

DETECTION OF *MYCOPLASMA HOMINIS* IN PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

Mycoplasma hominis is one of the well known genital mycoplasma agent that has been recognized as potentially pathogenic species and associated with various infectious diseases in adult men and woman. The organism could cause pelvic inflammatory disease, postpartum, postabortion fever and pyelonephritis. The Aim of this prospective study was to determine the occurrence and prevalence of *Mycoplasma hominis* in patients with urinary tract infections, since they are usually not detected by routine microbiological examination of urine samples in laboratory department. For this purpose, Culture method was to be attempted as the current laboratory methods of choice for detection urogenital mycoplasmas infections. This study was designed as a case-control study in which a total of 100 patients with mean age of the of 42.78 ± 22.49 (95% CI = 39-49.17) in case group as well as a mean age of 48.18 ± 18.81 ((95% CI = 42.83-53.53) in the control group. The patients of both sexes were investigated for the presence of *M. hominis* and they were admitted to the urology department at asser central hospital in Abha (a city in southwest region of Saudi Arabia), during over a period of one year (February, 2013-March, 2014). A detailed history with specific urinary symptoms and signs was obtained as a result. These patients were divided into two groups: The first group (or case group) and the second group (or control group): The case group, included 50 symptomatic patients who had been admitted in hospital and they were examined for the presence of one or more of the following specific urinary symptoms and signs: Urinary frequency, dysuria, suprapubic/pelvic pain. Unlike the case group, the control group included 50 asymptomatic patients who were free of any specific urinary symptoms and they were matched by the first group for their age index. First voided urine specimens were tested using urine microscopy, culture for *M. hominis* was performed and the isolates were identified serologically by growth inhibition test (disc method). In the case group, 8 cases out of 50 and in control group 1 out 50 were positive for *M. hominis* infection. The incidence of *M. hominis* was higher and statistically significant among case symptomatic patients group (8/50, 16%) than control asymptomatic patients group patients (1/50, 2%). On applying tests of significance to the observed data for isolation of *Mycoplasma hominis* among cases and controls, the values were found to statistically significant. Chi-square test with Yate's correction [χ^2 4.396; p value 0.036] and the Fisher's exact test (2 tailed) [p value = 0.0309] indicated the relevance of the findings. The Relative Risk (RR) for the occurrence of *Mycoplasma hominis* infection was estimated to be 8 (95% C.I = 1.0386 -61.6235). The odds ratio was determined to be 0.1071 (95% CI = 0.129-0.892) and the risk ratio was 0.2063 (95% CI = 0.0322-1.3224). This is the first report study for the detection of *M. hominis* in patients with urinary tract infections performed in Saudi

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Arabia. Infection caused by *M. hominis* was associated with higher incidence rate in patients with symptomatic urinary tract diseases. Further work studies is needed for their rapid detection of these organism in urine samples using other diagnostic methods such as PCR. Further knowledge of the role and the effect of this pathogen in patients with urinary tract infections can be a prospective field of study.

Keywords: *Mycoplasma hominis*, Urinary Tract Infections, Urine Culture

1. INTRODUCTION

Urinary tract infections are one of the most common health problem and they are the most nosocomial infections affecting people of all ages. In the United States, they are account for nearly seven million office visits and a million emergency department visits and one hundred thousand hospitalizations every year (Foxman, 2002). They are considered to be the most common bacterial infections in older adults as well as being the most frequent source of bacteremia (Mulholland, 1990; Esposito *et al.*, 1980). The most common microbial aetiology of urinary infections has been regarded as well established and reasonably consistent and these included *Escherichia coli* which remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, Other Gram-negative bacteria causing urinary tract infections include, *Klebsiella*, *Enterobacter* and *Proteus* species, Gram-positive bacteria such as *Enterococci fecalis*, *Staphylococcus saprophyticus* and group-B *Streptococcus* which infrequently can cause uncomplicated cystitis and pyelonephritis (Johnson, 1991; Ronald, 2002).

However, there are other studies reported that *Mycoplasma hominis* as one of the agent that is believed to be associated with urinary tract infections (Thomsen, 1978a; 1978b; Sai-Yin and Kwok-Yung, 1995; Satoshi *et al.*, 2006; Totten *et al.*, 2008; Taylor-Robinson and Furr, 2010; Humburg *et al.*, 2011). This pathogen has been recognized as potentially genital mycoplasma species; it is associated with various infectious diseases in adult men and woman. The organism could cause pyelonephritis, pelvic inflammatory disease, postpartum and postabortion fever in addition to extragenital diseases including septicemia, joint infections, central nervous system infections, respiratory tract infections and wound infections particularly after surgery (Thomsen and Lindskov, 1979; Taylor-Robinson, 1996a; 1996b; Stellrecht *et al.*, 2004; Waites *et al.*, 2005; Totten *et al.*, 2008; Patel and Nyirjesy, 2010; Taylor-Robinson and Furr, 2010; Taylor-Robinson and Jensen, 2011). In newborn children, this pathogen can also can cause meningitis, pneumonia and abscess (Pereyre *et al.*, 2009). The

organism been been recovered from the upper urinary tract in patients with acute pyelonephritis (Thomsen, 1978a; 1978b; Sai-Yin and Kwok-Yung, 1995), women with lower urinary tract (Humburg *et al.*, 2011). It has also been recovered from the urine of asymptomatic patients (Satoshi *et al.*, 2006). Despite of this, prevalence of this pathogen in patients with urinary tract infections in most developing countries is still to date with limited studies.

In Saudi Arabia, to our knowledge, there has been no previous reported cases and information studies regarding the association of *M. hominis* with urinary tract infections and there is lack of national data available from the community based on the prevalence of *M. hominis* infections. As a result of this, the present prospective study was designed to determine the occurrence and prevalence rate of *Mycoplasma hominis* in patients with urinary tract infections.

2. MATERIALS AND METHODS

2.1. Patients

This study was designed as a case-control study in which a total of 100 patients with mean age of the of 42.78 ± 22.49 (95% CI = 39-49.17) in case group as well as a mean age of 48.18 ± 18.81 ((95% CI = 42.83-53.53) in the control group. The The patients of of both sexes were investigated for the presence of *M. hominis* and they were admitted to the urology department at asser central hospital in Abha (a city in southwest region of Saudi Arabia), during over a period of one year (February, 2013-March, 2014). A detailed history with specific urinary symptoms and signs was obtained as a result. These patients were divided into two groups: The first group (or case group) and the second group (or control group): The case group, included 50 symptomatic patients who had been admitted in hospital and they were examined for the presence of one or more of the following specific urinary symptoms and signs: Urinary frequency, dysuria, suprapubic/pelvic pain. Unlike the case group, the control group included 50 asymptomatic patients who were free of any specific urinary symptoms

and they were matched by the first group for their age index (**Table 1**). A detailed history with specific Urinary symptoms and signs was obtained.

2.2. Clinical Specimen and Culture of *Mycoplasma hominis*

The first voided urine specimens were collected in a sterile plastic screw-cap containers from all of the patients that were included in the study. The urine specimens were centrifuged at 2000 g for a period of 10 min and the pellet was tested microscopically in order to count the White Blood Cells (WBCs) per High Power Field (HPF). The pellet was then suspended in 1 mL of a mycoplasma broth medium and serially 10-fold dilutions in a 10 B broth medium, supplemented with phenol red and arginine (0.1%) out to 10^{-3} (0.1 mL⁻¹ original specimen suspension into 0.9 mL broth) are made in screw-capped tubes for detecting *Mycoplasma hominis* as described previously (Abdul-Wahab, 2010). After inoculation, the caps were tightened and all inoculated broth tubes were incubated aerobically at 37°C under atmospheric conditions and the cultures showing an alkaline pH shift and a colour change from yellow to pink, resulting from arginine hydrolysis by *M. hominis* were sub cultured in agar plates and incubated an aerobically in a candle jar at 37°C in a period of up to 7 days. The agar plates were checked daily under stereomicroscope for the characteristic fried-egg appearance of *Mycoplasma hominis* colonies. Isolates

were confirmed serologically as *M. hominis* by a Growth Inhibition test (GI), using the disc method as described by Clyde, (1983). Antisera of *M. hominis*-Type Strain-PG21, which was used in this test was supplied as lyophilized compound from Animal Health and Veterinary Laboratories Agency, Surrey, UK.

2.3. Statistical Analysis

The statistical analysis was performed using the statistical package for the social sciences version 13.0 (SPSS software, Inc., Chicago, IL, USA). The tests of significance used were the Chi-square test (χ^2) and the Fisher's exact test (2 tailed) and the results obtained with a p value of ≤ 0.05 were considered to be statistically significant.

3. RESULTS

One hundred 100 patients were studied. The mean age of the patients in case group was 42.78 ± 22.49 (95%CI = 39-49.17) and in control group was 48.18 ± 18.81 ((95%CI = 42.83-53.53 of both sexes (**Fig. 1**).

The incidence of *M. hominis* was higher and statistically significant among case symptomatic patients group (8/50, 16%) than control asymptomatic patients group patients (1/50, 2%). In the case group, 8 cases out of 50 and in control group 1 out 50 were positive for *M. hominis* as identified by growth inhibition test (disc method). Zones of inhibition less than 1.5 mm in diameter were considered negative (**Table 1 and Fig. 2**).

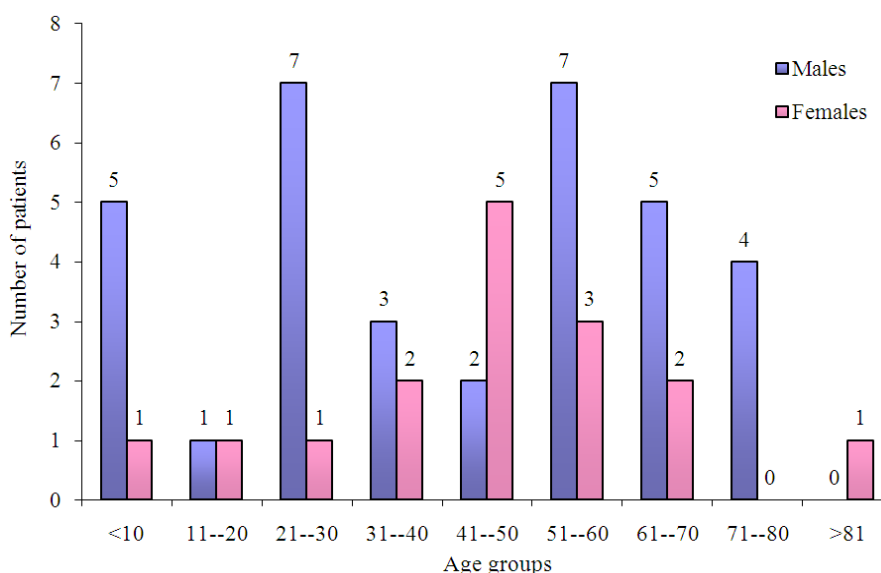


Fig. 1. Age and gender wise distribution in the patient group (n = 50)

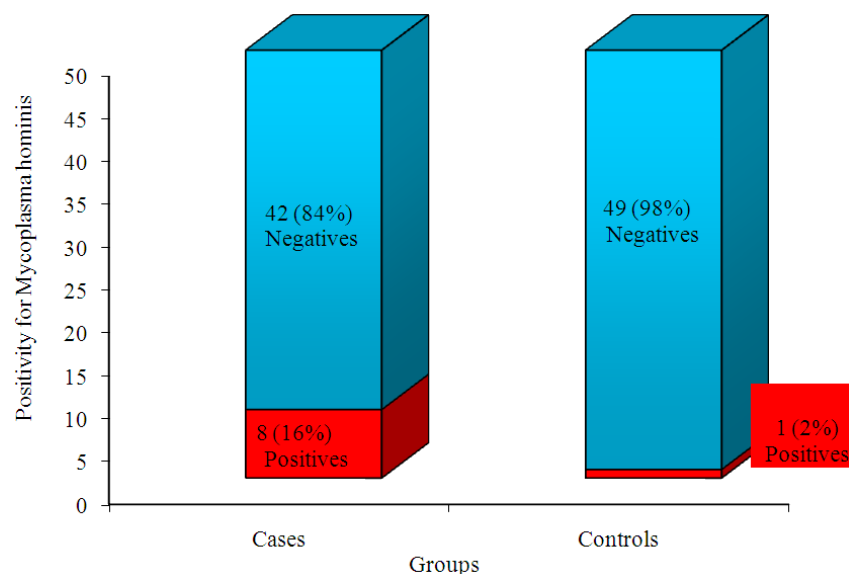


Fig. 2. Comparison of positivity between cases (n = 8/42) and Controls (n = 1/49) groups

Table 1. Distribution of patients and controls with and without specific urinary symptoms and positive *M hominis*

Patient group	Sex		Positive <i>M. hominis</i> N (%)	Total
	Males N (%)	Females		
Symptomatic ^a	34 (68)	16(32)	8 (16.0)	50
Asymptomatic	30 (60)	20(40)	1 (2.0)	50
Total	64 (64)	36(36)	9(9.0)	100

a: Each patient have one or more specific urinary symptoms (urinary frequency, dysuria, suprapubic/pelvic pain)

Table 2. Clinical manifestations in 8 symptomatic patients with positive *Mycoplasma hominis*

Patients	No. (%)
Total no.	8(100)
Mean Age	42.78 years
Sex	
Males	3 (38)
Females	5 (63)
Urine microscopy	
Numerous WBCs (>5/HPF)	3 (38)
Few WBCs (1-4/ HPF)	5 (63)
Urinary symptoms^a	
Urinary frequency	6(75)
Dysuria	5 (63)
Suprapubic/pelvic pain	3 (38)

^a: Most patients 6/8(75%) have at least two urinary symptoms

On applying tests of significance to the observed data for isolation of *M. hominis* among cases and controls, the values were found to statistically significant. Chi-square test with Yate's correction [χ^2 4.396; p value 0.036] and the Fisher's exact test (2 tailed) [p value= 0.0309]

indicated the relevance of the findings. The Relative Risk (RR) for the occurrence of *Mycoplasma hominis* infection was estimated to be 8 (95% C.I = 1.0386-61.6235). The odds ratio was determined to be 0.1071 (95% CI = 0.129-0.892) and the risk ratio was 0.2063 (95% CI = 0.0322-1.3224).

The incidence and characteristics of 8 symptomatic patients associated with positive *M. hominis* infections are shown in **Table 2**. Urinary frequency 6/8 (75%) and dysuria 5/8 (63%) were the most common symptoms among these patients.

4. DISCUSSION

The incidence and occurrence with clinical features that associated with *M. hominis* in patients with urinary tract infections in Saudi Arabia, is still to date with no information studies. To our knowledge, there has been no previous reported cases studies regarding the association of *M. hominis* with urinary tract infections; there is a lack of national data available from the

community based on the prevalence rate of *M. hominis* infections. One recent study reported the detection of *Mycoplasma hominis* from genital swabs of 0.8% of infertile females (Abdul-Wahab and Al Sunaidi, 2013). The present study indicated that *Mycoplasma hominis* is higher incident rate and statistically significant (8/50, 16%) among patients diagnosed with specific urinary symptoms and signs than in the asymptomatic control group. In the case group, 8 cases out of 50 and in the control group 1 out of 50 were positive for *M. hominis* infection. Occurrence of *Mycoplasma hominis* in urine with urinary tract infections have been described from other studies. In study (Humburg *et al.*, 2011), to evaluate and compare the accuracy of urethral swabs and urine specimens in the detection of *Mycoplasmas* in women with lower urinary tract symptoms during a urogynecological work-up, including cystometry, *Mycoplasma hominis* and *Ureaplasma urealyticum* as well as other microorganisms were detected by standard culture methods from 131 of 207 women reported with lower urinary tract symptoms and the other 76 patients was formed as controls group, of 207 women 50 (24.2%) had positive cultures for *Mycoplasmas*. The prevalence of *Mycoplasmas* in women with lower urinary tract symptoms (30.3%) was statistically significant and higher than in the group without lower urinary symptoms (14.5%) ($p = 0.011$). A study conducted by (Thomsen, 1978a; 1978b) resulted in *Mycoplasma hominis* being detected from the upper urinary tract in 7 out of a group of 80 infected patients with acute pyelonephritis and from 0 of 60 of the control patients group with noninfectious diseases of the urinary tract. On comparing the infected and control groups, the result was found to be statistically significant. In another case study of acute pyelonephritis with peritoneal signs and ascitis due to *Mycoplasma hominis* in an elderly male with no history of urinary tract instrumentation (Sai-Yin and Kwok-Yung, 1995), *Mycoplasma hominis* was reported from the culture of urine obtained during surgery from the renal pelvis of the patient with the right-sided acute pyelonephritis. *Mycoplasma hominis* is one of the well known genital mycoplasma agent that lives in the reproductive tract and it has been recognized as a potentially pathogenic species. It has been also been associated with various infectious diseases in adult men and women. Urinary tract infections caused by this pathogen in addition to *Chlymdia trachomatis* are less common than digestive tract bacterial causes that include mostly *Escherichia coli* and *Staphylococcus saprophyticus*, that cause most urinary tract infections. However, the exact statistics for both *Mycoplasma hominis* and *Chlymdia trachomatis* are unknown (West, 2006; Totten *et al.*, 2008).

In our study, the detection rate of urinary *Mycoplasma hominis* was low. This was similar to other previous reports (Thomsen, 1978a; 1978b; Thomsen and Lindskov, 1979). It might be due to the high sensitivity of mycoplasma (pH, temperature and materials present in culture media and clinical specimens; (Humburg *et al.*, 2011) as well as a loss of viability during specimen collection and/or transport, furthermore, mycoplasma antibody response is one of the criteria for diagnosing the disease and antibiotic inhibition is sometimes difficult to determine (Taylor-Robinson, 1996a; Amirmozafari *et al.*, 2009; Abdul-Wahab, 2010; Abdul-Wahab and Al Sunaidi, 2013). Further research studies are needed for rapid detection of the *Mycoplasma hominis* in patients with the urinary tract diseases using other diagnostic method such as PCR assay that was described as rapid alternatives to culture for the detection of *M. hominis* and *Ureaplasma* species (Cunningham *et al.*, 2013). It may will be necessary as it was found that the detection of genital mycoplasmas higher compared than culture method (Amirmozafari *et al.*, 2009). Nevertheless, detection of organisms through culture remains is considered as a gold standard method. It is also a worthy goal since quantitative results are more difficult to obtain with PCR and they do not permit the assessment of antibiotic sensitivity as well as other biological features (Taylor-Robinson, 1996a). Culture technique was found to be the most sensitive method for the isolation of both *M. hominis* and *U. urealyticum* (Abdul-Wahab, 2010).

In this study, the clinical manifestation in most symptomatic patients infected with *Mycoplasma hominis* (75%) have at least two urinary symptoms; urinary frequency and dysuria; there were three patients (38%) who had suprapubic/pelvic pain (Table 2). In addition to this, the presence of few pus cells (63%) has been detected more often than numerous pus cells (38%) in the urine of infected symptomatic patients (Table 2). The clinical symptoms in our patients will reveal that clinical diagnosis and confirmation of infection of *Mycoplasma hominis* will demand a series of full clinical and laboratory studies than the preset one. In the study performed by Thomsen, it has been described as cases of acute pyelonephritis and the presence of acute lumbar pain, pyuria and fever are associated with isolation of *M. hominis* from the upper urinary tract, only when there have been symptoms of acute infection. This was often in association with the development of a significant antibody response with obstruction or instrumentation of the urinary tract being considered as predisposing factors (Thomsen, 1978b; Thomsen and Lindskov, 1979; Taylor-Robinson, 1996b).

5. CONCLUSION

This is the first report study for the detection of *M. hominis* in patients with urinary tract infections performed in Saudi Arabia. Infection caused by *M. hominis* was associated with a higher incidence rate in patients with symptomatic urinary tract diseases. Further work studies are needed for their rapid detection of these organisms in urine samples using other diagnostic methods such as PCR. Further knowledge of the role and the effect of this pathogen in patients with urinary tract infections can be a prospective field of study.

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