table 1a) and a history of an STI within the 4 weeks prior to the current episode was significantly more frequent in women (Table 3).

An alternative explanation for this observed gender difference could be a difference in behaviour between men and women, implying less risky behavior of men. However, as shown in Table 1b, the men in our research population report a higher number of lifetime sexual partners as well as partners in the last 4 weeks than women. Although men report an overall higher condom usage than women (58 vs 27%), regular use was reported in a significantly lower percentage of men than women (1 vs 9%). In addition, because men and women in our study belong to the same population, high levels of condom use by men should protect women as well. This would however not hold true if these women are stable partners and the condoms are used with casual partners. However, qualitative research[4] as well as work on re-infection with *N.gonorrhoeae*[9] in our study population, suggest a network in which the stable partner of one is the casual partner of another. We therefore believe that the susceptibility of women to STIs and HIV cannot be explained by behavioural differences.

Although a previous history of discharge/dysuria was similar in both genders, a significantly higher proportion of men gave a history of treatment for their symptoms. (Table 3). This could mean that although women are aware of these symptoms, they are not recognised as being abnormal, and health care is not sought. And among those that do, the difficulty arises for the health care worker in the interpretation of these non-specific symptoms and signs which impacts on subsequent treatment. A history of previous genital ulcers was more frequent in men. This is in keeping with the gender distribution of patients attending the local STD clinic with genital ulcers. Ulcer pathogens have a predilection for keratinocytes , and the male genitalia provides a large keratinised area for these microbes. In addition, the male genitalia is more visible facilitating self-examination with easy recognition of ulcers, as opposed

Table 5a: Prevalence of non-ulcerative STIs and BV in HIV-1 infected (n=62) and uninfected (n=23) women who were previously treated for genital ulcers

	HIV-1 infected	HIV-1uninfected	p-value
N.gonorrhoeae (n = 13)	15%	17%	0.7
C.trachomatis (n = 5)	6%	4%	0.8
T.vaginalis (n = 23)	32%	13%	0.08
BV (n = 57)	73%	52%	0.08

Table 5b: Prevalence of non-ulcerative STIs and BV in HIV-1 infected (n=155) and uninfected (n=110) women who were previously treated for genital discharge/dysuria

	HIV-1 infected	HIV-1 uninfected	p-value
N.gonorrhoeae (n = 30)	14%	7%	0.08
C.trachomatis (n = 22)	12%	4%	0.02
T.vaginalis (n = 63)	26%	21%	0.3
BV (n = 185)	77%	59%	0.001

to the female genitalia where these lesions may go unnoticed. However, once aware of these ulcerative lesions, there was no difference in the rates of attendance between men and women seeking treatment for genital ulcer disease. This implies that ulceration of the genitalia is equally recognized by both genders as being abnormal.

HIV-1 infection is established by means of an antibody detection test. Detectable HIV-1 antibody levels only occur several weeks after exposure to the virus [10]. Therefore, HIV-1 seropositivity at this visit could not be due to the simultaneous acquisition of HIV-1 with the current acute infection with one of the other STI pathogens. The absence of an association between HIV-1 seropositivity and current non-ulcerative STIs among men (Table 2) indicates that the men who attend this STD clinic have had previous exposure to HIV-1. In the women however, we observed a significant association between HIV-1 seropositivity and current STIs. This implies that these women represent two sub-populations: one with a sexual behavioural pattern comparable to our male STD clinic attendees, that allowed them to become infected with HIV-1 and to get new STIs and the other with a behavioural pattern that provides minimal risk for any of these conditions.

We then postulated that the subpopulation of women with sexual behaviour comparable to the men, could be established by analysing data on history of previous episodes of ulcerative and non-ulcerative STIs. A history of treatment for a STI implies verification of STI symptoms by a health care worker and this may therefore be more accurate than a patient's report of such symptoms. We compared the association between HIV-1 infection and current STIs in women with a history of treatment for previous discharge/dysuria as well as genital ulcers.

In the women with a history of treated discharge/dysuria, the association between current gonococcal infection and trichomoniasis with HIV-1 infection disappeared as expected in a high-risk group (Table 5b). This did not happen with *C.trachomatis* infection or BV. From this it can be concluded that in patients with gonococcal disease or trichomoniasis, former treated episodes of discharge were likely to be of an STI nature. Infection with *C.trachomatis* and the syndrome of BV represent more chronic conditions. New episodes of these conditions may represent relapses without new exposure to the causative agents as well as HIV-1.

Lesions in the skin or mucosa of the genitalia unrelated to trauma, are almost exclusively caused by STI pathogens and a history of this is therefore a marker for sexual risk behaviour. A history of treatment for genital ulcer disease implies verification of this fairly specific STI symptom by a health care worker. When we analysed in the group of women with such a history the association of current infection with a discharge pathogen and HIV-1 infection, a pattern emerged that was comparable with that of the men attending the STD clinic i.e. no association was found (Table 5a). This is plausible, since a previous and not a current STI will influence the present HIV-1 serostatus. A history of previous genital ulcers is indicative of high sexual risk taking behaviour, which includes increased HIV-1 exposure. Therefore, in an equally exposed group of men and women, the distribution of current STIs remained independent of HIV-1 serostatus.

A previous history of specific STI symptoms ie. discharge/dysuria in male and genital ulcers in male and female was associated with HIV-1 infection (Table 4). The differential diagnosis for women presenting with discharge/dysuria on the other hand is broad. Therefore, previous history of discharge/dysuria does not necessarily imply a previous symptomatic STI, and would therefore not be expected to be associated with HIV-1 infection.

The second subpopulation of women with limited exposure to STIs and HIV-1 consists of two subsets of women, with no firm history of a previous STI. The first has genital symptoms of non-STI origin and these should not be at increased risk for HIV-1 infection. The second subset has a current STI but without a history of a former episode. Women in this subset are obviously part of a sexual network and might be exposed to HIV. Our data strongly suggest that it is this group of women that is responsible for the association of HIV-1 infection with current STIs.

Our data support the conclusions of La Ruche, *et al*. [11], who assessed the usefulness of data generated by the National Health Information System in Cote d'Ivoire. They concluded that non-ulcerative genital disease in the female are poorly instructive for the STD programme in terms of assessing the volume of STD drugs needed for public health services as well as monitoring the epidemiological trends of STDs. Genital ulcers in both genders, and non-ulcerative disease in the male on the other hand provide relevant information for the STD programme.

The epidemiological impact of syndromic management depends on the percentage of symptomatic infected individuals that are cleared of their infection for a period long enough to break the chain of transmission. In view of the non-specific nature of symptoms among women with non-ulcerative disease, an obvious limitation of syndromic management is the neglect of the infected female, who has symptoms but

does not recognise her symptoms as being abnormal, or is asymptomatic. Symptom awareness campaigns may improve syndromic management by bringing in infected women with unrecognised symptoms. Further research has to be considered on the effect on syndromic management of reduction of STIs in this population by mass treatment.

In conclusion, women with non-ulcerative genital disease attending our local STD clinics do not form a homogenous STD clinic population. We need to take heed of this in the interpretation and compilation of data.

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Chapter 7

STIs and adverse pregnancy outcome

Summary

In South Africa, as is the case in most developing countries, the burden of sexually transmitted infections (STIs) is borne by women. These infections are of concern to health care providers in that they carry an increased risk of reproductive tract sequelae among women, including adverse pregnancy outcome, and impact on the health of neonates. In addition, as with the other causes of STIs, they represent an elevated risk for infection with HIV-1.

The traditional splitting of STD services from reproductive health has resulted in neglect of these diseases in the pregnant women. In our setting, not only is the prevalence of STIs very high among this group of women, but the rate of re-infection is also unacceptable. Case-finding in an antenatal clinic would serve to improve the overall health of the community. Although case finding for syphilis is routinely performed at antenatal clinics, screening for the other STIs is not practiced. Point-of-care tests could potentially bridge this gap. Patients attending these facilities could be offered such tests to detect STDs.
This chapter reviews the current knowledge of STIs and the association between neonatal morbidity and adverse pregnancy outcome.

Sexually Transmitted Diseases, Adverse Pregnancy Outcome and Neonatal Infection

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ABSTRACT

Prevention and treatment of sexually transmitted infections (STIs) in the sexually active population are the main steps to prevent perinatal infection. However, the spread of STIs continues at an astronomical pace despite various attempts at controlling the epidemic. An important reason for this lack of STI control is that a large percentage of infected people go untreated because they have asymptomatic or unrecognised infections. The microbial differential diagnosis of STIs implicated in adverse pregnancy outcome is broad and includes viral, bacterial and protozoal infections. Infertility, ectopic pregnancy, pelvic inflammatory disease, chorioamnionitis, premature rupture of membranes, preterm birth and puerperal sepsis are some of complications seen in women as a result of infection with sexually transmitted pathogens. In addition, STIs may facilitate the transmission and acquisition of HIV. In the foetus or neonate, complications include abnormalities of the major organ systems. Infections in the form of pneumonia or conjunctivitis may also occur.Due to the lack of simple, inexpensive and sensitive point-of-care tests, screening for STIs in pregnancy is not performed routinely.

Introduction

Sexually transmitted infections (STIs) with an estimated number of 333 million new cases per year in adults (1), is one of the leading causes of morbidity worldwide. In men, these infections cause minimal complications. However, in women, pelvic inflammatory disease and decreased fertility are frequently seen (2,3). In addition, a large percentage of these infections in women either go untreated or are treated with substantial delay. This is largely attributed to the high frequency of asymptomatic or unrecognised infections. This is especially a problem in developing countries where STIs are more prevalent and access to health care is limited. Based on our own work we estimated that approximately 25% of women in rural Africa have an STI and that less than 10% of these are appropriately treated (4). Unrecognised STIs appear to be more common in antenatal clinic attendees (5). In addition, the problem is compounded by poor treatment seeking behaviour of individuals with STD symptoms. It is therefore not surprising that the world's burden of STIs is largely borne by women in the reproductive age group. These diseases not only threaten the health of women and their capacity to reproduce, but they may have detrimental effects on the foetus and neonate of infected individuals. Adverse pregnancy outcomes range from early abortion and premature births to congenital infections and death.

STIs in women are divided in four groups: vaginal infections, cervical infections, ulcerative infections and extra-genital infections. The organisms that cause these different infections are listed in table 1.Infection of the foetus occurs transplacentally via the mother's blood stream or as a result of ascending infection from the vagina and cervix into the uterus. Approximately 13 million infants are born prematurely in the world annually (6). Preterm delivery is the most important cause of perinatal morbidity and mortality. Prematurity accounts for at least 75 % of the perinatal mortality (7). There is increasing evidence that ascending infection from the lower genital tract is an important cause of preterm labour.

Our discussion will centre around current knowledge regarding the impact of STIs on outcome of pregnancy as well as the ability of the implicated microorganisms to cause infection in neonates.

Bacterial vaginosis

Bacterial vaginosis (BV) is the commonest cause of vaginal discharge in women of childbearing age. It is characterised by an increased proliferation of certain bacterial species in the vaginal ecosystem. Anaerobic bacteria, particularly *Gardnerella vaginalis, Mobiluncus spp, Bacteroides spp, Prevotella spp* and *Mycoplasma spp.* (8,9), replace the normally dominant and protective *Lactobacillus spp*.. The reason for this shift in the vaginal ecology is unknown.

There is increasing evidence linking bacterial vaginosis to adverse pregnancy outcome, including first trimester miscarriage, second trimester loss, late miscarriage, preterm labour, preterm delivery and low birth weight, premature rupture of membranes (PROM), chorioamnionitis and postpartum endometritis (8,9,10,11,12,13,14). To assess whether bacterial vaginosis affects the rates of conception and miscarriage in the first trimester, Ralph *et al*. (1999), screened 867 consecutive women undergoing in vitro fertilisation for the presence of BV (8). Although the rate of conception did not differ between the two groups, women with

TABLE 1: Sexually Transmitted Infections in Women and Adverse Pregnancy Outcome

	ORGANISM	ADVERSE PREGNANCY OUTCOME	NEONATAL INFECTION
Vaginal infection	Bacterial vaginosis (BV) complex	Yes	Probably
	Genital mycoplasmas	Yes	Probably
	Trichomonas vaginalis	Yes	No
Cervical infection	*Neisseria gonorrhoeae*	Yes	Yes
	Chlamydia trachomatis	Probably	Yes
	Mycoplasma genitalium	Unknown	Unknown
Ulcerative infection	*Treponema pallidum*	Yes	Yes
	Haemophilus ducreyi	No	No
	Calymmatobacterium granulomatis	Unknown	Yes
	Chlamydia trachomatis, L type	Unknown	Unknown
	Herpes simplex virus	Yes	Yes
	Human papillomavirus	No	Yes
Extra-genital infection	*Treponema pallidum*	Yes	Yes
	Hepatitis B Virus	No	Yes
	HIV	Unknown	Yes

BV who conceived had a significantly higher risk of miscarriage in the first trimester compared with women with normal vaginal flora (32% vs 18.5%). Seventy out of 239 patients with preterm labour developed PROM. Of the 70 patients with PROM, 51 (73%) had BV(11). A cohort study of 790 nulliparous Finnish pregnant women demonstrated a 7.3 fold risk of PROM in women with BV (15). Vaginal swabs taken at the time of admission from women in labour were evaluated for the presence of BV or normal flora. A 6.8 fold increase of chorioamnionitis was seen among women with BV as opposed to those with normal flora. In addition, patients with BV were 5.8 times more likely to develop postpartum endometritis (16). Between 46% and 61% of women with postpartum endometritis have organisms that is similar to the vaginal flora of patients with BV (17,18). Pregnant black women are at an increased risk, having a nearly three times higher prevalence of BV as compared to pregnant white women (14,19,20). A recent study reported on race/ethnicity and vaginal flora patterns in 842 pregnant women. Overall, 22% of blacks and 8.5% of whites had BV (21). Highly significant differences were also seen among 13 747 pregnant women, where 23% of black women and only 9% of white or Asian-Pacific Islander women had BV (22).

The mechanisms whereby microorganisms may initiate preterm labour and preterm delivery have not been fully established. Pathogenic mechanisms include an ascending infection that initiates a materno-foetal response with the production of prostaglandins and cytokines (23). It has been demonstrated that BV associated organisms produce mucinase and IgA protease (24) that may hydrolyse the protective cervical mucus and destroys mucosal membrane IgA (25). These factors could promote the entry of BV microflora or substances that these bacteria produce through the cervix into the lower uterine segment and possibly into the foetal membranes.

The production of sialidases by BV associated organisms has been associated with prematurity. Cervical mucus and amniotic fluid contain significant amounts of sialic acid and sialidases may therefore enhance the ability of BV organisms to adhere, invade and destroy mucosal tissue (26,27,28). However, in a recent nested case-controlled study, elevated cervical fluid sialidase activity at 22 to 24 weeks gestation did not distinguish women at increased risk for spontaneous preterm birth (29)

Bacterial sialidases have also been shown to decrease collagen synthesis in fibroblasts (30).In addition, many BV associated organisms produce proteases that may destroy or weaken collagen. Therefore, both these bacterial enzymes may contribute to the impairment of foetal membrane strength (25,31).

The anaerobic metabolism of the BV associated bacteria produces short chain fatty acid salts like butyrate and propionate. These substances are inhibitory to fibroblasts (32) and may also contribute to weakening or necrosis of foetal membranes.

Several organisms associated with BV produce large amounts of phospholipase A2, an enzyme capable of initiating prostaglandin synthesis (33,34,35,36). Prostaglandin E2 stimulates myometrial contractions and initiates cervical changes (37).

There is increasing evidence that inflammation of the upper genital tract may play a major role in the pathogenesis of preterm labor and premature rupture of membranes. Suggested mechanisms behind infection-associated preterm labour include the production of cytokines. Interleukin -1alpha (IL-1alpha) and tumor necrosis factor alpha which are secreted by stimulated monocytes and macrophages, stimulate prostaglandin production by human amnion and decidual cells (38,39). A strong association has been found between IL-1 beta and BV in pregnant women.(40). Higher levels of interleukin-6 (IL6) were found in the amniotic fluid of women in preterm labor with intra-amniotic infection than in women in preterm labor without

such an infection (41). Wennerholm *et al*. (1998), concluded that IL-1 alpha and IL-8, but not IL-6 were associated with bacterial vaginosis at the cervico-vaginal level (42).

The IL6/IL6 receptor pathway has recently been implicated in the development of intracerebral haemorrhage occurring in preterm infants (43). Women with BV have been shown to be more likely to have a positive fetal fibronectin test in vaginal fluid than uninfected women. (44) Foetal fibronectin has been reported to be a statistically significant predictor of preterm birth at < 32 weeks (44,45) and has been associated with a 16-fold increase in clinical chorioamnionitis and a 6-fold increase in neonatal sepsis

Various criteria have been previously used to diagnose BV. These include clinical observations as well as several point-of-care laboratory tests (46). These still play a role in the differentiation between different forms of abnormal vaginal discharge in women that seek medical attention. However, application of these criteria is difficult because factors unrelated to BV may influence the outcome of each of them. The Gram stain is currently the test of choice to evaluate the bacterial ecology in the vagina independent of clinical symptoms. The test is easily reproducible with a high specificity and sensitivity (47,48). Nugent's criteria should be employed in the reading of the Gram stained smears (49). This method of diagnosing BV allows for the distinction of various categories of abnormal vaginal flora, which may precede the development of clinical BV. This is important in screening during pregnancy because abnormal vaginal ecology in the absence of clinical symptoms may be related to adverse pregnancy outcome (50,51). PAP- stained cervical smears have also been successfully used to screen and manage BV (52). However, they are likely to produce results that are less reliable than those obtained by Gram staining. (53).

Current standard antenatal procedures do not provide for screening for BV. The findings from recent prospective randomised trials suggest that treatment of BV in certain women who are at high risk for preterm delivery decrease the rate of preterm birth (9). Screening for and treating pregnant patients with bacterial vaginosis early in pregnancy was shown to be more cost effective than dealing with the problems of preterm, premature and low birth weight infants (54).

The benefit of routine screening and treatment in the general population, is however, uncertain. If the prevalence of preterm delivery and BV is high in an area, then routine screening may be cost effective.

The increasing evidence suggesting an association between first (8) and mid trimester (14) miscarriages, raises the issue of 'when to screen and treat ?' Results from randomised controlled trials have shown that women with a history of second trimester loss should be screened for BV and treated with a course of oral metronidazole early in the second trimester of pregnancy (13,55). McGregor *et al*.(1997), evaluated the timing of initial antenatal visits to characterise the possible use of metronidazole in pregnancy. Most women booked after completion of nine weeks of pregnancy. The study concluded that pregnant women could be screened and treated at the initial antenatal visit (56). A recent meta-analysis as well as a population based case controlled study concluded that there is no evidence of teratogenicity from the use of metronidazole in women during the first trimester of pregnancy (57,58)

The current recommendation for the treatment of BV in pregnancy is 250mg metronidazole ,three times a day for seven days (12,59). Clindamycin 300mg twice daily for seven days may be used as an alternative to metronidazole (60). Treatment of BV with 2% clindamycin cream did not reduce preterm delivery nor low birth weight as compared to placebo cream. Intravaginal therapy appears not be effective against bacterial vaginosis-associated microorganisms harbored in the upper genital tract and systemic treatment is recommended to eradicate upper genital

tract infection to reduce preterm delivery (60).

Genital mycoplasmas

Three species of mycoplasma are associated with pathology in the genital tract: *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium*. While there is strong evidence that *M.genitalium* causes male urethritis, the causal relationship with infections of the female genital tract is inconclusive with no evidence to support a role in adverse pregnancy outcome (61).
M. hominis has been implicated as a cause of pelvic inflammatory disease as well as poor pregnancy outcome (62). Its strongest association however, is with bacterial vaginosis (61). Although its isolation as a single pathogen from the blood of patients with post partum and post-abortion fever indicates pathogenicity (63,64) its relation to adverse pregnancy outcome is only found in the context of BV (62).

The correlation between *U. urealyticum* and adverse pregnancy outcome is gaining prominence (65,66,67). However, the role of this microbe as a definitive pathogen is controversial since it is consistently present in the vagina in 80-90% of pregnant women from mid trimester till delivery (68). This lends support to the view that its role in any form of adverse pregnancy outcome is that of a bystander organism rather than a true pathogen. However, there is evidence to support the involvement of *U.urealyticum* in adverse outcome at most stages of pregnancy. Qi *et al*. (69), used PCR for the detection of *U.urealyticum* in cervical secretions as well as fallopian tube fluid and pelvic fluid from women with tubal pregnancy. They compared cervical infection in women with tubal pregnancy and normal early pregnancy. They found a significant correlation between *U.urealyticum* and tubal pregnancy. They also compared pelvic and tubal infection in women with tubal pregnancy and those undergoing tubal ligation. The difference observed at the cervical level was not confirmed in tubal or pelvic fluid. The weakness of this study is that the control group to establish the last relationship differed from the first and was not pregnant.

Several studies have shown that *U.urealyticum* may be found in amniotic fluid obtained by amniocentesis in early pregnancy and this seems to be related to preterm delivery and low birth weight (70). This is supported by the observation of Bashiri *et al*.(1999) (71), who reported that the 3/7 culture positive amniotic fluids taken for genetic amniocentesis harbored *U.urealyticum* with significantly elevated IL6 levels. Similar findings have been reported for women with BV. This condition may have coexisted with the amniotic fluid colonization. The remaining four patients had IL6 levels similar to the 23 amniotic fluids that were sterile. These four grew skin commensals. Because IL6 is a strong indicator of inflammation, this observation suggests that early amniotic fluid infection with *U.urealyticum* induces an inflammatory response, setting the scene for subsequent early termination of pregnancy and interference with fetal development and growth. Abele-Horn *et al*. (1997) (72), reported a significant correlation in a group of 170 women, between cervical and vaginal colonisation with *U.urealyticum* as the only potential pathogen, and an increased risk of amnionitis, chorioamnionitis, PROM and premature delivery.

The evidence for a causal relationship between *U.urealyticum* and infections in low birth weight infants is steadily increasing. The observation that this occurs in low birth weight infants suggests that the organism is responsible for both premature delivery and growth retardation as well as neonatal infection. Two of the three pregnancies reported by Bashiri *et al*. (1999) (71), resulted in foetal loss and one in preterm delivery. The organism has also been implicated in pulmonary and meningeal infections as well as in bacteraemias (73,74).

The offspring of women colonised with *U. Urealyticum* in Abele-Horn's study (1997) (72), had a significantly higher

chance of developing respiratory distress syndrome, pulmonary dysplasia and intraventricular haemorrhage. Infants with very low birth weight were more frequently infected as compared to term babies.

In conclusion, although direct proof is not as yet available, there is increasing evidence linking this organism to adverse pregnancy outcome including neonatal infection. Further work should focus on differences in virulence of the different biotypes of this organism (75). This has the potential to identify certain types as pathogenic and others as harmless colonisers. This would assist in the development of a diagnostic test. In addition, the effect of targeted antimicrobial interventions in well-designed clinical trials in sick infants born from women with genital colonisation with *U.urealyticum* needs to be studied.

Trichomoniasis

Like BV, trichomoniasis has been identified as a cause of preventable preterm delivery, premature rupture of membranes and low birth weight (76,77). Cotch *et al*. (1997)(77), prospectively studied the association between *Trichomonas vaginalis* and risk of adverse pregnancy outcome in a large cohort of ethnically diverse women. They concluded that pregnant women infected with *T.vaginalis* during the second trimester were statistically significantly more likely to have a low birth weight infant, to deliver preterm and to have a preterm low birth weight infant. The presence of *T. vaginalis* infection has also been associated with PROM (78).

Transmission of *T. vaginalis* to the neonate may occur during passage through an infected birth canal with female infants developing vaginal infections.

There are three different methods to diagnose trichomoniasis. These are in order of increasing sensitivity: microscopy on a wet mount of vaginal secretions in which the characteristic movements of *T. vaginalis* make them easily recognizable, culture in e.g. Diamonds media and PCR.

The recommended treatment of trichomoniasis is a STAT dose of 2g of metronidazole or tinidazole (79). However, with increasing levels of resistance of these organisms to metronidazole, an increase in the dosage may be warranted (i.e. 500mg bd X7 days; 250mg tds X 7 days) (personal communication). The treatment of neonates with genital tract infection due to *Trichomonas vaginalis* may not be necessary since the disease is self-limiting.

Gonorrhoea

Pregnant women infected with *Neisseria gonorrhoeae* may present with a vaginal discharge or the disease may go unrecognized (80). Pelvic inflammatory disease and perihepatitis may occur if cervical infection ascends into the adnexa during the first trimester, before the products of conception completely obliterate the uterine cavity (81). Septic spontaneous abortions may occur at this stage. Later on in pregnancy, gonorrhoea is associated with premature rupture of membranes, preterm labor, chorioamnionitis and post partum infection (82,83). There is no consensus on the role of pregnancy in disseminated gonococcal infection (84,85,86,87).

Gonococcal conjunctivitis (ophthalmia neonatorum) is the most common manifestation of perinatal infection due to *N. gonorrhoeae* (88,89). The infection is usually transmitted during delivery and the neonate presents two to three days later with a purulent conjunctivitis. If untreated, it may lead to perforation of the cornea and a panophthalmitis (88).

Rarely, newborns exposed to *N. gonorrhoeae* may develop disseminated infection with septicaemia and arthritis (88). Genital and rectal infections have been seen infrequently in neonates following delivery through an infected birth canal (90).

The diagnosis of ophthalmia neonatorum is generally made clinically and confirmed by a Gram stained smear and

culture of the conjunctival secretions. Culture remains the diagnostic standard for the diagnosis of *N. gonorrhoeae* infection (90). However, DNA based amplification techniques in the form of polymerase chain reaction and ligase chain reaction have been shown to be more sensitive than culture, but outside the research setting, clinical experience with these methods is limited.

Ceftriaxone (125mg imi stat) and spectinomycin (250mg po stat) are safe and effective for the treatment of uncomplicated gonorrhoea in pregnancy (92,93).

Disseminated neonatal gonococcal infections and gonococcal conjunctivitis should be treated with ceftriaxone, 25-50 mg/kg body weight imi/ivi for 7 days. The role of topical antibiotics does not seem to offer any added advantage (88). Irrigation of the eyes with normal saline is often performed. Silver nitrate (1%) eye drops, administered immediately postpartum, have been widely used for prophylaxis. Increased concentration of silver nitrate due to evaporation of water in old solutions, has been implicated in chemical cataracts (94). In addition, prophylaxis for neonatal conjunctivitis should include *C.trachomatis* infections and silver nitrate does not accomplish this. Tetracycline and erythromycin drops have been used as an alternative (95), but the rising prevalence of tetracycline resistance in *N.gonorrhoeae* makes this drug ineffective (96).

Chlamydia infection

Since the discovery of *Chlamydia trachomatis* as an important cause of STIs, the role of the urogenital serotypes in adverse pregnancy outcome has been studied. There is some evidence that infection predisposes to ectopic pregnancy (69), but involvement in adverse events later in pregnancy has not been established. In contrast to this, neonatal infections are seen in approximately two thirds of infants born by vaginal delivery from mothers with cervical chlamydia infection. The main manifestations of infection are conjunctivitis and pneumonia. Characteristically, both infections run a protracted course. The first ocular manifestations occur several days to one month after delivery. However, signs of infection may be found at birth if membranes have been ruptured long before delivery. The conjunctivitis first manifests as acute inflammation with a copious muco-purulent discharge but changes later to the more chronic follicular form. The respiratory manifestation of infant *C.trachomatis* infection is an afebrile interstitial pneumonia. This may manifest from 2 weeks to 3 months post delivery and is self-limiting. It is found in approximately half of the infants that have had conjunctivitis supporting an immunological pathogenesis in which antibodies against the 60 kD heat shock protein may play a crucial role (97). There are additional claims for a direct role of *C.trachomatis* in acute neonatal respiratory tract infections. This is supported by positive cultures in naso-pharyngeal bronchial secretions of such patients (98). However, in most cases other potential pathogens like CMV and *U.urealyticum* are also found (99,100).

A DNA amplification technique, like PCR or LCR, is the preferred diagnostic method for chlamydial infection of the female genital tract. Culture as well as antigen detection methods lack sufficient sensitivity in routine settings. Diagnosis of both neonatal infections is performed on conjunctival and nasopharyngeal secretions. DNA amplification technology is the method of choice (101, 102). Infected infants carry viable *C.trachomatis* in the conjunctiva for periods up till 9 months and in the naso-pharynx for more then a year.

Because tetracyclines are contra-indicated in pregnancy, pregnant women with *C.trachomatis* infection must be treated with an alternative (103). Azithromycin 500 mg daily for 3 days or the cheaper regimen of erythromycin 250 mg, 4 times daily for 2 weeks may be used. Although in-vitro susceptibility is very poor, several clinical trials suggest that amoxycillin can be successfully used to eradicate chlamydia in pregnancy. Timing of therapy as well as

partner treatment is important because reinfection before delivery will nullify treatment. Chlamydia conjunctivitis as well as pneumonia in newborns needs to be treated systemically. Erythromycin is the drug of choice and should be given orally in a dose of 50mg/kg-body weight in four divided doses for 10-14 days. Conjunctivitis may be prevented by installation of 0.5 % erythromycin drops.

Syphilis

Treponema pallidum infection is a well-established cause of adverse pregnancy outcome and remains an important cause of pregnancy loss and infant morbidity and mortality in many parts of the world. In sub-Saharan Africa the percentage of pregnant women with positive syphilis serology at the first antenatal clinic visit remains in the order of 8-10 % and many complete their pregnancy without adequate syphilis treatment.

The foetus is most likely infected transplacentally (104). This can occur any time during pregnancy because spirochetaemia exists in all active stages of syphilis (105). Early infection results in abortion and foetal loss while later infection may progress to early congenital syphilis. Another possibility is that the infection becomes latent, with late congenital syphilis manifesting years after birth.

Most infants born from untreated mothers with positive syphilis serology have no physical abnormalities at birth (106). Symptoms and signs of infection appear within a few days and full blown early congenital syphilis may develop (107,108). It usually starts with rhinitis (snuffles) followed by a generalised rash of a vesicular and bullous nature. Mucous patches develop and the skin starts to slough. The infant becomes progressively ill and may manifest with hepatosplenomegaly and neurological abnormalities. Many of these infants also have osteochondritis, which mainly affects the nasal bones and the lower extremities.

Late congenital syphilis manifests from between a few to 30 years after birth. It resembles the tertiary phase of acquired syphilis but with neurological and bone manifestations being more prominent then cardio-vascular disease.

The diagnosis of syphilis in the pregnant female is made by means of a test for non-treponemal anti-cardiolipin antibodies. The presence of significant amounts of such antibodies indicates disease activity. The most commonly used test is the Rapid Plasma Reagin (RPR) test. A positive reaction needs to be confirmed with a treponemal antibody test for which the Treponema Pallidum Haemagglutination Assay (TPHA) is used. However, in high prevalence areas the positive predictive value of a RPR test is high enough to start treatment without TPHA confirmation. This is of particular significance because the RPR can be done as a point-of-care test while this is not possible with the TPHA test(109).The problem with this approach is the difficulty in differentiation between low grade positive and a negative RPR test. The newer immuno-chromatography tests for syphilis are much easier to read but use treponemal antigens and are therefore unable to differentiate between active and non-active/treated syphilis. The development of a point-of-care test that identifies both anti-cardiolipin antibodies and specific treponemal antibodies is urgently needed.

Confirmation of the clinical diagnosis of early congenital syphilis can best be done by means of dark field microscopy on exudate of the skin lesions or the mucous patches. PCR on such specimens is a promising and likely more sensitive alternative. However its added value has still to be established. Diagnosis of syphilis in the asymptomatic infected newborn is still a challenge for test designers. Thus far, none of the available serological IgM tests is positive in all symptomatic newborns and its value in identifying infected asymptomatic infants is therefore questionable (110). The most reliable information can be obtained through monthly consecutive RPR tests. Each infant

starts off with an antibody level similar to its mother's. If it is infected, the titer will rise or remain stable for months. If there is no infection the titer will drop and become negative in 4-6 months. Unfortunately such long-term follow-up is difficult to achieve in developing countries where the problem of syphilis is most prominent.

Because of the difficulty of diagnosing congenital syphilis, the management is mainly based on a combination of clinical judgment, laboratory tests and pragmatism. Each child born with symptoms of congenital syphilis with positive dark field microscopy on a representative lesion should be admitted to hospital and treated with intravenous penicillin-G, 50.000 units per kg per day in 2 divided doses for 10 to 14 days. A symptom free infant born from a syphilis serology positive mother who has not received adequate treatment (3 weekly dosages of 2.4 MU benzathine penicillin imi) should be treated similarly.

Diagnosing infected women during pregnancy may prevent congenital syphilis. We found a strong inverse correlation between the number of penicillin doses received and symptoms of early congenital infection (110). However, foetal infection might still occur despite effective treatment (111)

Granuloma inguinale

Pregnancy does not seem to promote dissemination of granuloma inguinale (GI) or to affect the response to therapy (112). Congenital infections have not been documented. However transmission of the organism to the neonate may occur during delivery. Three cases of GI infection in infants have been reported recently. A 4 month old HIV positive child presented with a lateral neck mass which was diagnosed histologically to be (GI) (113). An 8-month-old infant and a 5-month-old infant presented with mastoiditis and external ear discharges. The 8-month-old went on to develop a temporal lobe abscess. The second child had a polypoid mass in the middle ear that was confirmed on histology to be GI. The mother of this infant had biopsy proven GI of the cervix (114). Extensive vulval granulomas may be an indication for caesarian section.

Herpes Simplex Virus (HSV) infection

The prevalence of genital herpes in the sexually active population is increasing worldwide and appears to have preceded the spread of HIV in most countries (115,116,117,118). It is therefore likely that vertical transmission of HSV infection will increase. Infection with HSV 2 is common among pregnant women infected with HIV, and HSV reactivation has been shown to complicate labor more often in this group than in other obstetric patients (119).

Infection due to HSV-2 and less commonly HSV-1 is usually acquired by the neonate during delivery via an infected birth canal or as a result of ascending infection following rupture of membranes (120,121,122). The disease manifests several days to weeks following exposure and prematurity is a recognised risk factor. Conjunctivitis is a common presenting sign with or without mucosal and skin lesions. CNS disease may occur as well as disseminated infection leading to life threatening complications, which include intravascular coagulopathies, hepatic, and adrenal necrosis (123,124,125).

HSV has the ability to infect the products of conception earlier on in gestation and causes congenital infection that may result in abortion, premature labor, skin vesicles, chorioretinitis, microcephaly, seizures, hepatosplenomegaly, bleeding diathesis or intrauterine growth retardation and death of an infected neonate (120,126,127,128,129). Congenital infection probably follows uterine infection as a result of haematogenous dissemination. To presume haematogenous dissemination, the infant has to have evidence of disease within 48 hours of rupture of foetal membranes or have a morphological lesion older than that

which could be accounted for by ascending infection.

Neonatal disease due to primary maternal infection is generally disseminated with a mean incubation time of 6 days. Transmission from recurrent maternal infection has a longer incubation period of about 14 days and is often localised to the brain (130).

Although HSV infections are common in pregnant women, they are rarely serious. However, disseminated infection may cause a fulminant hepatitis with maternal and perinatal mortality approaching 40% (131). To date only 56 cases of HSV hepatitis have been reported including 22 cases among pregnant women (131,132). On physical examination, half of the patients did not have mucocutaneous lesions, they were generally febrile and anicteric with markedly elevated aminotransferase values, without a corresponding elevation in the bilirubin level (133). Prompt administration of acyclovir is lifesaving and HSV infection should be considered as part of the differential diagnosis of acute hepatitis in pregnancy (132).

Clear definition regarding the identification of at risk pregnancies in the vertical transmission of HSV infection is lacking.

Brown et al. (1997), studied seroconversion prospectively during pregnancy in 7036 pregnant women in the US, in whom serological tests showed to be at risk for herpes simplex virus infection. Ninety-four of the women became seropositive for HSV, with 34 (36%) having symptoms consistent with herpes infection. Thirty percent of primary infections occurred in the first trimester, thirty percent in the second trimester and forty percent in the third trimester. Acquisition of infection and seroconversion completed by the time of labor was not associated with an increase in neonatal morbidity. Although 7% of the infants born to mothers who became HSV seropositive before the onset of labour but had subclinical viral shedding at the time of labour were exposed to HSV during delivery, none acquired HSV infection. However, 4/9 infants born to mothers who lacked type-specific antibodies to the homologous virus acquired HSV infection (134). It therefore seems that in this population, women at greatest risk to transmit HSV to their neonates are those who acquire their first episode of HSV during the latter stage of pregnancy (135). This observation may however vary in different populations depending on the prevalence of HSV infection.

The diagnosis of HSV infection can only be accurately established by clinical evaluation in conjunction with viral isolation. Serological testing by means of a type specific assay in the absence of disease differentiates infected from non-infected individuals. It is now generally accepted that such asymptomatic infections are accompanied by regular HSV shedding (118,128)

In the absence of skin lesions, congenital HSV infection may be difficult to differentiate from the clinical features of rubella, cytomegalovirus or toxoplasmosis, while neonatal HSV infections may only present with signs of sepsis. Prenatal ultrasound examination of the foetus may show multiple abnormalities, which are also associated with other in utero infections (136).

Laboratory diagnosis of HSV infection is based on direct detection of HSV from lesions or serology using type specific HSV antibodies. Viral cultures of amniotic fluid may be done when in utero infection is suspected (136). Immunohistochemistry of the extraplacental membranes and umbilical cord using herpes - specific antibodies has been used to diagnose congenital HSV infection (137).

The detection of IgM antibodies to HSV in infants may be helpful in the diagnosis of neonatal infection but are of little value in the management of genital HSV infection. However, specific commercial assays are now available and are either EIAs based on glycoprotein (gG1, gG2) or western blot. Although these tests may be used in patients with genital infections, the value of

screening all antenatal patients has not been established.

Currently, there is no specific program aimed at decreasing the transmission of HSV infection. The International Herpes Management Forum (IHMF) has suggested that pregnant women with primary genital herpes should be delivered by Caesarian section between the 34th week and term (124).

The pregnancy subgroup of the Herpes Simplex Advisory Panel in UK attempted to devise evidence based guidelines for management of HSV infections is pregnancy. The panel found that there is a paucity of literature addressing the issue of management of herpes in pregnancy.

Since women who acquire HSV treatment during the 3rd trimester of pregnancy are at increased risk of transmitting infection to their babies, the recommendation is aggressive intervention in this group to try and prevent the sequelae of infection. Routine caesarian section or sequential viral cultures during late gestation to predict viral shedding at delivery are unnecessary in women with recognised recurrent genital herpes since the risk of transmitting the infection is low.

Two thirds of women who acquire HSV infection during pregnancy will do so asymptomatically and the disease will therefore go unrecognised at the time of labour. The value of serological screening to identify asymptomatic primary infection in pregnancy has not been established. However serologic testing for HSV in the latter half of pregnancy could identify women who are susceptible to HSV infection, so that they may be appropriately counseled on the risk of acquiring genital herpes late in pregnancy (134).

Symptomatic patients, who acquire the infection during the 1st or 2nd trimester, may be treated with oral or intravenous aciclovir - 200 mg daily x 5 days. These is no indication for caesarian section at term.

Caesarian section should be considered in women acquiring the infection during the 3rd trimester, specifically in those developing symptoms within 6 weeks of delivery as the risk of viral shedding in labour is very high with no time to develop an adequate immune response (121). If vaginal delivery is unavailable, aciclovir treatment of mother and baby may be indicated.

Subclinical maternal shedding of the virus may occur at the time of delivery as a consequence of reactivation of disease (134). These women may potentially infect their infants intrapartum. Delivery by caesarian section is recommended for patients with perineal lesions. However, this mode of delivery does not protect if transplacental transmission has already occurred (138) or with asymptomatic HSV shedding (135).

The risks of vaginal delivery for the foetus in women with genital lesions at the onset of labour is small and must be weighed against the risks to the mother of caesarian section as well as the risks of antiviral treatment in pregnancy.

None of the antiviral drugs on the market are registered for use in pregnancy. However, aciclovir has been used in pregnancy with no evidence to show that it is teratogenic (139). The safety of valaciclovir and famciclovir in pregnancy has not been established.

Although the use of antiviral therapy has reduced the overall mortality of neonatal HSV infection, the mortality rates in CNS disease (15%) and disseminated disease (57%) remains high. (125,140).

Administration of oral acyclovir has been demonstrated to prevent cutaneous recurrences of HSV after neonatal skin, eye and mouth disease (125).

Human Papillomavirus

Laryngeal papillomatosis in young children is assumed to be acquired by passage through an infected birth canal. This assumption is based on the observation that similar HPV types have been seen in respiratory papillomatosis and anogenital warts (141). Treatment during pregnancy aims to reduce neonatal exposure to the

virus. Chemical agents viz. Podophyllin, 5-Fluorouracil and Imiquimod is not recommended for use in pregnancy (142). If there is extensive blockage of the vaginal outlet with warts, a caesarian section may be indicated (143).

HIV and Sexually Transmitted Diseases

HIV infection is a major cause of morbidity and mortality among women and young children (144,145) with the potential to be transmitted from the pregnant female to her offspring in utero, intrapartum or postpartum. In the absence of appropriate therapy, perinatal transmission of HIV has been reported to occur in 15% to 30% of births among HIV-infected women (144,146,147,148,149).

HIV infection and lower genital tract infection are inextricably linked since both are largely sexually transmitted. This relationship is a complex one with both disease entities having a potential role in the transmission, acquisition and clinical course of the other (150,151,152,153,154,155). The biologic mechanisms at play in this unique interrelationship are incompletely understood.

Genital ulcer disease has been shown to facilitate transmission of HIV (150,156,157,158,159). HIV nucleic acid was frequently detected in genital ulcer specimens from HIV infected men (160). A decreased rate of HIV nucleic acid was detected after successful treatment of these infections (158). It can therefore be hypothesised, that untreated genital ulcers in pregnant HIV infected females increases the intrapartum risk of HIV transmission to their neonate.

In most populations the genital discharge syndromes are far more prevalent than genital ulcers. However, data on non-ulcerative STIs and HIV infection are limited as compared with data for genital ulcer disease. The impact of cervical infections on the risk of acquiring and transmitting HIV infection has only recently been defined (154,155). The importance of the treatment of vaginal infections has been progressively highlighted by the association of BV and trichomoniasis as possible risk factors for the acquisition of HIV infection (151,161,162,163,164).

Although considerable evidence suggests that STIs may facilitate HIV transmission in the sexually active population, data linking vertical HIV transmission with STIs of the female genital tract are not available as yet.

Conclusion

The importance of early detection and treatment of STIs in pregnant women has been progressively accentuated by the implication of the various STI pathogens and adverse pregnancy outcome. Without specific screening procedures, a great majority of these infections will go undiagnosed. However, for the want of an appropriate point-of-care test with a high sensitivity and reasonable specificity, screening for STIs with the exception of syphilis, is not routine

Although the prevalence of STIs varies tremendously from area to area, the outcome of pregnancy is most likely microbe related and not area specific. In addition, some women are simultaneously infected with more than one sexually transmitted pathogen and this may also promote adverse pregnancy outcome.

Fundamental relationships between the STI pathogens and the various stages of pregnancy need to be established to enable effective intervention strategies.

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General Discussion

Sexually transmitted infections represent a major epidemic that affects in particular the uneducated and the poor. Policy makers and researchers alike have ignored this epidemic for decades. Reasons for this neglect have been the perception that these diseases do not have major complications, the judgemental attitude of the educated versus non-educated and the lack of economic incentives for the health care industry in return for efforts to develop better diagnostics. All of this has changed in the wake of the HIV epidemic during which it has become clear that spread of HIV and STIs is associated with the same behavioural patterns, that both infections often lack signalling symptomatology in infected individuals and that the presence of ulcerative as well as non-ulcerative STIs during sexual intercourse in at least one of the partners promotes the transmission of HIV.

Over the last 15 years, management of patients with symptomatic STIs (STDs) relies on syndromic management (SM). This is based on the non specific nature of the symptoms, the absence of diagnostic laboratories and other advanced health care facilities where the prevalence is highest and the lack of simple, sensitive and rapid point-of-care tests that reliable identify the often multiple aetiology. SM relies on symptom recognition and reporting by the patient and syndrome recognition followed by appropriate intervention by primary health care workers. These interventions consist of treatment of the index case by antibiotics and prevention of reinfection. The latter consists of counselling regarding risks and partner notification and treatment. It is obvious that, for SM to be successful as a tool for (STI) infection control, at least one partner in a sexual network needs to have symptoms that brings him/her into the health care system.

The work presented here addresses questions around the effectiveness of SM in the setting of KwaZuluNatal which is one of the worst hit parts in the world by both HIV infection and STIs. The first set of papers, collated in the first chapter, deals with the question whether HIV co-infection adversely affects the outcome of antimicrobial treatment of STDs. The findings strongly suggest that that is not so for clinical response of both non-ulcerative and ulcerative infections. This follows the general pattern in HIV infected patients indicating that short-term response to antimicrobial treatment of infections is not affected by HIV status or level of immune suppression. The latter is associated with relapses of diseases in which treatment does not lead to eradication of the microbe involved. Because the different STI pathogens are very diverse in nature, the question of endogenous relapse has to be addressed for each of these separately and this needs to be done in the context of levels of immune suppression. It can be hypothesised that acute infections like gonorrhoea and chancroid will not show immune status associated relapses, but that more chronic infections like genital herpes, granuloma inguinale and the different Chlamydia infections will. Data suggesting that this hypothesis might be true, are presented in chapters 1, 3 and 5.

The application of a combination of pheno- and genotyping methods on pre- and post-treatment isolates of *Neisseria gonorrhoeae* from women indicates that early microbiological failure of gonococcal disease results from rapid reinfection rather then persisting infection. On the other hand, the changing prevalence of the aetiology of genital ulcer disease with an increase of genital herpes and lymphogranuloma venereum, suggests persistent infection with concomitant increased transmission of HSV-2 and *C.trachomatis*, biovar LGV. Prospective studies with long-term follow-up in which patients are stratified according to levels of immune suppression, can provide definitive answers.

A ubiquitous problem with studies on STD patients is the high rates of loss-to-follow up. The studies presented here are no exception. Although the baseline characteristics for all three cohorts (male urethritis, female discharge and genital ulcer disease) did not significantly differ for patients with and without follow up, bias due to loss-to-follow up cannot be excluded. While it is likely that the lost patients did not return because their symptoms disappeared, the opposite with patients seeking care elsewhere because of disappointing outcome is an option as well. These issues are discussed at length in the different papers.

As with all infections in which antimicrobial treatment is started empirically, the drugs used for SM of STIs, have to cover the major aetiology of these infections and have to take into consideration antimicrobial susceptibility patterns of the microbes involved. Both prevalence of aetiological agents and susceptibility patterns change over time and vary in different geographical regions. Therefore, SM strategies have to be accompanied by a well-designed surveillance program. The work presented does not address the design of such surveillance programs but does address the issue of applicability of the current antimicrobial choices in the local SM protocols. While the availability of sensitive nucleic acid amplification tests for all but one (*Calymmatobacterium granulomatis*) of the recognised and potential causes of the three syndromes allow for an accurate inventory of the aetiologies, susceptibility testing is still a major challenge because of the fastidious nature of the organisms involved. Large scale testing is only possible for *N.gonorrhoeae* and *Haemophilus ducreyi*. Because of the virtual disappearance of *H.ducreyi* infection, the work on drug susceptibility was restricted to *N.gonorrhoeae*. While the data show that the use of ciprofloxacin or ceftriaxone for infections with this organism is still justified, a worrying trend of decreasing susceptibility to a variety of drugs was also observed.

The inventory of aetiologies of male urethritis syndrome shows that coverage of *N.gonorrhoeae* and *C.trachomatis* is still warranted. However, as in other studies, in a large proportion of patients no aetiology is found. While this is not an urgent problem from a patient management point of view, a situation in which treatment failures occur due to resistance in organisms that have as yet have not been identified as causative agents of this syndrome needs to be pre-empted. Therefore, establishing the role of potential pathogens is important. Two

papers presented in chapter 5 address this question. With respect to male urethritis and pelvic inflammatory disease.
The paper on urethritis aetiology also deals with the thorny issue of the nature of a control group for such studies. If one chooses individuals not at risk for acquiring STIs, it becomes impossible to differentiate between a readily transmissible coloniser that only indicates promiscuity and causative agents of infection. On the contrary, using men with GUD and no urethritis creates the possibility of underestimating the role of potential pathogens because these men could be asymptomatic carriers of a truly pathogenic organism. The study presented in this thesis, has chosen for a GUD control group, arguing that if with this study design, an association would be found between a microbe and urethritis the chances that this has no pathological significance becomes small. This design does not allow firm conclusions regarding the role of organisms that do not show an association. This work suggests that *M.genitalium* and *U.urealyticum* are aetiological agents of male urethritis. The role of *T.vaginalis* and HSV-2 remains unclear.

With the increasing prevalence of LGV and the persistent role of *C.trachomatis* in non-ulcerative disease, methodology to accurately measure susceptibility of *C.trachomatis* to antimicrobial drugs becomes a priority. In addition, susceptibility test methods for *T.pallidum*, *C.granulomatis*, *T.vaginalis* and HSV-2 that are reproducible and applicable on a large scale need to be developed. It is likely that such tests will be needed for *M.genitalium* and *U.urealyticum* in the near future.

While in male urethritis the lack of an aetiological diagnosis is frequently reported, in women this is obscured by a diagnosis of bacterial vaginosis. This label suggests an identified diagnosis but in reality the cause of this syndrome is unknown. The data presented in chapter 4 suggest that other STIs like trichomoniasis, might change the vaginal ecology and by doing so, inducing bacterial vaginosis. This is supported by data from papers in chapter 1 that indicate that outcome of treatment of bacterial vaginosis with metronidazole in the absence of other STIs is negatively affected by HIV status. This suggests a role for presence of HIV in the vaginal mucosa in the aetiology of bacterial vaginosis. This needs to be taken further in a long-term follow up study, that investigates the vaginal ecology in relation to the acquisition of STIs.

Treatment of infections has 2 different aims: curing the infected individual and controlling transmission. While chapter 1 shows that the first aim is met by means of SM strategies, but that is not the case for aim 2. Women become readily reinfected with *N.gonorrhoeae* and it is likely that that will be the case with the other pathogens that cause non-ulcerative STIs. Such rapid reinfection was not shown for men. This suggests that women become more readily infected with non-ulcerative STI pathogens then men. We postulate that this reflects the larger mucosal surface provided by vagina and cervix as compared to the urethral orifice. In addition, during intercourse the full content of the urethra, containing shedded pathogens if the man is infected, is deposited onto that surface. These rapid reinfections indicate that the

non-antibiotic components of SM strategies (counselling, condom promotion and partner treatment) are failing, at least in KwaZuluNatal. The uncontrollability of the STI epidemic impacts on HIV transmission. Therefore, alternative strategies are urgently needed. It seems that little can be gained from improved SM because that was done from the starting date of the studies onwards. Mass treatment has been used in a study in Uganda with little impact on STI prevalence and no impact on HIV incidence. However, the situation in Uganda was very different form KZN with fewer STIs and a significantly lower HIV incidence and prevalence. Modelling has shown that in a situation like in KwaZuluNatal, this type of intervention might work, especially when combined with optimised syndromic management and community education.

In conclusion, the work presented in this thesis highlights positive aspects of the application of SM strategies as well as the shortcomings of the STI control program in the region. It can be assumed that this can be applied to the rest of southern Africa. The work not only raises a number of scientific questions but also asks for an urgent review of intervention strategies.

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