Molecular Diagnosis of Sexually Transmitted Infections (STI) in Symptomatic Women of Puducherry by a Commercial Real Time Multiplex PCR, FTD Urethritis Plus - A Preliminary Report

Selvaraj Stephen1, Jothimani Pradeep1, Chinnadurai Rajkumar2, Rupal Samal3, Ashwini Vishalakshi3, Seetesh Ghose3, Sendhil Coumary3, Syed Habebullah3 and Balanehru Subramanian2

Department of Microbiology1, 3Obstetrics & Gynaecology, Mahatma Gandhi Medical College & Research Institute and 2Central Interdisciplinary Research Facility (CIDRF), Sri Balaji Vidyapeeth (SBV), Deemed-to-be-University, Puducherry - 607402, India.

Abstract

This study relates to the prevalence of common non-viral sexually transmitted infections in symptomatic women of Puducherry. Endocervical swabs were collected from 41 symptomatic women attending as outpatients in OB&G department of Mahatma Gandhi Medical College Hospital, Puducherry, using special swabs (eSwab) and transported to Microbiology department. DNA was extracted from these swabs and real time multiplex PCR was performed using FTD Urethritis plus in Rotor-Gene Q series 3000 (QIAGEN, Germany). The kit targets seven pathogens, viz., Chlamydia trachomatis, Neisseria gonorrhoea, Trichomonas vaginalis, Mycoplasma hominis, M. genitalium, Ureaplasma urealyticum and U. parvum. Among 41 symptomatic women, 17 were positive for single pathogen (U.parvum-11, U.urealyticum-4 and M.hominis-2) and six patients were positive for two or more pathogens. Interestingly, none of the patients were positive for genital chlamydiasis and gonorrhea. The significance of U.parvum as a potential pathogen needs to be confirmed. There was no statistical difference between the positive and negative patients among the different age groups.

Keywords: Chlamydia trachomatis, Neisseria gonorrhoea, Trichomonas vaginalis, Mycoplasma hominis, M. genitalium, Ureaplasma.
INTRODUCTION

Sexually transmitted diseases (STD) are considered as a stigma in third world countries. They have an adverse impact on the individual, community and public health of the nation1-4. Among the non-viral causes of Sexually Transmissible Infections (STI), syphilis and gonorrhoea are two major diseases, closely followed by genital chlamydiasis5-9. Syphilis is diagnosed on the basis of clinical findings and supported by serological test (VDRL/RPR). Either VDRL or RPR (VDRL/RPR) is being carried out in most of the laboratories in India. Chlamydia trachomatis requires chick embryo yolk sac or cell lines for isolation, Neisseria gonorrhoeae needs special culture medium and both organisms are also detectable by antigenic tests7-11. Other bacterial pathogens like Mycoplasma hominis, M. genitalium, Ureaplasma urealyticum and U. parvum grow on specialized media / Polymerase chain reaction (PCR) and their microscopic examination is also different from others11-16. The protozoan parasite Trichomonas vaginalis can be identified by microscopic examination17-20. Infections caused by the above seven pathogens have been reported throughout the world and in different parts of India2-4,6-8,10-20. A commercial multiplex real time PCR kit – FTD Urethritis Plus has the potential to detect the above seven pathogens21-23. This kit has been recently introduced in India and yet to be validated. According Pichon et al., eSwab® sampling seem to improve the pre-analytic phase compared to the dry swab24. Our preliminary findings on the performance of this kit are shared here.

MATERIALS AND METHODS

This preliminary research was carried out during the period of January 2019 – July 2019 in the departments of Obstetrics & Gynaecology, Microbiology and Central Interdisciplinary Research Facility (CIDRF), Mahatma Gandhi Medical College & Research Institute, Puducherry. Our Institutional Human Ethics Committee (IHEC) has approved this research work (Approval Number: Faculty Project/2018/09/17 dated 24/09/2018). Our Institutional Human Ethics Committee has forbidden us from collecting endocervical swabs from pregnant women for Research purposes.

Inclusion criteria

Patients presenting to Gynecology OPD with abnormal vaginal discharge, burning micturition, cervical fragility, cervical erosions, genital ulcers and lower abdominal pain.

Exclusion criteria

1. Antenatal women and also the patients who have received macrolides/ tetracyclines during the past 10 days because patients on antibiotics would have been cleared of STI infections and therefore they may give false negative results.
2. Women with cancerous or precancerous lesion of the cervix detected by clinical examination and/or Pap smear may bleed profusely while collecting endocervical swabs.
3. Age < 18 years or > 45 years. Patients older than 45 years are in the category of women with Menopause and the gynecologist may face difficulties in collecting the endocervical swabs.

Copan Liquid Amies Elution Swab (eSwab) Brescia, Italy were used. Endocervical Swabs were collected in the OPD by the Gynecologist on duty from 41 non-pregnant women who presented themselves during the above period with one or more signs and symptoms mentioned in the inclusion criteria. Swabs were transported to Microbiology laboratory within the same day and stored in the refrigerator. DNA was extracted from these swabs using QIAGEN DNA Blood Mini Kit (QIAGEN, Germany). The DNA extracts were kept frozen at -80°C and tested within 10 days in a Real Time PCR machine (Rotor-Gene Q series 3000, QIAGEN, Germany). A commercial multiplex real time PCR kit – FTD Urethritis Plus kit (Fast Track Diagnostics, Luxembourg) was used in the assay and Ct values less than 33.0 were considered as positive as per the technical brochure of the kit. Therefore Ct values above 33.0 were retested. The samples are analyzed in two different panels. Panel I has URScreen - N. gonorrhoeae, M. genitalium, C. trachomatis and Internal control – Murine Cytomegalo Virus (MCMV). Panel II has UTriMyc - T. vaginalis, U. urealyticum, U. parvum and M. hominis. Positive control for the respective targets and a common negative control for all seven pathogens were included in each run. We
have made use of two positive controls provided in the kit viz., Panel I (URScreen) has positive control for - *N. gonorrhoeae*, *M. genitalium* and *C. trachomatis*. Panel II (UTriMyc) has positive controls for - *T. vaginalis*, *U. urealyticum*, *U. parvum* and *M. hominis*. The negative control is common for all seven pathogens. Panel I has also contain an Internal control – Murine Cytomegalovirus (MCMV) to ensure the extraction of DNA and its quality are satisfactory. The PCR assay was performed in strict compliance with instructions given in the technical brochure: PCR reaction was performed with 25.0µl of total volume which comprises of 12.5µl Buffer, 1.5µl of primer probe mix, 1.0 µl of an enzyme and finally 10.0µl of extracted DNA was added to this PCR master mix. Cyclic conditions for Multiplex Real Time PCR were as follows: Holding Temperature at 50°C for 15 mins, initial denaturation at 94°C for 1 min followed by 40 cycles of annealing and extension at 94°C for 8 secs and 60°C for 1 min respectively.

**Statistical Analysis**

Percentages were calculated for categorical variables. Mean and Standard deviations are calculated with 95% confidence interval for the age of the patients. T-test was performed for positive and negative patients among different age groups. P value of d”0.05 was considered to be statistically significant. Statistical analysis was carried out using Graph Pad Quick Calcs (GraphPad Software, San Diego, CA).

**RESULTS**

Mean and SD age of the patients was 34.04 (SD=7.55) with 95% confidence interval (30.7 - 36.3). For positive patients, Mean and SD was 32.8 (SD=6.50) (95% C.I. 30.2 - 35.4) and for the negative patients 34.7 (SD=8.3) (95% C.I. 31.1 - 38.8). Seventeen patients had single pathogen isolation (*U. parvum* – 11, *U. urealyticum* – 4 and *M. hominis* – 2). Three patients had dual infections (*U. parvum* + *M. hominis* – 2 and *U. parvum* + *T. vaginalis* – 1). Another three patients showed evidence of infection with three pathogens viz., *U. parvum* + *U. urealyticum* + *M. hominis*. Ct values ranged from 19.00 to 32.00. Statistical significance was not observed between the multiplex qPCR positive and negative patients among the different age groups and the t-value is -0.87. (p=0.391217).

**DISCUSSION**

Four different commercial kits are available globally for diagnosing infection by the seven STI pathogens. These kits have been validated using appropriate controls. They include Seegene Allplex24-25, Anyplex24,26-28, Amplicor29 and Amplisens30. Regarding the Fast track FTD Urethritis Plus kit, this has been validated by overseas workers and found to be satisfactory21-23. These authors have compared the results of Multiplex qPCR with the gold standard culture for *C. trachomatis*, *N. gonorrhoea*, Mycoplasma and Ureaplasma. Microscopy was done for confirming *T. vaginalis*31-32. None of these five kits are available in India at present. Fast track FTD Urethritis Plus kit was imported and used by us for this preliminary research purposes. This kit has picked up 59.1% positivity among 41 symptomatic non-pregnant women of Pondicherry. It is interesting to note that none of our patients were positive for gonorrhoea or genital chlamydiasis. However, different authors across India reported a prevalence rate ranging from 6.8% - 7.7%7,8,31 for gonorrhoea and 1.6% - 23.0%30-32 for *C. trachomatis*. From Pondicherry itself 0%11 and 2.3%6 positivity was reported for CT and NG respectively by detection of their corresponding antigen in endocervical swabs. Regarding the role of other pathogens like Mycoplasma and Ureaplasma, Indian reports are few and far between. Saigal et al from Delhi reported detection of MG, MH, CT and UU in PCR, with the prevalence of 1.2%, 5.4%, 6% and 15.2% of symptomatic women respectively10. The role of Ureaplasma as a STI pathogen needs further debate and research since these organisms are also isolated from non-symptomatic healthy women and men15,29,30. Since FTD Urethritis Plus kit is for research use only (RUO) as indicated by the manufacturers of the kit, we have advised our Gynecologists to correlate our PCR results with clinical conditions. Our earlier finding of 0% positivity for *C. trachomatis* antigen in the endocervical samples of the women of Pondicherry in 2010-201211 is similar to the present scenario.

**CONCLUSION**

A major limitation of the study was the small number of samples (n=41). This is a time bound study over a period of seven months.
Convenient Sampling was carried out. Consecutive patients fulfilling the inclusion and exclusion criteria were enrolled in the study. A large number of symptomatic as well as asymptomatic women may be included in future studies for exploring the prevalence of the seven STI pathogens. This may be taken up as a follow-up research to get a better understanding of the burden of these pathogens in the women of Puducherry.

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CONFLICT OF INTEREST

The author declares that there are no conflict of interest.

AUTHORS' CONTRIBUTION

SS, SG, BS and RS did research conception and design work. PJ and RC performed the experiments. AV, SC and SH completed data acquisition. SS, SG, RS, PJ, RC, AV, SC and SH did data analysis and interpretations. SS and PJ drafted the manuscript. SS, SG, RS, PJ, RC, AV, SC and SH completed the critical revision of manuscript. All authors approved the final version of manuscript for publication.

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DATA AVAILABILITY

Data is available in the Department of Microbiology and Central Interdisciplinary Research Facility, all datasets could be provided if necessary to anyone on reasonable request.

ETHICS STATEMENT

This research work has been approved by Institutional Human Ethics Committee (IHEC) (Approval Number: Faculty Project/2018/09/17 dated 24/09/2018).

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