

Co-Infection with *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* in Symptomatic South African Men with Urethritis: Implications for Syndromic Management

M. De Jongh¹, M. Le Roux¹, A. Adam², A.M. Caliendo³ and A.A. Hoosen^{4,*}

¹Department of Microbiological Pathology, University of Limpopo, Medunsa Campus, Ga-Rankuwa, Pretoria, 0204, South Africa; ²Family Practitioner, Silverton, Pretoria, 0001, South Africa; ³Department of Pathology and Laboratory Medicine and Emory Center for AIDS Research, Emory University, School of Medicine, Atlanta, GA 30322, United States of America and ⁴Department of Medical Microbiology, University of Pretoria, Pretoria, 0001, South Africa

Abstract: *Background:* This study was undertaken to determine co-infection rates of common aetiological agents of urethritis and information regarding sexual contacts of symptomatic males presenting to a family practitioner.

Methods: Endo-urethral swab specimens were collected from 253 symptomatic men and tested for *N. gonorrhoeae*, *C. trachomatis* by the BD ProbeTec™ ET assay and for *T. vaginalis* by real-time PCR. Information regarding sexual behaviour was collected by the practitioner using a structured interview.

Results: Of the 253 patients investigated, 50 complained of urethral discharge with/without burning on micturition (BOM), the remaining 203 only complained of BOM. *N. gonorrhoeae* was isolated from 45%, *C. trachomatis* from 15% and *T. vaginalis* from 5.5%. *N. gonorrhoeae/C. trachomatis* co-infection was diagnosed in 7.5% and *T. vaginalis* was detected in a higher number of males presenting without visible discharge. Similar number of men reported sexual contact only with their wives compared to men having casual sexual contacts. There were fewer gonococcal infections in men reporting sexual contact with their wives alone compared to men reporting casual sexual encounters.

Conclusions: Treatment guidelines for men presenting to Primary Health Care facilities and Family Practitioners need to consider the evolving antimicrobial resistance profiles and the presence of mixed infections in symptomatic patients.

Key Words: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, urethritis.

INTRODUCTION

Adult male urethritis in South Africa is a common clinical condition with fairly large numbers of patients presenting to family practitioners and primary health care facilities [1]. The symptoms of this condition include urethral discharge and/or burning on micturition (BOM) and occasionally dysuria. The established sexually transmitted pathogens implicated in the aetiology of urethritis include *Neisseria gonorrhoeae*, *Chlamydia trachomatis* (D-K strains) and *Trichomonas vaginalis* whilst the pathogen status of *Mycoplasma genitalium* and *Ureaplasma urealyticum* is unclear [2, 3].

In South Africa, like many other developing countries, syndromic management of sexually transmitted infections is advocated and the National Department of Health formulated treatment guidelines in 1996 [4]. These guidelines are widely used by public health service providers working at Primary Health Clinics as well as private and family practitioners. The prevalence of co-infections with common aetiological agents of male urethritis as well as antimicrobial susceptibility profiles [5] informs therapeutic guidelines and especially syndromic management.

In a recent study we reported the antimicrobial susceptibility profiles of gonococcal isolates. The local isolates were resistant to the commonly used therapeutic agents and we advocated a change in the national treatment guidelines for *N. gonorrhoeae* [5]. To determine the prevalence of co-infections in men with urethritis, molecular assays were used in view of the higher sensitivity of detection.

METHODS

Endo-urethral swab specimens were collected from 253 consecutive symptomatic men presenting with urethral discharge with/without BOM to a family practitioner in Pretoria, South Africa. The urethral swab specimens were stored in 1 ml of sterile distilled water at -70°C for batch testing. Information regarding sexual behaviour was collected by the practitioner (AA) using a structured interview. HIV status was not determined.

C. trachomatis and *N. gonorrhoeae* testing was performed using the BD ProbeTec™ ET assay (Becton Dickinson, Sparks MD, USA). 200µl of the sample was used for testing according to the manufacturer's protocol. *T. vaginalis* testing was performed using a laboratory developed real-time PCR assay as previously described [6]. The target of the assay was a 67-base pair region of the repeated sequence of the *T. vaginalis* genome [7]. A clinical evaluation of the as-

*Address correspondence to this author at the Department of Medical Microbiology, University of Pretoria, PO Box 2034, Pretoria, 0001 Pretoria, South Africa; Tel: +27 12 319 2271; Fax: +27 12 321 9456; E-mail: anwar.hoosen@up.ac.za

say showed the real-time PCR test detected about 40% more positive samples than culture [6].

Statistical analysis was performed using Fisher Exact Test to determine whether there was any relation between two categorical variables.

Approval to perform this study was obtained from the Medical Research and Ethics committee (MCREC) of the University of Limpopo. (MP130/2005).

RESULTS

Two hundred and fifty three (253) specimens were collected from consecutive, symptomatic male patients. Their ages ranged from 15 to 73 years, with the mean age of 37 years. Of the 253 specimens collected, 50 were from patients

complaining of a urethral discharge with/without BOM. The remaining 203 patients complained only of BOM and there was no visible urethral discharge on examination.

The prevalence of single and mixed infections is shown in Fig. (1). *N. gonorrhoeae* was detected in a total of 113/253 (44.7%), *C. trachomatis* in 38/253 (15.0%) and *T. vaginalis* in 14/253 (5.5%) of the men recruited for the study. Co-infection with *N. gonorrhoeae* and *C. trachomatis* was found in 19/253 (7.5%); *C. trachomatis* and *T. vaginalis* in only 1/253 (0.4%) of the study subjects. No patient was infected with all 3 pathogens. None of the 3 pathogens were detected in 108 (42.7%) symptomatic men.

The breakdown of infections according to symptoms of only BOM or urethral discharge with/without BOM is shown in Fig. (2). In men who had a visible urethral discharge *N.*

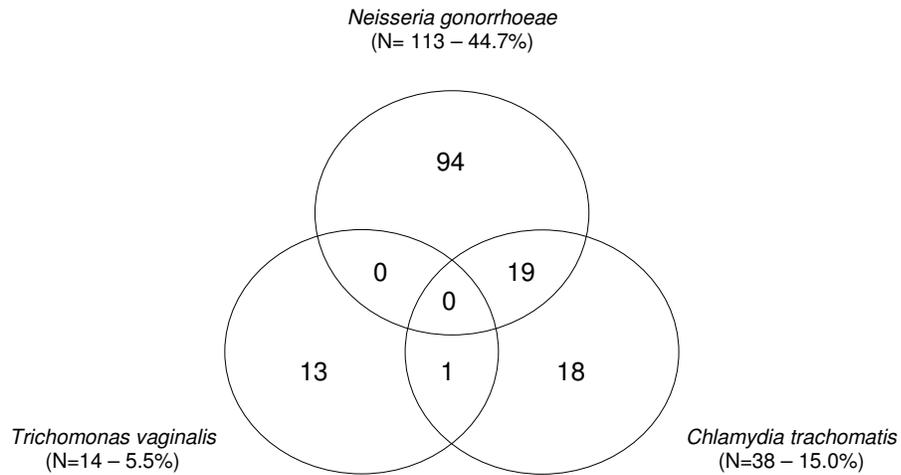
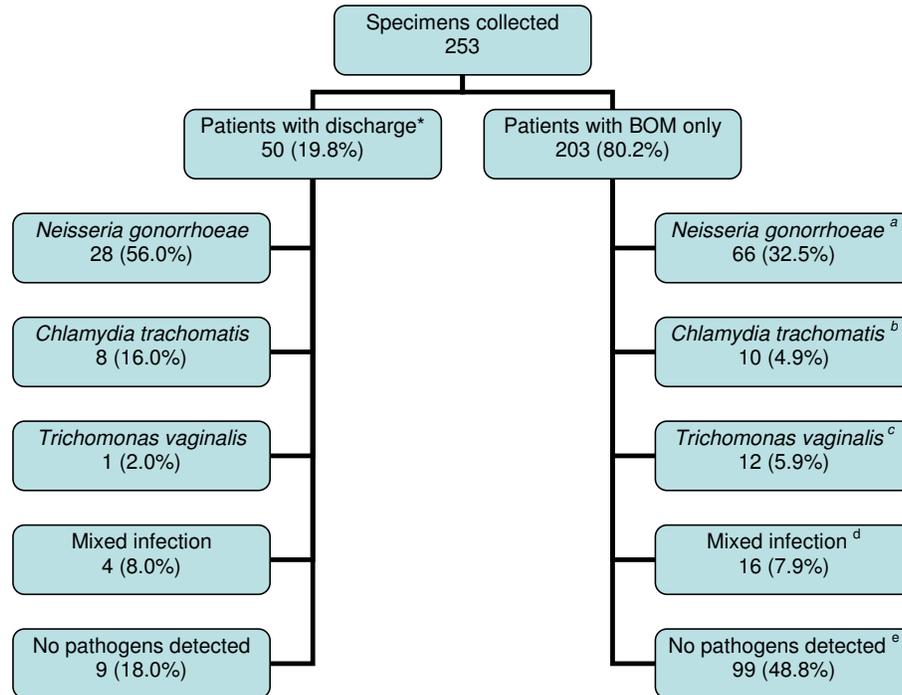


Fig. (1). Single and mixed infections in symptomatic men (N=253).



* with or without BOM

^ap=0.002; ^bp=0.006; ^cp=0.474; ^dp=1.000; ^ep<0.001

Fig. (2). Infections according to symptoms (N = 253).

gonorrhoeae was detected in 28 (56.0%); *C. trachomatis* in 8 (16.0%) and *T. vaginalis* infection in only one (2.0%). In four (8.0%) of the study subjects, co-infection with *N. gonorrhoeae* and *C. trachomatis* was found and in 9 (18%) none of the pathogens was detected.

Of the men who complained only of BOM, in 99 (48.8%) none of these pathogens were detected; *N. gonorrhoeae* was diagnosed in 66 (32.5%), *C. trachomatis* in 10 (4.9%) and *T. vaginalis* in 12 (5.9%) of the men. *N. gonorrhoeae* and *C. trachomatis* co-infection was found in 15 (7.4%) of the men and co-infection with *C. trachomatis* and *T. vaginalis* in only one (0.5%) person. There were significant differences for *N. gonorrhoeae* (56.0% vs 32.5%; $p=0.002$), *C. trachomatis* (16.0% vs 4.9%; $p=0.006$) and where no pathogens were detected (48.8% vs 18.0%; $p<0.001$) when comparing the groups.

A total of 54 (21.3%) study subjects suffered from other illnesses such as diabetes and influenza (diagnosed clinically). Seven patients (2.8%) were clinically diagnosed to have prostatitis, but no organisms were detected in the urethral specimens from these patients.

Regarding sexual behaviour, one hundred and fourteen (45.1%) men reported their wives as the only contact, whilst 112 (44.3%) of the men admitted to having sex with casual contacts (Table 1). Only one man (0.4%) admitted to sexual contact with his wife as well as casual acquaintances, whilst 24 (9.5%) of the men stated that they had steady relationships (with one partner) and for 2 (0.8%) of the participants no information was available.

For the pathogens identified according to sexual contacts, there were significant differences only where no pathogen was isolated when comparing men who had sex with their wives as sole partners versus those who had sex with casual partners.

DISCUSSION

This study determined the co-infection rate of *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* in adult males presenting with symptoms of urethritis to a family practitioner in the city of Pretoria, South Africa. The overall infection rate was 65% with the co-infection rate (i.e. more than one pathogen detected) of 8% (20 of 253). The infection rate in a similar study also using molecular diagnostic assays in a

high HIV prevalence area of South Africa for the same three pathogens was 74% [3]. The similarity between these studies is reflected by the diagnosis of *N. gonorrhoeae* being 45% vs. 52%; *C. trachomatis* 15% vs. 16% and *T. vaginalis* 6% in each study. It must be noted that the HIV status was not determined in our study. However, the study from KwaZulu Natal (KZN) reported a 45% HIV prevalence and the HIV status did not affect the prevalence of the urethral pathogens [3]. However, in the current study, we looked at signs and symptoms of men with urethritis and information on the sexual contacts of symptomatic males.

Gonococcal urethritis using the more sensitive molecular assay was diagnosed in 45% of the study participants, which is considerably less than the range of 64-96% reported in an earlier review of South African studies [1]. However, this lower prevalence has also been reported in more recent studies such as the one from KZN (52%) [3] and a recent study from our centre in Pretoria (39%) [5].

Chlamydial infection was found in 15% of the participants. This is very similar to what was found in the 1996 review by Pham-Kanter [1] where prevalences between 12–14 % were reported. This was in an era where laboratory testing for chlamydial infection was reliant on culture and antigen detection methods. It is also a striking similarity that using molecular techniques, the KZN study found a 16% rate of infection [3] and a more recent study from Johannesburg reported 19% [8]. When analyzing the type of symptoms, *N. gonorrhoeae* and *C. trachomatis* were detected from significantly higher number of individuals presenting with a visible urethral discharge (56.0% and 16.0%) as compared to those presenting with only BOM (32.5% and 4.9%).

There have not been many studies on the prevalence and role of *T. vaginalis* in adult male urethritis in South Africa. Our study showed a prevalence rate of 6% which compares favorably with the 6% found in KZN [3] and the 5% in Johannesburg [8]. In all these recent studies molecular diagnostic assays have been used. In a study by Crewe-Brown [9], a prevalence of 4% was reported. It is important to note that this earlier study used conventional methods such as culture and microscopy for the diagnosis of trichomoniasis. However, in our study there was a difference in the detection of *T. vaginalis* in men without visible discharge and only complaining of BOM as compared to those in whom there was a visible discharge. This needs to be confirmed by

Table 1. Sexual Contacts of Participants and the Pathogens Detected (N=253)

Contact/ Partner	No pathogen Number (%)	<i>N. gonorrhoeae</i> Number (%)	<i>C. trachomatis</i> Number (%)	<i>T. vaginalis</i> Number (%)	Mixed Number (%)	Total Number (%)
Wife	60 (52.6%)	38 (33.3%)	5 (4.4%)	6 (5.3%)	5 (4.4%)	114 (45.1%)
Casual contact	37 (33.0%) ¹	50 (44.6%) ²	9 (8.0%) ³	6 (5.4%) ⁴	10 (8.9%) ⁵	112 (44.3%)
Steady partner	9 (37.5%)	5 (20.8%)	4 (16.7%)	1 (4.2%)	5 (20.8%)	24 (9.5%)
Not recorded	1 (50%)	1 (50%)	0	0	0	2 (0.8%)
Wife & Casual	1 (100%)	0	0	0	0	1 (0.4%)
	108 (42.69%)	94 (37.15%)	18 (7.11%)	13 (5.14%)	20 (7.91%)	253 (100%)

¹ $p=0.003$; ² $p=0.081$; ³ $p=0.255$; ⁴ $p=0.975$; ⁵ $p=0.170$.

studying and following-up a larger cohort of patients as this may have implications for therapy. There may be a need to add an anti-trichomonocidal agent in men presenting with complains of BOM in the absence of visible discharge. Co-infection was detected in a total of 20 individuals; the majority being *N. gonorrhoeae* and *C. trachomatis* (19 persons) and only one with *C. trachomatis* and *T. vaginalis*.

The pathogen status of *M. genitalium* is unclear and no investigation for this organism was performed in the current study. In a review on urethritis in men, *M. genitalium* was implicated as the causal agent in 18-46% of men with urethritis [2] and local South African studies reported overall prevalence of 5% [3] and 7-14% [8]. For syndromic management the antibiotic used for treating chlamydial infection invariably covers the genital mycoplasmas and this may be a factor in this potential pathogen not being investigated in many epidemiological studies for implementation of syndromic management guidelines. Furthermore, one has to use molecular diagnostic methods to diagnose *M. genitalium* as it takes a very long time to culture.

With regard to sexual behavior, differences were found when comparing sexual partners and pathogens isolated. Significantly fewer pathogens were detected in males that had their wives as sole contact, when comparing men who had sex with casual contacts, reflecting high sexual risk behavior.

More than ten years ago, in 1996 the National Department of Health of South Africa implemented guidelines for the syndromic management of sexually transmitted diseases (STDs). For urethritis (discharge with/without BOM), a two agent regimen to cover for gonorrhoea and chlamydia was advocated viz. ciprofloxacin plus doxycycline. In view of the results obtained in this study, where *T. vaginalis* was detected in a higher number of males presenting without a visible discharge, the question is raised whether metronidazole should be included in the syndromic regimens for patients presenting with BOM only, or whether it should only be administered to patients with persistent urethral symptoms. There needs to be further studies to answer these questions. However, it is heartening to note that metronidazole is still the drug of choice for the treatment of trichomoniasis, as most local isolates remain susceptible [10].

In a recent article [5], we advocated a change in the syndromic management of patients with urethritis from ciprofloxacin to ceftriaxone as local isolates have become resistant to the quinolones [11]. The drugs of choice for the treatment of chlamydial infection remain the tetracyclines (doxycycline) or azithromycin. Azithromycin is favoured because of the ease of administration of a single dose.

LIMITATIONS OF THIS STUDY

HIV status of participants was not determined.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Z Adam for assisting in the collection of specimens, Jessica Ingersoll and Tameka Bythwood for performing the molecular testing. This work was supported in part by the Emory Center for AIDS Research (P30 AI050409).

ABBREVIATIONS

BOM	=	Burning on micturition
<i>C. trachomatis</i>	=	<i>Chlamydia trachomatis</i>
<i>M. genitalium</i>	=	<i>Mycoplasma genitalium</i>
<i>N. gonorrhoeae</i>	=	<i>Neisseria gonorrhoeae</i>
PCR	=	Polymerase chain reaction
STD's	=	Sexually transmitted diseases
<i>T. vaginalis</i>	=	<i>Trichomonas vaginalis</i>
USA	=	United States of America

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