Stephen *et al. J Pure Appl Microbiol,* **13**(**3**), 1791-1796 | September 2019
Article 5801 | https://doi.org/10.22207/JPAM.13.3.55

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

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 Stephen[1], Jothimani Pradeep[1], Chinnadurai Rajkumar[2], Rupal Samal[3], Ashwini Vishalakshi[3], Seetesh Ghose[3], Sendhil Coumary[3], Syed Habebullah[3] and Balanehru Subramanian[2]

Department of Microbiology[1], [3]Obstetrics & Gynaecology, Mahatma Gandhi Medical College & Research Institute and [2]Central 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.*

***Correspondence:** stephens4950@gmail.com; +919894383368

(Received: 01 August 2019; accepted: 08 September 2019)

Citation: Selvaraj Stephen, Jothimani Pradeep, Chinnadurai Rajkumar, Rupal Samal, Ashwini Vishalakshi, Seetesh Ghose, Sendhil Coumary, Syed Habebullah and Balanehru Subramanian, 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, *J Pure Appl Microbiol*., 2019; **13**(**3**): 1791-1796. https://doi.org/10.22207/JPAM.13.3.55

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 nation[1-4]. Among the non-viral causes of Sexually Transmissible Infections (STI), syphilis and gonorrhoea are two major diseases, closely followed by genital chlamydiasis[5-9]. 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 tests[7-11]. 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 others[11-16]. The protozoan parasite *Trichomonas vaginalis* can be identified by microscopic examination[17-20]. Infections caused by the above seven pathogens have been reported throughout the world and in different parts of India[2-4,6-8,10-20]. A commercial multiplex real time PCR kit – FTD Urethritis Plus has the potential to detect the above seven pathogens[21-23]. 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 swab[24]. 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 Cytomegalo Virus (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 Allplex[24-25], Anyplex[24,26-28], Amplicor[29] and Amplisens[30]. Regarding the Fast track FTD Urethritis Plus kit, this has been validated by overseas workers and found to be satisfactory[21-23]. 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*[17-23]. 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%[7]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 respectively[10]. 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 men[15,29,30]. 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 2010-2012[11] 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.

ACKNOWLEDGMENTS

The authors acknowledge with thanks the financial support and encouragement provided by the Chairman, Vice-Chancellor, Dean of Faculty, Dean Research and Dean, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth (SBV) Deemed-to be- University for providing SBV Faculty Research Fund to the First author (SS).

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.

FUNDING

The first author (SS) has received Faculty Research Fund from Sri Balaji Vidyapeeth (SBV), Deemed-to-be-University. Grant No: Dean (R) Res. grants-Seed money – Faculty/2018/445 dated: 19.12.2018.

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).

REFERENCES

1. World Health Organization (WHO). Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections. Overviews and Estimates. Geneva, Switzerland: WHO; 2001.
2. Hemalatha R, Ramalaxmi BA, Swetha GK, Rao DM, Charyulu S, Kumar D. Nutritional status, bacterial vaginosis and cervical colonization in women living in an urban slum in India. *Int. J. Nutr. Metab.*, 2012; **4**: 77-82.
3. Preethi V, Mandal J, Halder A, Parija SC. Trichomoniasis: An update. *Trop. Parasitol.*, 2011; **1**: 73-5. https://doi.org/10.4103/2229-5070.86934.
4. Sapna G, Ankur G, Saroj S, Agrawal BM. Outcome of routine microbiological screening for lower genital tract infections in symptomatic non-pregnant females complaining infertility. IOSR *Journal of Dental and Medical Sciences*, 2014; **13**: 26-29. https://doi.org/10.9790/0853-13132629.
5. Luijt D, Di Lorenzo C, van Loon AM, Unemo M. Most but not all laboratories can detect the recently emerged *Neisseria gonorrhoeae* porA mutants – results from the QCMD 2013 N. gonorrhoeae external quality assessment programme. *Euro. Surveill.*, 2014; **19**: 20711. https://doi.org/10.2807/1560-7917.ES2014.19.8.20711.
6. Muthusamy S, Elangovan S. A study on prevalence and antibiotic sensitivity testing methods for *Neisseria gonorrhoeae* isolates among female outpatients of sexually transmitted infection clinic. *Int. J. Health Allied Sci.*, 2017; **6**: 11-4.
7. Sharma M, Rizvi M, Gupta R, Azam M, Khan HM, Parvez A, Shukla I. Alarming resistance of *Neisseria gonorrhoeae* in a tertiary care hospital of North India. *Indian J. Med. Microbiol.*, 2018; **36**: 285-8. https://doi.org/10.4103/ijmm.IJMM_18_123.
8. Sood S, Verma R, Mir SS, Agarwal M, Singh N, Kar HK, Sharma VK. Nucleic acid amplification tests (NAATs) for gonorrhoea diagnosis in women: experience of a tertiary care hospital in north India. *Indian J. Med. Res.*, 2014; **140**: 649–52
9. Kriesel JD, Bhatia AS, Barrus C, Vaughn M, Gardner J, Crisp RJ. Multiplex PCR testing for nine different sexually transmitted diseases. *Int. J. STD AIDS*, 2015; **0**: 1–8.
10. Saigal K, Dhawan B, Rawre J, Khanna N, Chaudhry R. Genital Mycoplasma and *Chlamydia trachomatis* infections in patients with genital tract infections attending a tertiary care hospital of North India. *Indian J. Pathol. Microbiol.*, 2016; **59:** 194-6. https://doi.org/10.4103/0377-4929.182019.
11. Sivaranjani V, Stephen S, Ghose S, Umadevi S. Poor performance of Point-of-Care (POC) kits for *Chlamydia*

trachomatis antigen detection in endocervical specimens. *Indian Journal of Basic and Applied Medical Research*, 2017; **6:** 630-39.

12. Mikolajczyk M, Wristlien PK, Wrobel M, Mazela J, Chojnacka K, Skrzypczak J. Prediction of short-term newborn infectious morbidity based on maternal characteristics in patients with PPROM and Ureaplasma species infection. *Ginekol Pol.*, 2015; **86**: 685-88. https://doi.org/10.17772/gp/57857.
13. Dhawan B, Gupta V, Khanna N, Singh M, Chaudhry R. Evaluation of the diagnostic efficacy of PCR for *Ureaplasma urealyticum* infection in Indian adults with symptoms of genital discharge. Jpn. *J. Infect. Dis.*, 2006**; 59**: 57-58.
14. Gossי M, Lysvand H, Pukstad B, Nordbר SA. A Novel Simple Probe PCR Assay for Detection of Mutations in the 23S rRNA Gene Associated with Macrolide Resistance in *Mycoplasma genitalium* in Clinical Samples. *J. Clin. Microbiol.*, 2016; **54**: 2563-67. https://doi.org/10.1128/JCM.01233-16.
15. Badami N, Salari MH. Rate of *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* in Infertile Females and Control Group. *Iranian J. Publ. Health*, 2001; **30**: 57-60.
16. Lee MY, Kim MH, Lee WI, Kang SY, Jeon YL. Prevalence and Antibiotic Susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Pregnant Women. *Yonsei Med. J.*, 2016; **57**: 1271-5. https://doi.org/10.3349/ymj.2016.57.5.1271.
17. Fule SR, Fule RP, Tankhiwale NS. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J. Med. Microbiol.*, 2012; **30**: 314-6. https://doi.org/10.4103/0255-0857.99493.
18. Kaur S, Khurana S, Bagga R, Wanchu A, Nancy M. Trichomoniasis among women in North India: A hospital based study. *Indian J. Sex Transm. Dis.*, 2008; **29**: 76-81. https://doi.org/10.4103/0253-7184.48729.
19. Purnima M, Bartman MT, Pasutti L, Krupp K, Arun A, Reingold AL, Klausner JD. Prevalence of *Trichomonas vaginalis* infection among young reproductive age women in India: implications for treatment and prevention. *Sex Health*, 2009; **6**: 339–44. https://doi.org/10.1071/SH09038.
20. Muthusamy S, Elangovan S. A study on the prevalence of genital trichomoniasis among female outpatients attending sexually transmitted infection clinic in a tertiary care hospital. *J. Lab. Physicians*, 2017; **9**: 16-9. https://doi.org/10.4103/0974-2727.187920.
21. Ashshi AM, Batwa SA, Kutbi SY, Malibary FA, Batwa M, Refaat B. Prevalence of 7 sexually transmitted organisms by multiplex real-time PCR in Fallopian tube specimens collected from Saudi women with and without ectopic pregnancy. *BMC Infect. Dis.*, 2015; **15**: 569. https://doi.org/10.1186/s12879-015-1313-1.
22. Sendzik T, Balbier S, Hofmann M, Steimer M, Carman WF, B ch S, Kneweler K. Evaluation of the Fast-track Diagnostics Real time multiplex PCR for simultaneous detection of *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Mycoplasma hominis, Ureaplasma urealyticum* and *Ureaplasma parvum.* http://www.fast-trackdiagnostics.com/media/102060/2013_05_asm_poster_std.pdf
23. Harrison M, Harding-Esch E, Broad C, Soares C, Fuller S, Okala S, Saunders J, Barber T, Hay P, Sadiq ST. Comparison of the FTD™ Urethritis Plus (7-Plex) Detection Kit With Routine Sexual Health Clinic Nucleic Acid Amplification Testing For Detection Of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* In Urine, Vaginal, Pharyngeal And Rectal Samples. *Sex. Transm. Infect.*, 2016; **92**(Suppl 1): A76–A77. https://doi.org/10.1136/sextrans-2016-052718.219.
24. Pichon M, Gebeile R, Lina B, Jacquet G, Gaymard A. Which sample for the transport of mycoplasma, eSwab® or dry swab? *Ann. Biol. Clin. (Paris)*, 2019; **77**(1): 95-98.
25. Garcia M, Chloי P, Perraud E, Bourgoin A, Lיvרque N, Defaux AB. Comparison of Anyplex II STI-7 detection and Allplex STI Essential PCR assays for detection of seven sexually transmitted infections on urine and vaginal specimens. The Congress of ESCMID. 2017: **e5964**: EV0240.
26. Goldenberger D, Paladin S, Feuz K, Derengowski B, Courtet V, Egli A. Single center validation of the Seegene Allplex™ GI Bacteria and STI Bacteria multiplex real-time PCR panels. Joint annual meeting 2017. (August 30 - September 01, 2017).
27. Berחot B, Amarsy R, Goubard A, Aparicio C, Loeung HU, Segouin C, Gueret D, Jacquier H, Meunier F, Mougari F, Cambau E. Assessment of Coinfection of Sexually Transmitted Pathogen Microbes by Use of the Anyplex II STI-7 Molecular *Kit. J. Clin. Microbiol.*, 2015; **53**: 991-993. https://doi.org/10.1128/JCM.03370-14.
28. Choe HS, Lee DS, Lee SJ, Hong SH, Park DC, Lee MK et al. Performance of AnyplexTM II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. *Int. J. Infect. Dis.*, 2013; **17**: e1134–e1140. https://doi.org/10.1016/j.ijid.2013.07.011.
29. Prete RD, Ronga R, Lestingi M, Addati G, Angelotti UF, Di Carlo D, Miragliotta G. Simultaneous detection and identification of STI pathogens by multiplex Real Time PCR in genital tract specimens in a selected area of Apulia, a region of Southern Italy. *Infect.*, 2017; DOI 10.1007/s15010-017-1002-7.
30. Joyee AG, Thyagarajan SP, Rajendran P, Hari R, Balakrishnan P, Jeyaseelan L, Kurien T; STD Study Group. Chlamydia trachomatis genital infection in apparently healthy adult population of Tamil Nadu, India: a population-based study. *Int. J. STD AIDS*, 2004; **15**: 51–5. https://doi.org/10.1258/095646204322637272.
31. Sethi S, Roy A, Garg S, Venkatesan LS, Bagga R. Detection of *Chlamydia trachomatis* infections by polymerase chain reaction in asymptomatic pregnant women with special reference to the utility of the pooling of urine specimens. Indian J Med Res. 2017; **146**: S59–63. https://doi.org/10.4103/ijmr.IJMR_981_15.
32. Preena B, Baveja UK, Chawla R, Saini S, Khaki P, Bhalla K, Mahajan S, Reddy BSN. Simultaneous detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by PCR in genitourinary specimens from men and women

attending an STD clinic. *J. Commun. Dis.*, 2007; **39**: 1-6.

33. Patel AL, Sachdev D, Nagpal P, Chaudhry U, Sonkar SC, Mendiratta SL, Saluja D. Prevalence of Chlamydia infection among women visiting a gynaecology outpatient department: evaluation of an in-house PCR assay for detection of *Chlamydia trachomatis. Annals Clin. Microbiol. Antimicrob.*, 2010; **9:** 24. https://doi.org/10.1186/1476-0711-9-24.