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Original Article

Molecular prevalence of eight different sexually transmitted infections in a Lebanese major tertiary care center: impact on public health

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Abstract: Background: Sexually transmitted diseases (STD) are caused by a variety of pathogens transmitted by sexual activity. Untreated infections can cause major complications with a substantial high cost on health sector. With the development of molecular techniques, STD screening became easier with a high sensitivity and specificity. Objectives: In Lebanon, official data regarding STD trends are scarce. This study elucidates the STD molecular profile at a tertiary care center, American University of Beirut Medical Center (AUBMC), its distribution among gender and age groups, with a comparison to international studies. Methods: A retrospective data analysis was conducted on all STD panels performed at AUBMC from January 2017 till December 2019 to determine the molecular prevalence of eight different sexually transmitted organisms. Results: Our samples belonged to 248 females (41.5%) and 349 males (58.5%). Only 53.5% of the samples tested positive for one or more organisms. *Ureaplasma urealyticum/parvum* was found to be the most common pathogen (49.3%), followed by *Gardenerella vaginalis* (33.5%), *Chlamydia trachomatis* (5.36%), *Mycoplasma genitalium* (5.16%), *Neisseria gonorrhea* (2.5%), Herpes simplex virus (2.5%), and Trichomonas vaginalis (1.39%). Age was distributed between 5 and 80 years old. Regarding the pathogen's distribution among gender, *Ureaplasma urealyticum/parvum*, Herpes simplex virus, and *Gardenerella vaginalis* were more common in females, the rest was more detected in males. Conclusion: Data will be of great importance for clinicians, in terms of diagnosis and treatment. It will help adopting an evidence based STI control programs in Lebanon, and it is essential for future larger studies and sexual health awareness programs.

Keywords: STI, STD panel, Lebanon, molecular prevalence, multiplex PCR

Introduction

Sexually transmitted diseases (STD) or sexually transmitted infections (STI) are infections commonly spread by sexual activity including vaginal intercourse, anal or oral sex [1]. STD are considered a major health burden, imposing a substantial cost on the health sectors [2, 3], that reached around $15.9 billion in the United States in 2018, with chlamydial infections causing the biggest burden if HIV and human papillomavirus (HPV) viruses were excluded [4]. STD symptoms can vary from mild suprapubic pain to dysuria, vaginal or urethral discharge and ulcerative lesions but unfortunately, most infections are asymptomatic and if left untreated, may lead to serious complications such as chronic pelvic inflammatory disease, ectopic pregnancy, infertility, neonatal mortality and many genital/oropharyngeal cancers [5]. Many recent studies also state that STD amplify the risk of human immunodeficiency virus HIV transmission [6-8].

Increased rates can be attributed to social and legal acceptance of same-sex sexuality (Lesbian, Men who have sex with men), in addition to non-controlled prostitution and a sexual health freedom delineated by the availability of oral contraceptive pills and condoms [9]. The global surge in infection rates affected many countries, for example, in the USA, there is an 82.6% increase in the rate of *Neisseria gonorrhoeae* since the historic low in 2009, and a

2.9% increase in the rate of *Chlamydia trachomatis* from 2017 to 2018 as reported by the CDC [10]. Greenland [11] and United Kingdom [12] had similar rise in gonorrhea and chlamydia cases, as well as other STI organisms. In a study published in 2019, the annual incidence rate of *C. trachomatis, N. gonorrhea, T. pallidum and T. vaginalis* in the Middle East and North Africa between the years 1990 and 2006 was estimated to be 60 per 1000 [13]. Furthermore, a systematic review published in 2019 determined the prevalence of *C. trachomatis* to be 3% in the Middle East population [14]. This rate was on part with the World Health Organization's (WHO) estimates observed in the Pacific and European regions; where the capacity for surveillance is higher and STI control programs are well established in comparison to the Middle East and Northern Africa.

Recently, molecular testing has replaced many ancillary laboratory tests, and the impact of molecular testing on STI diagnosis has been substantiated worldwide. For decades, the laboratory diagnosis of STI consisted of microbial antigen detection, serology for antibodies detection, e.g. kits for *Chlamydia trachomatis* [15], routine culture for some organisms (selective media for *Neisseria gonorrhea*), tests detecting microbial metabolites (Whiff test) with a variable sensitivity and specificity. STD panels consist of nucleic acid amplification technique (NAAT) followed by the detection of specific sequences in the DNA or RNA organisms using PCR, real time PCR, or reverse transcription PCR [1]. The introduction of NAAT for STD detection using different body fluids (vaginal swab, urine, etc...) were found to be more sensitive than the conventional subjective, microscopic examinations or the time-consuming bacterial culturing methods [16]. Thus, STD panels provide a cost-effective diagnostic panel allowing for a faster detection and a considerable reduction in reagents costs and turnaround time [17]. This will reduce the time from diagnosis to treatment, and accelerate the process of partners tracing and treatment, especially for asymptomatic patients [1]. Moreover, many patients present to the Emergency Department with ambiguous symptomatology, suggestive of an overlapping/coexistent STD and urinary tract infection, which necessitates the implementation of such STD panels in many hospitals, for accurate and fast diagnosis [18]. Such steps are essential for an ongoing STD-screening programs, objective epidemiological data, and subsequently a targeted treatment. Molecular diagnostic techniques have resulted in reduction of STD transmission rates [19]. The use of non-invasive specimens were more convenient and encouraged patients to get tested [5], especially in groups who are at high risk of infection but tend to hide their symptoms or fear the invasive genital examinations. Rapid testing also decreased the risk of developing STD-associated complications and reduced the overall costs to both patients and healthcare facilities [20].

In Lebanon, only scarce data characterizing STD epidemiology can be found, official reports are not available. Data from our country published in 2016, showed a significant increase in the prevalence of *N. gonorrhea* in semen samples collected over a period of seven years in a northern Lebanese district, with a 20% surge in the prevalence of *N. gonorrhea* between 2015 and 2016 [21]. Another study reporting on the molecular epidemiology of STI among Lebanese women, revealed that amongst women experiencing no symptoms at all, 61% were identified to be carrying one of the pathogens of interest knowing that asymptomatic infections are considered a big threat since they are responsible of the sustained transmission in the community [22]. In addition, coinfection with two or more pathogens accounting for more than 40% of the cases [23]. The high prevalence of STI in the region as well as that identified in specific populations in Lebanon, combined with an increased antibiotic resistance of several bacterial strains [21] necessitate the generation of basic epidemiological data to assess the burden of STD in the country. As such, routine monitoring of STD epidemiological trends using molecular diagnostic assays is necessary to understand the impact of this interplay and provide insights into the STD profile and trends in Lebanon, and adopt evidence based STI control programs. In our study, we will determine the prevalence of STD pathogens using the multiplex real time PCR technique, we will analyze data in correspondence to age and gender, and we will compare our results to international studies, stressing on how such new molecular data will affect STD trends in Lebanon, and future clinical decisions.

Table 1. Distribution of detected pathogens among different age categories

Pathogen	Age categories				Total
	<25	25-35	36-49	≥50	
Ureaplasma urealyticum/parvum	15%	44.5%	35.5%	5%	N=248
Herpes simplex virus	7.5%	38%	46%	7.5%	N=13
Trichomonas vaginalis	0%	28.5%	43%	28.5%	N=7
Chlamydia trachomatis	11%	26%	55.5%	7.5%	N=27
Mycoplasma genitalium	15.5%	46.5%	38%	0%	N=26
Neisseria gonorrhea	0%	69%	31%	0%	N=13
Gardenerella vaginalis	18%	41.5%	34.5%	6%	N=169

Materials and methods

After Institutional Review Board (IRB) approval, we conducted a retrospective data analysis on all STD panels performed at the Department of Pathology and Laboratory medicine at the American University of Beirut Medical Center (AUBMC) from January 2017 till December 2019. Our data consisted of 597 specimens that were collected and tested over 2 years.

STD panel collection

Specimens consisted of vaginal/endocervical swabs from women, urethral swabs from men, and first catch urine from both women and men. Handling of these swabs followed strict adherence to testing and quality assurance protocols.

STD panel laboratory performance

Regarding the workflow, all STD panels were performed using Fast Track Diagnostics (FTD STD9 on Rotor-gene 3000, 6000) which consists of a multiplex real-time PCR technique, for detection of pathogen genes by TaqMan® lyophilized technology. Primer, probe mix, enzyme, and buffer were all present in a single Fast Track lyophilized tube, into which nucleic acids were added. A normal PCR run was started on the Fast Track cycler, and results were analyzed instantly using the automated platform "FastFinder".

Data retrieval

We retrieved all the STD panel reports, and we pooled the results into an excel sheet. We checked for the presence/absence of eight organisms (*Ureaplasma urealyticum/parvum*, *Gardenerella vaginalis*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhea*, Herpes simplex virus, and Trichomonas vaginalis) in each sample, the presence of more than one pathogen in each sample, and we retrieved the corresponding age and gender of each sample.

Statistical analysis

All results were analyzed using IBM SPSS software version 19. We only used the descriptive statistics (frequencies and cross-tabulation). The STD molecular prevalence of different organisms was stratified according to gender, age, and the number of pathogens detected in a single specimen.

Results

We present below the results of the pathogens detected in 597 specimens tested for STD panel, and their distribution among gender and age. Results are expressed as frequencies, percentages or means.

Distribution among age groups

Age was distributed between 5 and 80 years old with a mean of 35.5 years old, pathogens distribution among different age categories is presented in **Table 1**.

Distribution among gender

Tested specimens belonged to 248 females (41.5%) and 349 males (58.5%). *Ureaplasma urealyticum/parvum*, Herpes simplex virus, and *Gardenerella vaginalis* were more common in females, the rest was more detected in males (**Table 2**).

Frequency of co-infections

Only 53.5% of the samples tested positive for one or more organisms. *Ureaplasma urealyticum/parvum* was found to be the most common pathogen (49.3%), followed by *Gardenerella vaginalis* (33.5%), *Chlamydia trachomatis* (5.36%), *Mycoplasma genitalium* (5.16%),

Table 2. Distribution of pathogens according to gender

Gender	Pathogen						
	Ureaplasma urealyticum/parvum	*Herpes simplex virus*	*Trichomonas vaginalis*	*Chlamydia trachomatis*	*Mycoplasma genitalium*	*Neisseria gonorrhea*	*Gardenerella vaginalis*
Female	59%	77%	43%	15%	31%	7.5%	68.5%
Male	41%	23%	57%	85%	69%	92.5%	31.5%

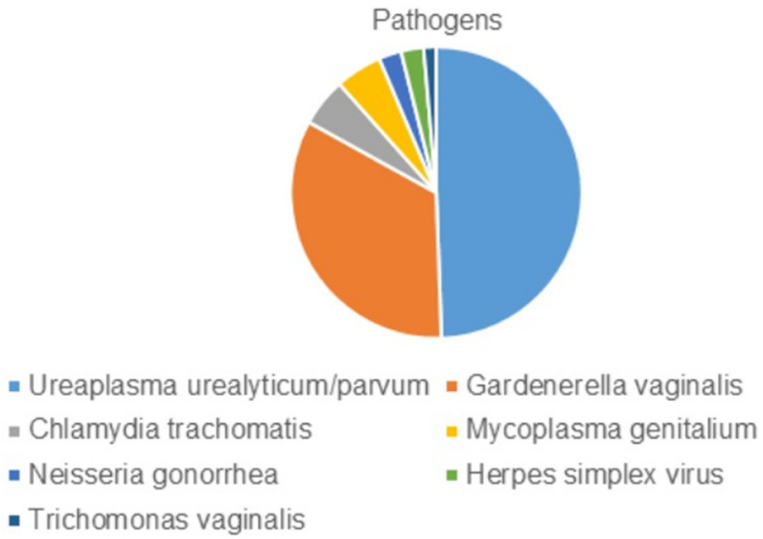

Figure 1. Prevalence of STD pathogens in positive specimens.

Neisseria gonorrhea (2.5%), Herpes simplex virus (2.5%), and Trichomonas vaginalis (1.39%) (**Figure 1**).

The multiplex technique was able to detect more than one pathogen in some specimens, the results are shown in **Table 3**. To note, in 75.5% of positive specimens, *Ureaplasma urealyticum* and *Gardenerella vaginalis* were present simultaneously, the rest was a non-significant combination of organisms.

Discussion

To date, with the advancements of molecular techniques, molecular STD epidemiological studies have been conducted in several countries. In our study, the most common encountered pathogen is *Ureaplasma urealyticum/parvum*, with the highest prevalence in females (59%) and among young people aging 25-35 years old, which correlates well with similar results obtained from Israel [24], Korea [17], Italy [25], Sub-Saharan Africa [26], Australia [27] and France [28] using different multiplex PCR assays. Although a recent statement from the European STI guidelines mentioned that routine testing and treatment of patients with *Ureaplasma urealyticum/parvum* is no longer recommended and with no benefit [29], other studies showed that *Ureaplasma urealyticum/parvum* carriage correlates with higher numbers of preterm delivery and vacuum-assisted delivery, with 68% of newborns born to carrier mothers tested positive for *Ureaplasma urealyticum/parvum*, with a statistically significant risk factor for Arab ethnicity [24]. Thus, continuous screening of *Ureaplasma urealyticum/parvum* is important in order to avoid such complications in our country.

Regarding *Gardenerella vaginalis*, the second most common encountered pathogen, it was 2 times more recovered in females, and like *Ureaplasma urealyticum/parvum*, it was more prevalent among young patients (25-35 years old), which is concordant with other epidemiological data from Australia (50.9%) [5]. To note, *Gardenerella vaginalis* is not tested in all commercial multiplex PCR assays, the majority of studies detected it by sequencing or singleplex PCR assays. The role of *Gardnerella vaginalis* in vaginal disease is still controversial due to its presence in both healthy and bacterial-vaginosis type vaginal microflora [30]. Such information justifies the high prevalence of *Gardenerella vaginalis* in our samples but does not necessitate prompt intervention to treat such infections.

Chlamydia trachomatis, and *Mycoplasma genitalium* showed a prevalence of 5.36% and 5.16% respectively, with a predominance in males especially for *Chlamydia trachomatis* (85% of positive samples are from male patients), which is similar to Korean data [17] and Japanese data [31] but lower than what was reported in an Australian research where the

Table 3. Number of detected pathogens in each specimen

Distribution of pathogens among specimens						
Number of detected pathogens	0	1	2	3	4	Total
Number of specimens	46.5%	50.2%	1.65%	1.5%	0.15%	N=597

study population consisted of sexually active women; this is not surprising since most chlamydial infections in women are usually asymptomatic, and pass undetected. Apart from *Ureaplasma urealyticum/parvum* and *Gardenerella vaginalis*, which are not considered serious STD pathogens but are usually screened in some STD panels, *Chlamydia trachomatis* ranks among the most recovered pathogens in males aging between 36-49 years old. According to CDC, *Chlamydia trachomatis* is the most common notifiable STD infection in the United States [10], with an 82% prevalence rate in a French study [28]. It is twice more reported in females than males, which doesn't correlate with our epidemiological results. Another Australian study, targeting women attending sexual health clinics, reported a similar *Chlamydia trachomatis* detection rate of 3.7% in women less than 34 years old [5, 32]. *Chlamydia trachomatis* does not seem to be that prevalent in our young population compared to other ones [33], and this might be due to an under-diagnosis in our young population or it can reflect a real absence of chlamydial infection and requires further investigation (prospective study).

Concerning *Mycoplasma genitalium*, it is a bacterium, extremely difficult to culture, that we suspect in cases of non-gonococcal non-chlamydial cervicitis or urethritis. With the development of NAAT, it is debatable whether to routinely screen or not for *Mycoplasma genitalium*. According to Baumann et al, *Mycoplasma genitalium* prevalence was similar between males and females [34], the global prevalence is higher among females than males (1-4% versus 1-6.4%) [35-37] and found to be 0.3% versus 0.2% in a Japanese study [31] but our results show more prevalence and particularly among males, which may be explained by more men presenting to do the test, with no significant difference among age groups less than 50.

On the other side, *Neisseria gonorrhea* and *Herpes simplex virus* had a similar detection rate of 2.5%, with a predominance among patients aged between 25-35 years old for *Neisseria gonorrhea*. Higher rates of *Neisseria gonorrhea* cases were found in other studies: 4.9% in a Korean study [17], 4.3% in a Japanese study [31] and 11.2% in a French study [28]. 92.5% of the positive *Neisseria gonorrhea* samples belonged to male patients, which is concordant with a Lebanese study showing that *Neisseria gonorrhea* prevalence was only 0.2% in women [23], which is also similar to CDC reports regarding increased gonorrhea cases among males more than females [10].

Regarding *Herpes simplex virus*, many multiplex PCR assays are currently used to detect HSV type I and II, instead of real time PCR assays, with the advantage of minimizing the sample amount and detecting other causative agents simultaneously. The *Herpes simplex virus* detection rate in our population (2.5%) was similar to a women Australian population using another PCR assay (2.5% for *Herpes simplex virus* type I and 0.8% for *Herpes simplex virus* type II) [27], but lower than other populations [5, 38]. *Herpes simplex virus* is more prevalent among females, similar to data from United States [10, 39].

Trichomonas vaginalis is a non-notifiable sexually transmitted protozoal infection, resulting in limited epidemiological data compared to other pathogens. Our results showed a 1.39% prevalence, comparable to the 2% prevalence found among Lebanese women [23], but higher than the detection rate of a Japanese study [31] and lower than what was detected in Australia using another multiplex assay (VDL07) [27]. Our multiplex technology detected the simultaneous presence of *Ureaplasma urealyticum* and *Gardenerella vaginalis*, both are among the most common detected pathogens but causing less severe infections and complications, and they are part of the normal flora as mentioned before [30].

Out of 597 samples, only 320 turned out to be positive, and infection with multiple pathogens accounted for 6.25% only, a very low percent-

age compared to other studies [17, 40], which indicate the low prevalence of STD in the studied population or the underreported/diagnosed infections. Although STD panel test is commercially available, the test's cost remains a limitation for some Lebanese patients, with no insurance coverage, who cannot afford molecular testing, and the physician ends up ordering routine bacteriology tests with a long turnaround time, and further treatment delay, with great chances of missing other concomitant pathogens.

Limitations

This study has a number of limitations, only the molecular prevalence was reported, and our samples do not represent the whole Lebanese population, however, our aim was to calculate the prevalence at a tertiary care center and the relationships to selected demographic characteristics like age and gender. Results interpretation according to sexual behavior (number of partners, condom or contraception use) was bypassed because of the retrospective type of the study. The comparison of the multiplex PCR method to gold standard laboratory methods necessitate more microbiological/clinical data and can be another research project on its own.

Conclusion

Our results shed the light on the STD situation in Lebanon, which appears to be acceptable in the absence of elevated numbers of STI, unless many patients are not seeking medical care. These results might help physicians, to guide their diagnosis and their approach for future lab tests order especially when it comes to atypical case presentations or asymptomatic ones.

The data available on STD trends in Lebanon and specific social determinates that drive such trends is limited if not absent. As such, clinical practice and diagnosis is currently guided by imported recommendations from countries with major differences in social factors that drive specific tendencies in prevalence rates.

In this study, we provide the first report on STD prevalence within a major Lebanese tertiary care center. The high rate of *Ureaplasma urealyticum/parvum* elucidated along with the associated higher rates of preterm delivery and vacuum-assisted delivery, especially among Arab ethnicities, suggest the need to re-evaluate current screening decisions, and introduce routine testing and treatment for this particular pathogen in our population.

Moreover, as our samples showed lower rates of *Chlamydia trachomatis* in comparison to those reported in the United States, Europe and Australia, further research should be done to support this finding since this low rate may be attributable to under reporting in asymptomatic individuals.

Finally, the prevalence of *Mycoplasma genitalium* obtained in this study is five times higher than that observed worldwide, suggesting the need to collect more data to indicate targeted demographics and investigate whether local routine screening is prompted.

Our results shed light on the importance of contextualizing our current practice within a framework that takes into consideration local social determinants that influence the underlying prevalence of these STD and highlight the need to undergo larger systematic data collection and analysis to fight this burden and improve clinical management.

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Disclosure of conflict of interest

None.

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