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# The Role of Multiplex Polymerase Chain Reaction in Detecting Etiological Causes of Bacterial Prostatitis Associated Benign Prostatic Hyperplasia

***Bramastha A Rosadi[1], Tjokorda GB Mahadewa[2], Gede Wirya K Duarsa[3]***
[1]Department of Surgery Udayana University, Sanglah General Hospital Denpasar Bali
[2]Department of Neuro Surgery Udayana University, Sanglah General Hospital Denpasar Bali
[3]Department of Urology Udayana University, Sanglah General Hospital Denpasar Bali
Correspondence: Tel (+62) 361227 911. E-mail: rosadie.bramastha@gmail.com

**Background:** Benign Prostatic Hyperplasia (BPH) has been correlated with chronic prostatitis according recent study. Chronic pelvic pain is the chief complain of BPH followed by prostatitis. The gold standard of the etiological diagnosis is urine culture, but the negativity rate is still high. Multiplex polymerase chain reaction (PCR) as a diagnostic tool in search of etiological causes could identify microorganism on DNA level. This research aims to find out the role of multiplex polymerase chain reaction as diagnostic tools on prostatitis patients. **Material and Method:** A total of 12 samples collected during the TURP procedure in Sanglah General Hospital Denpasar – Bali from February until May 2015. All of the samples has been diagnosed prostatitis clinically and perform urine culture test. The prostate specimen taken was sent to the Pathological anatomy for histopathology diagnostic and underwent multiplex PCR for etiologic diagnostic. **Result:** 12 samples have been declared as prostatitis based on histopathology examination, and then were analyzed using multiplex PCR. 10 samples were positive (6 were *E. coli*, 2 were *C. trachomatis*, the rest were *N. gonorrhea* and *P. aeruginosa*). The urine culture revealed 9 positive, within the result 6 were E. coli, and the others were *P. aeruginosa, M. morganii* and *A. haemolyticus*. **Conclusion:** In prostatitis patient, the etiological diagnostic was important. Multiplex PCR as diagnostic tools could detect the microorganism on a negative urine culture. The combination of the urine culture test and multiplex PCR revealed a better result on etiologic diagnosis which leads to a better management of the disease.

**Keywords:** Prostatitis, Urine Culture, Multiplex Polymerase Chain Reaction

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## INTRODUCTION

*Benign Prostatic Hyperplasia* (BPH) regard as the most common of the degenerative diseases on male and followed by lower urinary tract symptoms. The symptoms although not life threatening but troublesome, this symptom significantly affect the quality of life of the patients. Histologically on group of 41 – 50 years old patient, 20% has been diagnosed as BPH, at the age of 51 – 60 years old the prevalence was higher up until 50% and the highest incidence on the age of 80's within 90%. In the world, the incidence in between 0,5 – 1,5 / 100.000 but the mortality is very low[1].

The risk factor of BPH were unclear, newest research leads to inflammatory diseases, genetic predisposition and races. Approximately 50 % male under the age of 50's with BPH has a congenital factor with the probability of autosomal dominance, and the risk factor are 4 times fold[1,2]. The AUA guidelines state that 90% of male on BPH within age 45 – 80 years old has lower urinary tract symptoms[3]. These symptoms were the chief complaint of the patients and commonly followed by chronic prostatitis. 45 – 98 % prostate specimen post operative were histopathology diagnosed as chronic prostatitis [4].

The prostatitis was combination of infection, chronic pelvic pain, and asymptomatic inflammation. Prostatitis commonly under diagnosed, and accidentally revealed on post operative histopathology examination [5]. Prevalence prostatitis in the world were vary, from 2% until 13 %[5,6,7,8,9], In USA, approximately 5 – 12 million peoples were diagnosed as prostatitis[10]. The cost to treat prostatitis were abundant, there were 8.021.396 prostatitis cases and costing USD 3017 – 6534 per patient with the sum of USD 84 million[6,10,11]. The etiological microorganism of prostatitis was *E. Coli,*

*Pseudomonas Spp., Chlamydia trachomatis, Neisseria gonorrhea, Ureaplasma urealyiticum* and *Mycoplasma genitalium*[12]. There was outcome controversy of the operative procedure of BPH patient with prostatitis. Jonas et al state that BPH patient with prostatitis still had a LUTS after the surgery[9,10], on the contrary Yalcinkaya et al state that there were no significant differences of the cases[4].

Diagnostic procedure of prostatitis was based on 4 tube examination and the etiological diagnostic tools were urine culture[5]. The urine culture has many limitation and revealed only *P. aeruginosa* and *E. coli*. If the culture failed to detect the microorganism, the diagnosis was straight to type III prostatitis and treated by broad spectrum antibiotics[5]. The invention of DNA hybridization and nucleic acid amplification could detect *N. gonorrhea*, the recent discovery of polymerase chain reaction (PCR) was a bright light on detecting etiological diagnosis of prostatitis[12,13].

PCR as a diagnostic tool was a one step examination, using one tube sample that simultaneously detect vary of microorganism including viral infection. PCR method were more sensitive and cost effective compared to the others traditional method[13]. This research aims to find out the role of multiplex polymerase chain reaction (mPCR) as diagnostic tools on prostatitis patients.

## MATERIAL AND METHODS

Pure cultures of submitted strains from *E. coli, C. trachomatis, N. gonorrhea, U. urealyiticum, M. genitalium*, *Corynebacterium* spp. and *P. aeruginosa* were maintained on Luria agar slants at 4°C for working purposes, while stock cultures were stored in 15% glycerol at -80°C. Briefly, 10 ml aliquots of cells were centrifuged at 12,000×g for 3 min at 4°C and subjected to DNA extraction. The DNA was then purified and recovered in 200 µl of elution buffer followed by dilution in 1 ml of phosphate-buffered saline (PBS) containing 1 mg/ml chenodeoxycholate and stored at -80°C until further use. Upon thawing, the specimens were divided into aliquots for assessment and comparison to cloned markers and a 3.0 kb pGEM-T easy vector to ensure the DNA had originated from the correct bacterial species.

A total of 12 consecutive samples from February to May 2015 that has been diagnosed prostatitis clinically collected, midstream urine was taken. The urine specimens were collected into sterile 20 ml conical tubes on-site and taken to the microbiology division and plant to blood sheep agar media for culture. Positive culture regard as bacterial growth more than 100.000 colony forming unit.

All 12 samples underwent operative procedure, during the TURP procedure in Sanglah General Hospital Denpasar – Bali the prostate specimen taken from the first resection and sent to the pathological anatomy for histopathology diagnostic and underwent multiplex PCR for etiologic diagnostic. The prostate specimen stores on Eppendorf tube and sent directly to preservation at the temperature of -80°C until further use.

The specimen undergo centrifugation at 3,000 rpm for 20 minutes, the pellets were washed in 5 ml of sterile water and then centrifuged again at 2,000 rpm for 10 minutes. The DNA in the pellet was then extracted by three rounds of resuspension in 30% polyethylene glycol and 3 ml NaCl, followed by incubation on ice for 30 min, and centrifugation at room temperature for 5 min at 15,000 rpm. The final pellet was frozen at -20°C. To confirm the pathogenic bacterial infection, an initial 16S rDNA consensus primer from the causative bacteria in all specimens was investigated via PCR and automated sequencing analysis. The amplified PCR products were electrophoresed on 1.5% agarose gels containing ethidium bromide in buffer and were photographed with an Image Master.

## RESULT

To establish the PCR conditions which permit the amplification of sequences from *E. Coli, C. trachomatis, N. gonorrhea, U. urealyiticum, M. genitalium, Corynebacterium spp.* and *P. aeruginosa*, the laboratory strains of these organisms were cultured, followed by extracting their DNA and performing PCR assays. The optimal results were obtained at an annealing temperature of 54°C or 56°C. These temperatures allowed for the amplification of the seven specific bands along with reduced levels of non-specific amplification products. These assays revealed that the 54°C annealing temperature efficiently generated all products.

The mean age of the sample was 68,8 ± 6,9 years old within a range of 60 until 78 years old. All of the sample were married and has no history of diabetes, Tb infection and another systemic inflammation nor malignancy. The characteristic of the sample was presented on table 1.

Table 1. Subject Characteristic

| **Subject Characteristic** | | **n = 12** |
|---|---|---|
| Age | | |
| | Mean± SD | 68, 8 ± 6,9 |
| | Min | 60 |
| | Max | 79 |
| Educational Status | | |
| | Elementary | 6 (50,0) |
| | Junior High | 1 (8,3) |
| | High | 2 (16,7) |
| | Post Graduate | 3 (25,0) |
| Marital Status | | |
| | Marriage | 12 (100,0) |
| Occupation | | |
| | State Government | 2 (16,7) |

| | |
|---|---|
| Farmer | 7 (58,3) |
| Private | 3 (25,0) |

All of the samples were analyzed using multiplex PCR that has been establishing using the previous state methods. 10 samples (83,3%) were positive (6 were *E. coli*, 2 were *C. trachomatis*, the rest were *N. gonorrhea* and *P. aeruginosa*). The urine culture revealed 9 positive (70%), within the result 6 were *E. coli*, and the others were *P. aeruginosa, M. morganii* and *A. haemolyticus*. There was interesting result from the examination, we found concordance (agreement) of 58,3% from the 2 examination and 41,7% disconcordance. Both of the procedure successfully detect *E. coli and P. aeruginosa.* The agreement between 2 examinations presented on Table 2 and mapping of the result were presented on Table 3.

Table 2. Agreement of the Procedure

| Urine | PCR | | |
|---|---|---|---|
| | Negative | Positive | |
| Negative | 0 (0,0) | 3 (25,0) | *Disconcordance* 41,7 % and |
| Positive | 2 (16,7) | 7 (58,3) | *Concordance* 58,3 % |

Table 3. Result of Procedure

| Subject | Urine Result | PCR Result | Microorganism |
|---|---|---|---|
| 1 | Positive | Positive | *Escherichia coli* |
| 2 | Negative | Positive | *Chlamydia trachomatis* |
| 3 | Positive | Positive | *Escherichia coli* |
| 4 | Positive | Positive | *Pseudomonas aeruginosa* |
| 5 | Positive | Positive | *Escherichia coli* |
| 6 | Positive | Positive | *Escherichia coli* |
| 7 | Positive | Positive | *Escherichia coli* |
| 8 | Positive | Negative | *Morganellamorganii* |
| 9 | Positive | Positive | *Escherichia coli* |
| 10 | Negative | Positive | *Chlamydia trachomatis* |
| 11 | Negative | Positive | *Neisseria gonorrhea* |
| 12 | Positive | Negative | *Acinetobacterhaemolyticus* |

## DISCUSSION

The diagnosis of prostatitis has traditionally been largely dependent on methods such as culture, enzyme immunoassay, DNA hybridization, antibody staining as well as the examination of rectal and prostatic fluids for the signs of inflammation and infection[5,6,12,13]. The previous methods did not profound as standard diagnostic studies, mPCR described as being a more sensitive detector of pathogenic bacteria compared to traditional tests involved in the detection process[13,14].

This study describes a rapid one step method for the detection of pathogenic bacteria in prostatitis associated BPH patients by mPCR. We have tested the 12 specimens by urine culture and found 58,3% concordance with the mPCR results. Moreover, mPCR had higher positivity rate (83,3%) compared to urine culture (70%). The mPCR supremely detect the STD microorganism that failed detected by urine culture. The downside of the mPCR were mainly based on primary DNA, consequently special attention must be paid in the primer design[13,14]. Moreover the amplification conditions must be optimized since the reactions can be affected by several factors, including temperature and duration of DNA denaturation, primer annealing, extension as well as the concentrations of the polymerase, MgCl2 and primers[13,15-17]. This comes up to the negative result like *Morganella morganii* and *Acinetobacter haemolyticus.* We don't have the primary DNA of both of the microorganism. At present, the universal features of mPCRs lead to highly reliable, sensitive and specific multiple target gene detection is not known. Nevertheless, our sequencing data showed that our mPCR method yields highly reliable, pathogen-specific information

## CONCLUSION

In prostatitis associated benign prostate hyperplasia patient, the etiological diagnostic was important. Multiplex PCR as diagnostic tools could detect the microorganism on a negative urine culture. The combination of the urine culture test and multiplex PCR revealed a better result on etiologic diagnosis which leads to a better management of the disease.

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