academicJournals

Vol. 8(8), pp. 830-838, 19 February, 2014
DOI: 10.5897/AJMR2013.6537
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Novel simple diagnostic methods compared to advanced ones for the diganosis of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasmas hominis* in patients with complicated urinary tract infections

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Accepted 23 January, 2014

Chlamydia trachomatis **is responsible for the most common sexually transmitted bacterial infection worldwide. Infections with** ***Ureaplasma urealyticum*** **and genital** ***Mycoplasma hominis*** **have been recognized for about a decade as common sexually transmitted infections (STIs) in developed countries. The aim of this study was to assess the diagnostic value of novel simple diagnostic kits used in the detection of** ***C. trachomatis*****,** ***U. urealyticum*** **and** ***M. hominis*** **in urine of patients with complicated urinary tract infections (UTIs) and to compare these simple methods to molecular and cultural ones as gold standards. This study was conducted on two male patient's groups; group I: 30 patients with compilcated UTIs and Group II: 50 patients with non complicated UTIs. Twenty (20) healthy male control subjects (Group III) were also included. The patients were chosen from those admitted to the urosurgery department or attending the urosurgery outpatient clinic in Theodor Bilharz Research Institute (TBRI). First voided urine (FVU) samples and mid-stream urine (MSU) were collected from patients and controls. FVU samples were investigated for** ***C. trachomatis*** **plasmid DNA using polymerase chain reaction (PCR) and for** ***C. trachomatis*** **antigen using Chlamyfast OIA test. MSU samples were used for inoculating of conventional culture media and three culture kits; Mycoplasma DUO, Mycoplasma IST and Mycokit NUM. Blood samples were investigated for the presence of** ***C. trachomatis*** **IgG antibodies using enzyme-linked immunosorbent assay (ELISA) and ImmunoComb Chlamydia Kit and for mycoplasmal antibodies using mycokit sero.** ***C. trachomatis*** **infection was found in 35% (28/80) of patients in both groups, 56.7% (17/30) was detected in group I and 22% (11/50) in group II compared to none of the controls.** ***C. trachomatis*** **infection was significantly higher in group I versus group II (P<0.05). Chlamyfast OIA test was less sensitive but more specific than serological assays. ImmunoComb assay had a higher sensitivity but lower specificity than ELISA. A total of 34 cases (42.5%) were positive in a pathogenic level for U.** ***urealyticum*** **and or** ***M. hominis*** **and 30% were positive for** ***U. urealyticum*** **only, 7.5% were positive for** ***M. hominis*** **only and 5% had mixed infection with both organisms. Mycoplasmas infection in group I was found to be significantly higher than in group II (P<0.05). Mycoplasma IST has the highest sensitivity (100%) andin identification of** ***U. urealyticum*** **while both mycoplasma ISTand mycoplasma DUO showed the highest sensitivity in identi-fication of** ***M.hominis.*** **Serological evidence was detected in 16/24 (66.7%), 2/6 (33.3%) and 4/4 (100%) of** ***U. urealyticum, M. hominis*** **and mixed infections respectively. The serological response to each infection is significantly higher in group I than in group II (P value <0.05). Our study detects a high prevalence rate of** ***C. trachomatis*****,** ***M. hominis*** **and** ***U. urealyticum*** **in cases with complicated UTI. Commercially available kits are simple and sensitive methods to use in laboratories which do not routinely test for these pathogens.**

Key words: *Chlamydia trachomatis*, urinary tract infections (UTIs), non gonorrheal urethritis (NGU).

INTRODUCTION

Urinary tract infections (UTI) are frequent type of infectious pathology treated in primary care clinics. The participation of microorganisms associated with sexually transmitted infection has been reported recently as possible cause of UTI (González-Pedraza et al., 2003). According to a World Health Organization (WHO, 2001) report, *Chlamydia trachomatis* is responsible for the most common sexually transmitted bacterial infection world-wide, affecting more than 90 million people and has been known to have a significant effect on human reproduction (Paavonen and Eggert-Kruse, 1999; Al-Sweih et al., 2012). The prevalence of *C. trachomatis* in woman under 25 years of age is very high (up to 30%) hence, young sexually active women are the most at risk (Mangin et al., 2012). *C. trachomatis* is one of the causes of acute and chronic urinary tract infections and acute or silent salphingitis (Wanic-Kossowska et al., 2001) that if not treated in an early stage can lead to severe compli-cations, such as pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility (Marrazo and Stamm, 1998; Gaydos et al., 2004).

Most *C. trachomatis* genital tract infections in men and women are asymptomatic, so the opportunity for unchecked transmission is high even in countries with advanced public health care systems (Dean et al., 2012; Vicetti Miguel et al., 2013). Dysuria and increased urinary frequency associated with presumed urinary tract infection are extremely common reasons for seeking treatment in primary care. About 10% of women suffer from symptoms of a urinary tract infection in any year, and seeking treatment provides a potential opportunity to detect both symptomatic and asymptomatic infection with *C. trachomatis* (Mangin et al., 2012).

C. trachomatis infection is easily treatable with antibiotics, so detection and treatment of infected indivi-duals is a key element of Chlamydia control programs (Gaydos et al., 2004; Van der Helm et al., 2013). Although there are a variety of methods for testing for *C. trachomatis*, no single test is ideal (Mangin et al., 2012). Culture, nucleic acid hybridization tests, and nucleic acid amplification tests (NAATs) are available for the detection of *C. trachomatis*. Culture and hybridization tests require urethral swab specimens, whereas NAATs can be performed on urine specimens. The sensitivity and specificity of the NAATs are clearly the highest of any of the test platforms for the diagnosis of chlamydial infections (Lee and Lee, 2013).

Infections with *Ureaplasma urealyticum* and genital *mycoplasmas* (*M. hominis and M. genitalium*) have been recognized for about a decade as a common STD in developed countries (Yoshida et al., 2002). Genital urea-plasmas and mycoplasmas are natural inhabitants of the male urethra, contaminating the semen during ejacu-lation. However, *U. urealyticum*, are implicated in invasive diseases such as urethritis, postpartum endometritis, chorioamnionitis, spontaneous abortion and premature birth, as well as low birth weight, pneumonia, bacteremia, meningitis and chronic lung disease in prematurely born infants (Dhawan et al., 2012; Al-Sweih et al., 2012). *Mycoplasma hominis* is also associated with bacterial vaginosis, pelvic inflammatory disease, arthritis and even neonatal meningitis (Hopfe et al., 2013).

The aim of this study was to asses diagnostic value of novel simple diagnostic kits used in the detection of *C. trachomatis*, *U. urealyticum* and *M. hominis* in urine of patients with complicated urinary tract infections (UTIs) and to compare these simple methods to molecular and cultural ones as gold standards.

MATERIALS AND METHODS

Patients

This study was conducted on 80 patients that were divided into two patient's groups; group I: 30 male patients with clinically diagnosed complicated UTIs complained of recurrent UTI that resist treatment with conventional antibiotics (cystitis, prostatitis, epididymititis, cancer bladder, urethral stricture,stone fomation, hydronephrosis and urinary bilharziais), their Group II: 50 male patients with non complicated UTIs. Twenty male healthy control subjects (Group III) were also included. The patients were chosen from those admitted to urosurgery department or attending the urosurgery outpatient clinic in TBRI. The controls were selected from those admitted, during the same period, for Surgery Department with normal urine analysis. The patients diagnosis was based on complete history taking including increased frequency of micturation, dysuria, haematuria, uretheral discharge, antischistosomal treatment, instrumentation or any previous pelvic troubles. Complete clinical assessment, radiological investigation, cystoscopy and histopa-thological examination, urine analysis and culture were also performed. The patients or controls did not receive antibiotics two weeks before the study.

Sample collection

Early morning first voided urine (FVU) samples and mid-stream urine (MSU) were collected from patients and controls. FVU samples were stored at -70°C untill investigated for *C. trachomatis* antigen using enzyme immunoassay (EIA) and *C. trachomatis* plasmid DNA using PCR. MSU samples were centrifuged and the pellets were then used for inoculating of three culture kits; Mycoplasma DUO, Mycoplasma IST and Mycokit NUM and conventional culture media. Blood samples were collected and sera were stored at -70°C to be investigated for the presence of *C. trachomatis* antibodies and mycoplasmal antibodies using ELISA and ImmunoComb Chlamydia Kit and Mycokit Sero respectively.

Detection of *Chlamydia trachomatis*

PCR analysis of C. trachomatis plasmid DNA in urine specimens

Eight (8) ml of FVU samples were mixed thoroughly and centrifuged

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at 3000 rpm for 30 min at room temperature. The precipitate was transferred to 1.5 ml microcentrifuge tube and centrifuged at 14000 rpm for 30 min. The pellets were stored at -20°C until DNA extraction. DNA extraction was performed using QIA amp Viral RNA Mini kit (Qiagen GmbH, Germany) because the AVL buffer used in this kit, inactivates the numerous unidentified PCR inhibitors found in urine. The extraction was done following the QIA amp using spin-column protocol with slight modification as follows: The urine pelet was dissolved in 1ml PBS. 250 µl of urine solution were added to 560 µl of the buffer AVL/carrier RNA followed by vortexing and incubation for 15 min at room temperature. 750 µl of absolute ethanol were then added and mixed by pulse-vortex for 20 s. The mixture was then applied to the QIA amp spin column and centrifuged at 8000 rpm for 2 min followed by washing with buffer AW1 then AW2. DNA adsorbed onto the QIA amp silica gel membrane was eluted by adding 60 µl buffer AVE followed by incubation for 5 min and centrifugation for 5 min at 8000 rpm. The elution step was repeated to increase the yield of DNA. The eluted solution was stored in aliquots at -20^{o}C untill performance of amplification. PCR amplification: performed with PCR kit (DiaSorin, Italy-UAS). Primers set used : It delineated PCR amplification product of 207 bp within the cryptic plasmid of *C. trachomatis* : PC_{24} : 5`GGG ATT CTT GTA ACA ACA AGT CAGG 3` and PC_{27} : 5`CCT CTT CCC CAG AAC AAT AAG AAC AC 3`. The 207 bp product obtained with primers PC24 and PC27.

Reaction mixture: The reaction volume was 100 µl containing 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 2.5U Taq DNA polymerase (Promega, Madison WI, USA), 50 P mole of each primer. Ten microliters of extracted DNA were added to 100 µl of the PCR mixture in the reaction tube. Enzymatic amplification was performed using a programmable thermal cycler 480 version (Perkin Elmer Ceteus, Norwalk, Conn). Amplification started with an initial denaturation step at 94°C for 5 min followed by 35 cycles. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and primer extension at 72°C for 1 min. The reaction was stopped by cooling at 4°C.

Detection of amplification product: Fifteen microliters of the PCR product were electrophoresed through 2% agarose gel containing ethidium promide (0.5 mg/ml) at constant current 100 volt for 45 min. Bands were observed under UVR light and photographed.

Optical immunoassay (Chlamyfast OIA, International Microbio, France) in urine

Optical immunoassay is a two-step procedure involving antigen extraction and detection. The test surface is a silicon water reflecting white light into a predominant gold color. The sample was placed on the test surface. If it contains the antigen, binding would occur between the antigen and the water surface and it was then recognized by the anti-LPS conjugate. After washing to remove unbound components, an optical enhancer was applied to the test surface. Its precipitation leads to an increased thickness in the molecular thin film with subsequent change in the color of the reflected light from gold to blue/purple. If chlamydial antigen was not present in the sample, there would be no change in color.

Enzyme-linked immunosorbent assay for detection of Chlamydia IgG in serum

Enzyme-linked immunosorbent assay (ELISA) (DRG Diagnostics, Germany) was performed and interpreted according to manufacturer's instractions. IgG titer greater than 1:512 was a criterion for serodiagnosis of acute or current infection.

EIA ImmunoComb Chlamydia Kit for semi-quantitative determination of Chlamydia IgG in serum

The ImmunoComb Chlamydia Bivalent IgG Kit (ImmunoComb, PBS, Orgenics, France) is an EIA test intended for the semi-quantitative of IgG antibodies to *C trachomatis* in serum. ImmunoComb consist of a solid-phase EIA. The solid phase is a comb with 12 projections. Each tooth is sensitized at two positions; upper spot: goat antibodies to human immunoglobulin (Internal Control), lower spot: inactivated antigens of *C. trachomatis.* The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test was performed stepwise, by moving the comb from row to row, with incubation at each step. Serum specimens were added to the diluent in the wells of row A of developing plate. The comb was then inserted in the wells of row A. Anti-*C. trachomatis* antibodies, if present in the specimens, will specifically bind to the respective chlamydial antigens on the lower spot of each tooth of the comb. Simultaneously, immunoglobulins present in the specimens would be captured by the anti-human immunoglobulin on the upper spot (Internal Control). Unbound components would be washed away in row B. In row C, the human IgG captured on the teeth will react with alkaline phosphatase-labeled anti-human IgG. In the next two rows, unbound components were removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results were visible as gray-blue spots on the surface of the teeth of the comb. A positive control (anti- *C. trachomatis* IgG) and a negative control were included in each assay run. Upon completion of the test, the tooth used with the positive control should show 2 gray-blue spots. The tooth used with the negative control should show the upper spot and either no other spot or a faint lower spot. The upper spot should also appear on all other teeth, to confirm the addition of the specimen, proper functioning of the kit and correct performance of the test.

Detection of mycoplasmas

Conventional Culture for Mycoplasmas

Urine sediment was cultured for *M.hominis* by direct plating on enriched heart infusion (HN) mycoplasma agar and indirect inoculation of HN mycoplasma broth, which were then incubated at 37°C in a candle Jar. It was also cultured for *U. urealyticum* on U_9 broth and A_7 agar (Int-mycolpasma-France) and incubated anaerobically according to El-Mishad et al. (1992). The plates were then examined under a dissecting microscope for up to 10 days. The results have been determined in terms of identification and quantification of *U. urealyticum* and *M. hominis*. For quantification; two different levels have been determined for both *U. urealyticum* and *M. hominis*: a non-pathogenic level which was determined as $<10^4$ colour changing units (CCUs)/ml and a pathogenic level which was determined as $\geq 10^4$ CCU/ml.

Detection and Antibiotic Susceptibility Testing of Urogenital Mycoplasmas

The centrifuged pellets were resuspended in sterile saline and then processed with the following 3 kits:

Mycoplasma DUO (Sanofi, Diagnostics Pasteur, France): The kit composed of microplates, each of 6 wells containing substrates and growth factors for mycoplasma to ensure optimal sensitivity, besides inhibitors for polymorphic flora to ensure optimal selectivity. It was designed for identification and enumeration of mycoplasmas. Identification was based on specific metabolic properties; hydrolysis

of urea by *U. urealyticum* or arginine by *M.hominis* which was indicated, after 24 or 48 h of incubation, by a change in colour of the well containing the relevant substrate without clouding of the medium. Titration was based on applying successive dilutions of the specimens to the lower three wells. The titer was expressed as the number of CCUs/ml specimen. The technique allowed titration around the level of 10^3 and 10^4/ml which were the accepted pathogenicity levels

Mycoplasma IST (bioMerieux, France): The principle and components were similar to those of DUO with the addition of eleven more wells for determining the susceptibility of the strain to six antibiotics including doxycycline, josamycine, ofloxacine, erythromycine, tetracycline and pristinamycin.

Mycokit-NUM (PBS-Orgenics, france): Serial tenfold dilutions were made from the urine and inoculated into the transport medium; urea broth (U_9) for *U. urealyticum* in the first column and into arginine broth (M_{42}) for *M. hominis* in the second column; in presence of appropriate PH indicator. After 48 h incubation, the titer was estimated in CCU/ml as the reciprocal of the highest dilution revealing a colour change.

Serological assessment for mycoplasmas (Mycokit-SERO, PBS-Orgenics, France)

For detection of mycoplasmal antibodies; anti-*U.urealyticum* and anti-*M.hominis;* in sera. The principle and components of the kit were similar to those of Mycokit-NUM, except that lyophlized *U.urealyticum* and *M.hominis* were added to U_9 and M_{42} columns respectively. After incubation for 48 h at 37°C, growth of mycoplasma induced a pH change of the medium, turning its colour from yellow to blue. If serum contained specific antibodies, inhibition of multiplication of mycoplasma would occur preventing the colour change (metabolic inhibition test). The antibody titer was the least dilution showing no colour change (remained yellow).

Statistical analysis

The statistical analysis was performed using SPSS version 10.0 statistical software (SPSS Inc, Chicago, IL). Data were presented as mean ± SD and range or as an absolute number and percentage. Sensitivity, specificity and predective values were calculated. Chi-square tests were used for the analysis of the categorical variables and Quantitative data with uneven distribution were analyzed with the Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

RESULTS

Two groups of patients were included in the present study; Group (I): consists of 30 male patients with clinical diagnosis of complicated UTIs. Their ages were ranged from 29 to 54 years with a mean of 30 ± 9.8 years (average ± SD) (Table 1). Group (II): consists of 50 patients with simple non complicated UTIs. Their ages were ranged from 32 to 59 years with a mean of 32 ± 8.7 years (average ± SD). Using amplified PCR assay, *C. trachomatis* infection was found in 35% (28/80) of patients in both groups, 56.7% (17/30) was detected in group I and 22% (11/50) in group II compared to none of the controls. Serological evidence was detected in 76% (13/17) and in 27% (3/11) of positive cases of group I and group II respectively. *C. trachomatis* infection was significantly higher in group I versus group II ($P<0.05$).

Table 1. Distribution of 30 patients Group I among different complicated clinical condition.

Complicated Clinical Status*	No.	%
Cystitis	8	27
Prostatitis	15	50
Epididymitis	13	43
Cancer bladder	9	30
Urethra stricture	6	20
Stone formation	11	37
Hydronephrosis	4	13
Urinary Bilharzial	16	53

*One patient may have more than one clinical status

Table 2 shows the diagnostic performance of different tests used for the detection of *C. trachomatis* infection among all studied patients using PCR assay (Figure 1) as reference standard. It was noticed that Chlamyfast OIA test which detects chlamydial antigen in urine specimens was less sensitive but more specific than serological assays that detect IgG antibodies in serum. ImmunoComb assay had a higher sensitivity but lower specificity than ELISA.

In this study, out of 80 urine specimens of infected groups, a total of 34 cases (42.5%) were positive in a pathogenic level for *U. urealyticum* and or *M. hominis* by at least one of the diagnostic procedures used. It was found that 30% were positive for *U. urealyticum* only and 7.5% were positive for *M. hominis* only and 5 % had mixed infection with both organisms. In the control group, *U. urealyticum* and *M. hominis* were isolated from 5 and 2.5% of normal subjects respectively in a non-pathogenic level. The distribution of different types of mycoplasma among the studied groups are shown in Table 3. Mycoplasmas infection in group I was found to be significantly higher than in group II ($P<0.05$).

The diagnostic value of different identification procedures for *U. urealyticum* and *M. hominis* among studied groups were shown in Table 4. It was observed that Mycoplasma IST has the highest sensitivity (100%) and absolute negative predictive value (NPV) in identification of *U. urealyticum* while both Mycoplasma ISTand Mycoplasma DUO showed the highest sensitivity and absolute NPV in identification of *M. hominis.*

The serologic reactivity to *U. urealyticum* and *M. hominis* infection among positive cases was illustrated in Table 5. Serological evedince was detected in 16/24 (66.7%), 2/6 (33.3%) and 4/4 (100%) of *U. urealyticum, M. hominis* and mixed infections respectively.

Serologic reactivity to *C. trachomatis*, *U. urealyticum,*

Table 2. Diagnostic performance of recent tests used for detection of *C. trachomatis* among all studied patients with UTI.

Test result	*C. trachomatis* PCR On urine sediment		Sensitivity	Specificity	PNV*	NPV**
	Pos. (No=28)	Neg. (No=52)				
Chlamyfast OIA						
Pos	17	9	61	83	65	80
Neg	11	43				
ELISA IgG						
Pos	16	11	57	79	59.5	77.4
Neg	12	41				
ImmunoComb IgG						
Pos	26	14	93	67	61	95
Neg	2	38				

*NPV: Negative predictive value. **Positive predictive value.

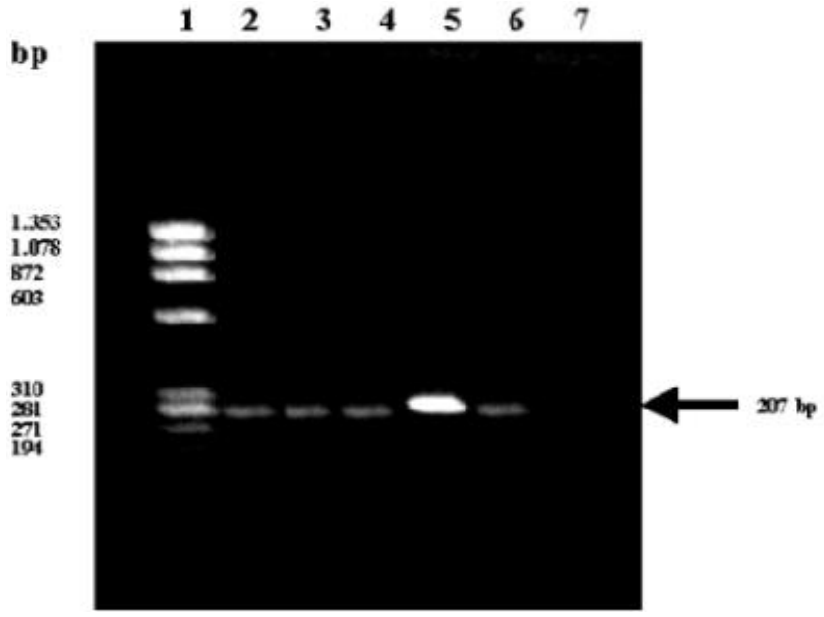

Figure 1. PCR Results for FVU specimens from male patients with recurrent urogenital infections. The 207 bp product obtained with primers PC_{24} and PC_{27}. Lane 1: molecular weight marker, Lanes 2-5: amplification products of four *Chlamydia trachomatis* positive specimens, Lane 6: a positive control and Lane 7: negative control.

M. hominis among studied groups are shown in Table 6. It was noted that the serological respond to each infection is significantly higher in group I than in group II (P value <0.05).

DISCUSSION

Chlamydia trachomatis is one of the most common sexually transmitted pathogens of humans. According to the Centers for Disease Control (CDC), *C. trachomatis* infection is among the most prevalent of all STDs, and since 1994, has comprised the largest proportion of all STDs reported to CDC (CDC, 2010). Since most of infected men and women are asymptomatic, this high number of unrecognized infected individuals may provide the reservoir for spreading the infection to other men and women via sexual intercourse

Table 3. Distribution of different types of mycoplasmas among disease groups.

Group isolates	M. hominis		*U. urealyticum*		Mixed		Total	
	No.	%	No.	%	No.	%	No.	%
Group I (No=30)	4	13.3	18	60	2	6.7	24*	80
Group II (No=50)	2	4	6	12	2	4	10	20
Total (No=80)	6	7.5	24	30	4	5	34	42.5

*P value <0.05 versus group II.

Table 4. Diagnostic value of different identification for *U. urealyticum* and *M. hominis* in disease groups.

Culture system	Diagnostic performance			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Mycoplasma IST				
U. urealyticum	100	89.7	78.6	100
M. hominis	100	98.6	90	100
Mycoplasma DUO				
U. urealyticum	90.9	89.7	76.9	96.3
M. hominis	100	97.2	80	100
Mycokit - NUM				
U. urealyticum	80	90	72.7	93.1
M. hominis	70	100	100	95.9

Table 5. Serologic Reactivity to *U.urealyticum* and *M.hominis* among positive cases using Mycokit-NUM.

Positive case	Positive		Negative	
	No.	%	No.	%
U. urealyticum (No. = 24)	16	66.7	8	33.3
M. hominis (No = 6)	2	33.3	4	66.7
Mixed infection (No.= 4)	4	100	0	0
Total (No. =34)	22	64.7	12	35.3

(Nassar, 2007).

Reported rates of chlamydial infection have increased dramatically over the past decade, reflecting expansion of chlamydial screening activities, highly sensitive nucleic acid amplification tests and improvements in information systems used for reporting rather than a true increase in disease burden (Workowski and Berman 2007 ; Geisler, 2010). However, screening and treatment are focused on females, with the burden of this infection and infertility sequelae considered to be a predominantly female problem, although the prevalence of chlamydial infection is similar in males and females (Cunningham and Beagley 2008; Fernández-Benítez et al., 2013). Prevalence of C. trachomatis varies according to geographic area, age and status of patients (Ghazvini et al., 2012; Fernández-Benítez et al., 2013). In developing countries, infections caused by Chlamydia and Mycoplasma have not been adequately studied and the prevalence of C. trachomatis, genital ureaplasmas and genital mycoplasmas has not been determined so infections by these pathogens may be higher than those reported (Gdoura et al., 2008; Ghanaat et al., 2008).

In our study C. trachomatis infection was found in 35% (28/80) of patients in both groups, 56.7% (17/30) was detected in group I and 22% (11/50) in group II compared to none of the controls. These results were similar to a study done by Vasic et al. (2004) who reported that Chlamydia infection has been determined in 35.55% (128/360) of male patients with urethritis symptoms. Our results was comparable to a previous study in Egypt by El Sayed et al. (2006) where *C. trachomatis* was detected in 25 out of 50 (50%) of examined urine samples. In a Japanese study, 47.7% (73/153) of FVU specimens in men with urethritis were positive for *C. trachomatis*

Table 6. Serologic reactivity to *C. trachomatis*, *U. urealyticum, M. hominis* among studied groups.

Test result	Group I (No. = 30)		Group II (No. = 50)		Total (No. = 80)	
	No.	%	No.	%	No.	%
C. trachomatis						
Positive	18	60	9	18	27	33.8
Negative	12	40	41	82	53	66.2
U. urealyticum						
Positive	17	56.7	3	6	20	25
Negative	13	43.3	47	94	60	75
M. hominis						
Positive	4	13.3	2	4	6	7.5
Negative	26	86.7	48	96	74	92.5

(Maeda et al., 2004). Wiggins et al. (2006) reported that up to 30% of urethritis cases were due to a *C. trachomatis* infection, compared to only 4% in healthy control subjects.

C. trachomatis is a frequent pathogen associated with upper and lower urinary tract infections (El-Sayed et al., 2006). Chlamydial infection in the male urethra can be complicated by urethritis, epididymitis and prostatitis (Gdoura et al., 2008), Although the role of Chlamydia in the development of prostatitis is controversial, it is highly suggested that Chlamydia is considered to be an etiological agent, with incidences of up to 39.5% reported in patients with prostatitis (Cunningham and Beagley, 2008). This may explain the higher prevalence of C. *trachomatis* in the group of complicated UTI, as 50% of our patients suffer from prostatitis, 43% suffer from epididymitis and 30% had urethral stricture.

In this study, the diagnostic performance of different tests used for the detection of C. trachomatis infection among all studied patients was compared using PCR as a reference standard. Chlamyfast OIA test which detects chlamydial antigen in urine specimens was less sensitive but more specific than ImmunoComb assay that detect IgG antibodies in serum. ImmunoComb IgG had a higher sensitivity but lower specificity than ELISA IgG. Vasic et al. (2004) reported that Chlamyfast OIA test detected significant percentage of the Chlamydia infections in patients, which emphasizes the diagnostic importance in diagnosing STIs. Chernesky et al. (1998) concluded that supplemental to the *C. trachomatis* antigen detection, the easily performed ImmunoComb IgG is of great value in routine diagnosis of genital chlamydial infections. However, Bakardzhie et al (2011) noted a lack of specificity of the ImmunoComb IgG compared to PCR. In an Egyptian study performed by El-Sayed et al. (2006), ELISA IgG showed high level of specificity (100%) and low level of sensitivity (40%) when compared to the cell culture method. As sensitivity of the ELISA test is low, this can assist but cannot replace direct antigen detection or isolation of the organism by the culture technique (Kamel, 2013).

Mycoplasma pathogens have been discovered in the urogenital tract of patients suffering from PID, urethritis and other urinary tract diseases (Goulet et al., 1995). *M. hominis* is frequently recovered from the genitourinary tract. In addition, *M. hominis* causing bacterial vaginosis in women, has been proposed as one cause of NGU in men (Workowski and Berman, 2007).

In our study pathogenic level for *U. urealyticum* and or *M. hominis* was found in 42.5% of our patients; 30% were positive for *U. urealyticum* only and 7.5% were positive for *M. hominis* only and 5 % had mixed infection with both organisms. *U. urealyticum* and *M. hominis* were isolated in a non-pathogenic level from 5% and 2.5% of normal subjects respectively. Our results were comparable to those in a previous study from Turkey in which *U. urealyticum* was found in 48% of patients (24/50) with non-gonococcal urethritis. Thirteen (13) of these patients had only *U. urealyticum*, and the rest had mixed pathogenic organisms; 14% (7/50) *U. urealyticum* with *M. hominis*; 6% (3/50) *U. urealyticum* with *C. trachomatis* and 2% (1/50) *U. urealyticum* with *M. hominis* and *C. trachomatis* (Kılıç et al., 2004). Also Takahashi et al. (2006) in Japan detected *Mycoplasma* and *Ureaplasma* in FVU of young men at comparable rates of 4% for *M. hominis* and 12% for *U. urealyticum.* Whereas in Tunisia genital *M. huminis* and genital ureaplasmas affected a large number of infertile male patients, reaching 10.6% and 15.4% respectively (Gdoura et al., 2008).

Mycoplasma IST and Mycoplasma DUO showed high sensitivity compared to culture as gold standard method of detection. This finding was similar to previous studies which found no difference between Mycoplasma IST and culture regarding isolation of *Mycoplasma* (Kılıç et al., 2004) and considered Mycoplasma Duo assay as a simple and a sensitive method comparable to culture and

PCR for the detection of *Ureaplasma* spp. (Cheah et al., 2005; Ekiel et al., 2009). Biernat-Sudolska et al. (2013) reported that the sensitivity and specificity of the Mycoplasma IST test when compared to the culture method as a “gold standard” were determined as 91 and 96%, respectively, while the positive and negative predictive value of the commercial test was 27 and 99%, respectively for detection of *M. hominis* in clinical samples. Clegg et al. (1997) demonstrated sensitivity of 93% and specificity of 87% of Mycoplasma IST kit compared to the culture of *M. hominis*. These findings make the current tests suitable for use in diagnostic laboratories that do not currently test for *Ureaplasma* spp. or *Mycoplasma.*

It is interesting that 53% of our patients were suffering from urinary bilharziasis. This coexistence between urogenital schistosomiasis and sexually transmitted infection including *Chlamydia trachomatis* and *Mycoplasma genitalium* has been previously reported in the area of Madagascar, where S*chistosoma haematobium* is endemic. Authors in that study suggested that similar situation can be anticipated in most other schistosoma-endemic areas in sub-Saharan Africa presenting the two disease entities as a diagnostic challenge for the local health care providers in these areas (Leutscher et al., 2008).

Conclusion

Our study detects a high prevalence rate of *C. trachomatis*, *M. hominis* and *U. urealyticum* in cases with complicated UTI. Patients with urinary symptoms should be evaluated for three pathogens as common causes of NGU. Commercially available kits are simple and sensitive methods to use in laboratories which do not routinely test for these pathogens. The association between STD and *S. haematobium* requires further studies.

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