Direct detection of *Mycoplasma* species from male with sterile Pyuria by Polymerase Chain Reaction

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الخلاصة

استهدفت الدراسة تحديد نسبة تواجد بكتريا المايكوبلازما في عينات البول القيحية سالبة المزرعة البكتيرية وتقييم تقنية سلسلة تفاعلات البلمرة eMyco PCR كتقنية حساسة وسريعة للتحري المباشر.

تم جمع 55 عينة من البول من مرضى يعانون البيلة القيحية سالبة المزرعة البكتيرية بالإضافة إلى 20 عينة بول من اشخاص اصحاء كمجموعة ضابطة حضروا الى المستشفى التعليمي في الديوانية والمختبرات الخاصة المختلفة في محافظة الديوانية وتم اختبارها بتقنية سلسلة تفاعلات البلمرة (PCR) لوجود المايكوبلازما .

كانت أعمار المرضى (20-57)سنة والذين كانوا يعانون من بول قيحي سالب المزرعة البكتيرية. والفئة العمرية (20-30 سنة) سجلت أعلى نسبة اصابة بالبكتريا 55/31 (56.36٪) . من مجموع 55 مريضا يعانون من البيلة القيحية العقيمة كان 21 (38٪) من المرضى قد اعطوا نتيجة موجبة لوجود المايكوبلازما SPP من خلال الكشف المباشر عن طريق PCR مع فروق معنوية (P <0.05). من ناحية أخرى كان 21/7 (12.7٪) من المرضى يعانون من التهاب المسالك البولية المتكررة، 11/4 (7.2٪) من المرضى يعانون من العقم الأولي والثانوي، في 3/3 (5.5٪) من المرضى يعانون من سرطان المثانة البولية، في حين 3 / 6 (5.5٪) من المرضى يعانون من التهاب البروستات المزمن و 14/4 (7.2٪) من المرضى لاتوجد لديهم مضاعفات اظهروا نتاج موجبة الى أنواع المايكوبلازما SPP

اظهر فحص eMyco PCR كونه تقنية سريعة وفعالة للكشف عن أنواع المايكوبلازما في عينات البول القيحية سالبة المزرعة البكتيرية . كما ان نسب الاصابة بالبكتريا تقل بتقدم العمر.

Abstract

To determine the percent of occurrence of *Mycoplasma* spp. in sterile pyuria and the evaluation of e-Myco PCR as rapid and sensitive technique for identification. Sterile pyuria urine samples collected from 55 patients attending to Al-Diwania Teaching Hospital and different private laboratories in Al-Diwania province were tested by polymerase chain reaction (PCR) for the presence of *Mycoplasma* spp. by a new kit called e-Myco in addition to 20 healthy individual as control group.

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In the present study the ages of patients with sterile pyuria were (20-57) year old and the age group (20-30 years) recorded the highest incidence of sterile pyuria 31/55 (56.37%). From total 55 sterile pyuria patients 21 (38%) patients were positive for *Mycoplasma* spp. by direct detection by PCR with significant difference (P<0.05). On the other hand 7/21(12.7%) patients were with recurrent urinary tract infection, 4/11 (7.2%) patients with primary and secondary infertility, in 3/3 (5.5%) patients with urinary bladder cancer, in 3/6 (5.5%) patients with chronic prostitis and 4/14 (7.2%) in non complications patients were positive for *Mycoplasma* spp.

PCR assay provide a rapid and effective measure to detect *Mycoplasma* spp. in sterile pyuria. The incidence of sterile pyuria decrease with progress in age.

Introduction

Sterile pyuria is a condition in which WBC are present in the urine(≥ 10/HPF)) without bacterial growth in a standard culture (1). Sterile pyuria is associated with a number of infective agents including viruses, fungi and a typical or fastidious organisms such as Mycoplasmas, Ureaplasmas and *Chlamydia trachomatis* and non- infectious conditions like of calculi, anatomic abnormalities, nephrocalcinosis and polycystic kidney disease (2).

Mycoplasma spp. are associated with various diseases of genitourinary tract in human (3), in male it causes urinary tract infections, chronic prostitis (4) , prostate cancer (5), epididymitis (6) non-gonococal urethritis (7) and male infertility (8,9), but they are usually not detected by routine microbiological diagnosis (10,11).

Molecular techniques such as polymerase chain reaction (PCR) are useful for the identification of microorganisms that are difficult to cultivate and for those that are grow slowly (12, 13,14).

In this study a new kit called e-Myco plus mycoplasma (Intron/ Korea) was used for direct detection of mycoplasmal growth within broth after incubation 48-72 hours. This kit used usually for the detection of mycoplasmas contamination in cell

culture by detection of 8 genus, 209 species of *Mycoplasma* including *M.pneumoniae*, *M.hominis*, *M.penetrans*, *M.fermentans* and *M.genitallium* due to used 16S rRNA . This kit contains all the components for the PCR reaction template DNA, distilled water also 8-methoxypsoraen (8-MOP)was added to prevent cross-contamination by PCR product due to its ability to intercalate into double-stranded nucleic acids and form a covalent inter-strand cross link after photo-activation.

Materials and methods

Specimens: This study was carried out on 55 patients whom diagnosed with sterile pyuria, 21 patients with recurrent urinary tract infection, 11 patients with primary and secondary infertility, 3 patients with urinary bladder cancer, 6 patients with chronic prostitis, and 14 without any complications. Their age ranged from 20 to 57 years and 20 healthy individual as control attending to Al-Diwania Teaching Hospital and private laboratories.

Urine samples from patients with symptoms (discharge, dysuria, pain, frequent urination), these samples were analyzed as follow: Routine urine examination, any sample containing more than 10 leukocytes was culture on MacConkey agar, Blood agar and Sabouraud agar to detect the presence of bacteria and Candida, samples negative for culture (no significant growth after 24 hours) were stored frozen at (-70℃) for 4-6 days until processing by PCR.

Extraction of DNA: DNA was extracted from urine specimens as follow: the urine specimens were thawed at room temperature, then 1 ml of urine was transferred to micro centrifuge and centrifuged at 13000 rpm for 30 minutes. Supernatant was discarded and the pellet was washed with 1 ml of phosphate buffer saline and centrifuged again at 13000 rpm for 15 minutes. Supernatant was discarded and DNA was extracted from pellet by washed in phosphate buffer solution and resuspended in 50 µl of distilled water. After boiling for 10 minutes, an aliquot of **7** µl was used directly in PCR experiments (15).

Detection and measurement of extracted DNA: The quality of the isolated DNA was determined by running 7 µl of each extracted sample and 3 µl of loading buffer on ethidium bromide-stained 1.5% agarose gel.

Amplification of 16S rRNA: amplification was performed in 20 µl of reaction mixture. Each tube of kit contain DNA polymerase, chemical stabilizer, loading buffer, dNTPs, Tris Hcl (pH 8.3), Kcl, Mgcl2, *Mycoplasma* primer set, internal control and 8-MOP. The extracted templet DNA 7 µl and distilled water was added. The reaction mixture were placed in thermal cycler as recommended by (Intron/Korea).

Table (1) Protocol of e-*Myco* plus Mycoplasma PCR Detection Kit.

	PCR	Temp.	Time
	Initial denaturation	94 c°	1 min
35 cycles	Denaturation	94 c°	30 sec
	Anneating	58 c°	20 sec
	extension	72 c°	1 min
	Final extension	72 c°	5 min

Detection of product: Aliquots of amplified samples 10 µl were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide

Results

In the present study the ages of patients with sterile pyuria were above 20 year old and the age group (20-30) recorded the highest incidence of sterile pyuria 31/55 (56.37%) compared with other age groups and the cases of sterile pyuria decrease with progress in age as shown in table (1)

Table (1) : Distribution of patients with sterile pyuria according to age

Age group	Number of patients with positive *Mycoplasma* spp	% of patients with positive *Mycoplasma* spp
20-29	31	56.37
30-39	16	29
40-49	6	10.9
50-57	2	3.64
Total	55	100

Results were represented in figure (1) showed that from total 55 sterile pyuria patients *Mycoplasma* spp. were detected directly by PCR in 7/21(12.7%) recurrent urinary tract infection, 4/11 (7.2%) primary and secondary infertility, 3/3 (5.5%) in complications patients.

Of the 55 sterile pyuria patients studied 21 (38%) were positive for *Mycoplasma* spp. with significant differences ($pP < 0.05$), while *Mycoplasma* spp. were detected in 2/20 (10%) of healthy control non significant differences($P < 0.05$) table (2).

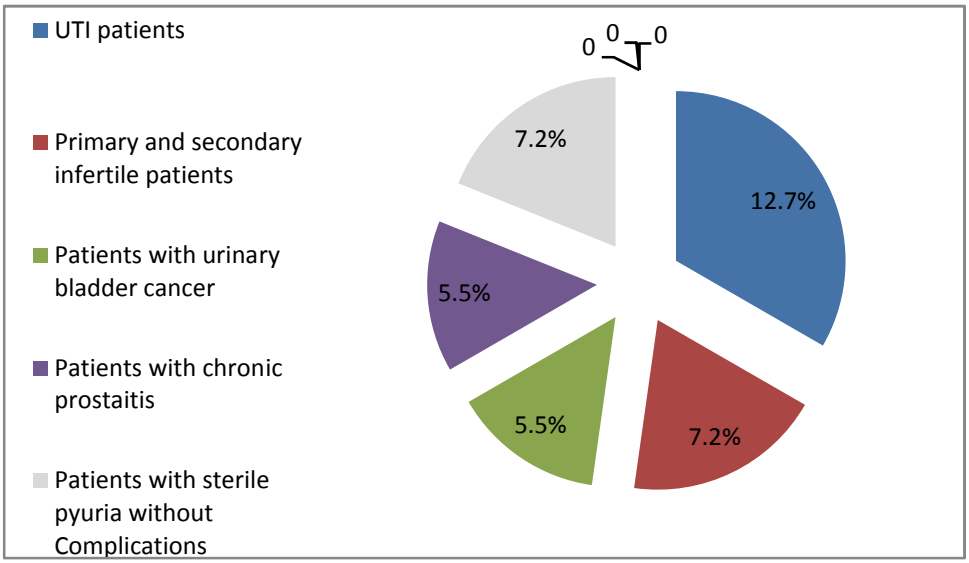

Figure (1) Positive detection of *Mycoplasma* spp. by PCR from patients with sterile pyuria

Table (2) Results of *Mycoplasma* spp. by PCR

Results of PCR	Patients with sterile pyuria N= 55		Control healthy patients N= 20	
	Number	%	Number	%
Positive results of *Mycoplasma SPP* by PCR	21	38	2	10
Negative results of *Mycoplasma SPP* by PCR	34	62	18	90
Statistical analysis	Significant P<0.05		Non significant	

A photograph of electrophoresis based on bromide-stained agarose for PCR-amplified products from *Mycoplasma* spp .is presented in figure (2).

Figure (2) indicated that the presence of 160bp band (internal control) only, that means no *Mycoplasma* growth, while the presence of 160bp and 260 bp band (internal and target band) means the growth of *Mycoplasma* was low, while if 160, 260, and 570 bp it means the growth of *Mycoplasma* were high.

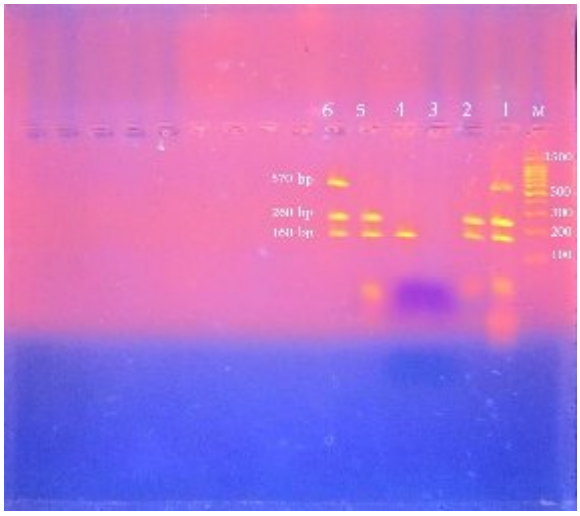

Figure (2) Electrophoretic analysis of PCR products for *Mycoplasma* spp. 16S rRNA from urine samples.

M 100 bp size marker, lane(1,6) positve patient samples, lane (2,4) negative patient samples, lane 4 negative control (distilled water).

Discussion

Mycoplasma spp. are common pathogen in male urogenital tract and considered as one of etiological factor for sterile pyuria. However, it is commonly found in healthy people . Many studies have shown that there is a higher rate of sterile pyuria in male with *Mycoplasma* spp. infection in compared to those who did not have *Mycoplasma* spp. (16).

In this study (12.7%) of patients with recurrent urinary tract infection due to ability of *Mycoplasma* spp. to cause many infections in genitourinary tract (17).

In this study, we found a high number of infertile men (7.2%) with *Mycoplasma* spp. may be due to ability of these microorganisms to deterioration of spermatogenesis, impairment of sperm, function or obstruction of seminal tract (18). *In vittru* studies show that overnight incubation of sperm with Mycoplasma spp. impairs sperm physiology, especially it has negative influence on sperm motility, morphology and vitality (19).

Also *Mycoplasma* spp. are present in (5.5%) pateints with bladder cancer. Recent epidimiologic, genetic and molecular studies suggest that infection and inflamation due to *Mycoplasma* spp. initiate certain cancers (20,21).

This study line with other study In Japan, *Maedo et al*. (2004) reported that the prevalence of *M. genitalium* only was 17.0% in first-voided urine specimens from153 male patients with NGU (22) however, Our results are considerably lower than reported in Japan, China and Austria (23,24).

The difference between our results and those reported by previous studies may be due to the difference in the populations studied and the prevalence of the microorganism in the study area.

Finally, PCR assay by used e-myco Mycoplasma kit provide a rapid and effective measure to detect fastidious and atypical microorganisms in sterile pyuria cases which is useful for identification of etiological agents and the consequent management and treatment of patients.

Conclusion

Significant numbers of *Mycoplasma* spp. which are not screened for routine examinations of urine samples were present in sterile pyuria cases.

e-Myco PCR assay is a rapid and sensitive technique for the detection of *Mycoplasma* spp.

Recommendations

It is recommended to use the e-Myco PCR technique as a routine test for diagnosis of *Mycoplasma* of sterile Pyuria in our clinical laboratories.

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