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MULTIPLEX PCR FOR THE DIAGNOSIS OF RTI/STI INFECTIONS AMONG WOMEN ATTENDING TERTIARY CARE CENTRE IN MUMBAI, INDIA

Medical Microbiology

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ABSTRACT

RTI/STIs continue to present as major public health, social, and economic problem in developing countries leading to considerable morbidity, mortality and stigma. STIs refer to the mode of transmission, whereas RTIs refer to the site of infection. This prospective study was undertaken in a tertiary care hospital among women complaining of abnormal vaginal discharge using Gram stain and Multiplex Real time Polymerase Chain Reaction. Bacterial vaginosis was detected in 37% cases on the basis of Nugent scoring. Multiplex PCR of endocervical swabs detected presence of organisms in 81 % cases, with detection of Gardnerella vaginalis being highest as a sole pathogen (30%) and coinfection detected in (24%) with Ureaplasma urealyticum. Incorrect treatment due to similar symptoms by different microbes further complicates the disease management by syndromic approach as it can result in misdiagnosis. The introduction of molecular diagnostic tests which gives accurate etiological diagnosis provides an important step to address the burden of RTI/STIs. A relook of modification of syndromic management is the need of the hour as different microbes mimic symptoms. Hence molecular diagnostic tests enable accurate etiological diagnosis, thereby specific treatment can be given.

KEYWORDS

RTI (Reproductive tract infection), STI (Sexually transmitted infections), Bacterial vaginosis

INTRODUCTION-

RTI/STIs are the most common infectious diseases worldwide, they present a huge burden of disease and adversely impact reproductive health of women. These infections often go undiagnosed and untreated, and when left untreated, they lead to complications such as infertility; ectopic pregnancy and cervical cancer. Some of the RTIs are associated with poor pregnancy outcome and high morbidities and mortalities among neonates and infants. About 40% of women in India are estimated to have RTI/STI at any given point of time, but only 1% complete the required treatment of both partners. (1). Accurate and early etiological diagnosis of the infectious agent is of critical importance for appropriate treatment and timely intervention, thereby preventing further transmission of infection.

MATERIALS AND METHODS –

This open labelled, prospective study was carried out among Female patients attending Obstetrics & Gynaecology OPD with complaint of abnormal vaginal discharge from October 2016 to September 2017. Required demographic data of participants like age, occupation, marital status, age of sexual activity initiation etc. were collected based on structured questionnaire. Abnormal vaginal discharge was considered as discharge that is different from usual with respect to colour/odour/consistency (e.g. discoloured or purulent or malodorous). (2). Women with genital warts, labial ulcerations, vaginal lesions and pregnant women were excluded. The study was initiated after the receipt of Institutional Ethics Committee approval and eligible participants were enrolled after obtaining written informed consent. Total 3 swabs were collected from each participant. So after swab sample collection wet mount was prepared to check presence of motile *Trichomonas vaginalis*. Smear was prepared from vaginal discharge and evaluated for Nugent score, clue cells, leukocyte and budding yeast cells for diagnosis of Bacterial vaginosis (BV) or candida using Gram staining. (3). Same smear was also evaluated for presence of *Neisseria gonorrhoeae.* Wet Endocervical swab was collected using flocked swab and qualitative assay of organisms was done using commercial FTD STD9 an in vitro test, multiplex real time PCR for the simultaneous detection of pathogens including *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis, Gardnerella vaginalis, Ureaplasma urealyticum/parvum, Herpes Simplex Virus 1/2.* The primer probe sequences, controls and cycling conditions for PCR were as per kit insert. The flowchart of sample collection and laboratory investigations is presented in Fig.1

Fig 1. Flowchart of sample collection and laboratory investigations

RESULTS –

Patients were divided in the different age group. The average age of the study population was 30.71 years. Maximum age was 50 years and minimum age was 21 years. Among these, 77 % females were homemaker, 15 % were skilled workers, 5 % were students and 2 % were unskilled workers – maid.

On analysis, 97% were sexually active and 95 % of the females were married, 5 % unmarried. Out of 5% unmarried women, 2% were sexually active. Among all these females, 97 females were sexually active. Observed age of initiation of sexual activity was found to be less as 15 years and maximum 33 years. Average age of initiation of sexual activity was 21.95 years.

31 % females complained of vaginal discharge with odor and in 34 % females abnormal vaginal discharge was associated with pain. Previous history of same complaints was given by 47 % of females.

Among these, treatment was extended to 51.06 % females. Out of which only 42.66 % females completed the treatment given.

Microscopy of gram stained smear of endocervical swabs showed presence or absence of gram negative cocci, gram positive bacilli, gram negative bacilli and clue cells (Table 1) on the basis of which Nugent score is calculated and interpreted. We didn't find gram negative cocci in a single smear. Gram negative bacilli were found in 79 % smears. Clue cells were seen in 22 % smears. Pus cells were seen in 27 % smears. Budding yeast cells in only 3 % smears.

According to Nugent scoring, 37 % cases had bacterial vaginosis and 33 % cases were negative. In 30 % cases reports were intermediate and repeat sample was requested.

Multiplex PCR using FTD STD 9:
Multiplex PCR of endocervical swabs detected presence of organisms in 81% cases. The distribution of pathogens detected is as under (Fig 2):

Sole Pathogen –
- *Neisseria gonorrhoeae* – 1%
- *Gardnerella vaginalis* – 30%
- *Ureaplasma urealyticum/parvum* – 15%
- *Trichomonas vaginalis* – 3%

Co-infections –
- *Gardnerella vaginalis* + *Ureaplasma urealyticum / parvum* – 24%
- *Gardnerella vaginalis* + *Trichomonas vaginalis* – 4%
- *Ureaplasma urealyticum / parvum* + *Trichomonas vaginalis* – *3%*
- *Nesseria gonorrhoeae* + *Ureaplasma urealyticum / parvum* – 1%

Overall, *Gardnerella vaginalis* infection found was 58 % of women, 42% women were positive for *Ureaplasma urealyticum/parvum* and 10% women were positive for *Trichomonas vaginalis* infection

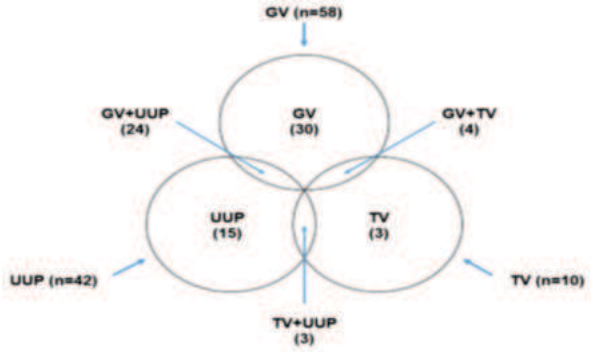

Fig 2. Distribution of major organisms detected by Multiplex PCR

GV as sole pathogen (Table 1) – Matching results in
- Positive NS and PCR positive – 18/30 (60%) cases
- Intermediate NS and PCR positive – 5/30 (16.67%) cases
- Negative NS and PCR positive – 7/30 (23.33%) cases

GV + UUP coinfection (Table 1) – Matching results in
- Positive NS and PCR positive – 9/24 (37.5%) cases
- Intermediate NS and PCR positive – 6/24 (25%) cases
- Negative NS and PCR positive – 9/24 (37.5%) cases

So, the intermediate and negative Nugent score categories cannot exclude the possibility of Bacterial vaginosis due to GV (*Gardnerella vaginalis*) and GV + UUP coinfection.

Table 1: Distribution of organisms with respect to Nugent score and PCR result

Organisms detected by Multiplex PCR test	Nugent Score			Total
	Positive	Intermediate	Negative	
GV	18	5	7	30
TV	0	1	2	3
UUP	2	9	4	15
NG	1	0	0	1
GV + UUP	9	6	9	24
GV + TV	3	1	0	4
TV + UUP	0	1	2	3
NG + UUP	0	0	1	1
Nil	4	7	8	19
Total	37	30	33	100

Table 2: *Gardnerella vaginalis* Detection Distribution by microscopy and PCR

		Microscopy result for GV			
		Present	Absent	Total	p-Value by Fisher's exact Test
PCR Result for GV	Present	30	28	58	
	Absent	7	0	7	0.03
	Total	37	28	65	

On comparing the results of Microscopy and Multiplex PCR for detection of *Gardnerella vaginalis* (Table 2), *p*-Value by Fisher's exact test was 0.03 ($p < 0.05$) so this distribution was statistically significant. Sensitivity of Multiplex PCR (FTD STD9) was 81.08% (95% CI = 67.19% - 93.63%) and Positive Likelihood Ratio is 0.81 (95% CI = 0.72 – 0.96). Multiplex PCR has proven to be more significant than routine procedures.

DISCUSSION –
The prevalence of RTI/STIs is high in many countries including India. However, the exact figures are rarely available as various studies quote variable prevalence rates. (4). Their profile varies with changes in socioeconomic, cultural, geographic and environmental factors prevalent in different parts of the country. However, due to lack of adequate laboratory backup in the country, information regarding the profile of RTI/STIs relies essentially on syndromic diagnosis. (5). Sexually active females have the risk of acquiring infections from infected partners. During treatment of STIs, partner treatment is important in order to prevent recurrence.

In the present study, the majority of the study subjects were in the sexually active age group of 26-30 years (35.0%). Mean age of subjects in our study was 30.71 years. Similar findings are reported in studies by Esen et al (6) and Mammen et al (7) which is 32.5 years and 34.7 years respectively. In 34% women abnormal vaginal discharge was associated with lower abdominal pain where as it is observed 4.9% by Sridevi et al (8) and 6.34% by Ganta et al (9). As compared to other studies, our study has shown a higher rate of detection of Bacterial Vaginosis using Nugent score for diagnosis of Bacterial Vaginosis.

Our study by multiplex PCR (FTD STD 9) shows high prevalence of *Gardnerella vaginalis* which is different from other studies eg Esen et al (6).However, prevalence of *Ureaplasma urealyticum* is high in both studies.

Comparing the results of Microscopy and Multiplex PCR for detection of *Gardnerella vaginalis*, by Fisher's exact test, Multiplex PCR has proven to be more significant than routine procedures.

Multiplex PCR holds promise in detecting coinfections in Nugents score Intermediate/Negative samples.

Limitations of our study:
- *Trichomonas vaginalis* was not detected by wet mount in the present study and it is probably due to the delay of sample transport to laboratory.
- In the present study, *Chlamydia trachomatis* and *Mycoplasma genitalium* were not detected by Multiplex PCR test, probably because of low prevalence of these pathogens in the study population.

Syndromic approach is based on subjective judgment which may lead to more disruption, misdiagnosis and mistreatment.

Syndromic Case Management Protocol (10)

Kit No.	Syndrome	Colour	Contents
Kit 2	Vaginal Discharge (VD)	Green	Tab. Secnidazole 2 g (1) and Tab. Fluconazole 150 mg (1)

Our results emphasize that syndromic case management (SCM) is imprecise and results in treatment errors. Unnecessary treatment in some patients and inadequate treatment in others will contribute to an ever increasing burden of antibiotic resistance. The study underscores the need to implement diagnostic assays like multiplex PCR for etiologic diagnosis & pathogen identification prior to treating patients with vaginal discharge with antibiotics.

CONCLUSION –

Detection of Bacterial vaginosis by both microscopy and molecular test denotes highest prevalence of *Gardnerella vaginalis*. Using molecular test, high prevalence of *Ureaplasma* was reported but their pathological significance needs more exploration. To conclude, in resource-poor countries, which lack trained personnel and laboratory facilities, SCM remains a rational approach to RTI/STIs care. However, its successful implementation requires regular evaluation of its algorithm. It should cover variables related to the etiological agents for a given syndrome using highly sensitive and specific diagnostic techniques like Multiplex PCR and other sophisticated methods.

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