Detection of *Trichomonas vaginalis* using urethral, vaginal and cervical swabs with standard diagnostics from males and females in Canton Sarajevo

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**ABSTRACT**

**Objectives:** Trichomoniasis is a non-viral sexually transmitted infection (STI) caused by the protozoan parasite *Trichomonas vaginalis* (TV). The aim of our study was to identify the most frequent TV isolates using urethral, vaginal and cervical swabs with standard diagnostics from males and females.

**Material and methods:** The analysis of collected swabs (urethral, vaginal and cervical) was performed at the Department of Microbiology, Institute for Public Health of Canton Sarajevo, from June 2016 to March 2017. Microscopic evaluation of wet preparations and cultivation were demonstrated as the most common method for TV diagnostics. The urethral swabs were collected all together with vaginal and cervical in absolute value of 4784 from males and females.

**Results:** *T. vaginalis* was identified in the frequency of 269 isolates. The highest frequency of positive isolates was in June (N=69) and July (N=45) in 2016 while the lowest rate was in January 2017 (N=8). There were 53 cases of total positive swabs among males and females from January to March 2017 in Canton Sarajevo and median age was 29 year (interquartile range 25.0-37.5). Out of them 30 were males and 23 females with no statistical significance.

**Conclusions:** *T. vaginalis* was identified from urethral, vaginal and cervical swabs with standard diagnostics in high frequency from males. Our data suggest that wet mount examination of fresh samples contribute to increase the sensitivity in the diagnosis of trichomoniasis.

**Key words:** *T. vaginalis*, STI, prevalence.

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**INTRODUCTION**

Trichomoniasis is the most prevalent non-viral sexually transmitted infection (STI) affecting 276 million new infections annually (1). It is caused by the flagellate protozoan parasite *Trichomonas vaginalis* (TV) that exists as a trophozoite. TV has four flagella that arise from a single stalk and fifth flagellum, which forms an undulating membrane. It is approximately 20µm in length and 10 µm wide and infection which caused is more common in females than in males. Mostly, infections are asymptomatic or mild for both but health consequences for females are more serious and might include pelvic inflammatory disease (PID) with infertility. Infections in males are recognized mostly as urethritis with possible complications such as prostatitis and epididymitis.

The standard method used in diagnosis of trichomoniasis is wet mount examination, based on motility and morphology of the parasite. Inspite of convenience and low cost the microscopic evaluation of wet preparations might have a limit of low sensitivity (51%-65%) in vaginal samples as well as in males (2). Culture was considered the gold standard method for diagnosing TV infection before molecular detection methods become available. Culture has sensitivity in range of 75%-96% and a specificity up to 100% (3). Nowadays the culture was substitute by the nucleic amplification tests (NAATs) in many laboratories with increased the sensitivity and specificity in the detection of TV. Practitioners who use wet mounts should evaluate slides immediately because of sensitivity which decreases up to 20% (4). Metronidazole in therapy in a single dose of 2 gram per day or 500 mg orally twice a day for 7 days reduces symptoms and signs of TV infection and also reduce transmission. Tinidazole is a FDA-approved alternative that is equally effective. Sex partners of patients with TV should be also treated. Reinfection rate among females treated for trichomoniasis is very high (17% within 3 months) (5), so retesting for TV is...
recommended for all sexually active females and their partners as well. Condoms should be used during intercourse until the infection is eradicated in partners.

National STI surveillance data in Bosnia and Herzegovina (B&H) does not exist when it comes to TV.

The aim of our study was to identify the most frequent TV isolates from urethral, vaginal and cervical swabs with standard diagnostics from males and females in Canton Sarajevo. In the study, standard tool was applied for TV detection in B&H which includes staining techniques in diagnosis of TV and culture. The accurate identification of TV trophozoites considers an important approach in the diagnosis of trichomoniasis.

**Material and Methods**

The analysis of collected swabs (urethral, vaginal and cervical) was performed at the Department of Microbiology, Institute for Public Health of Canton Sarajevo in the period from June 2016 to March 2017.

The urethral swabs were collected all together with vaginal and cervical in Canton Sarajevo in absolute value of 4784 (N=4784) from males and females (Figure 1). Microscopic evaluation of wet preparations and cultivation were demonstrated as the most common method for TV diagnostics in B&H. The morphologic criteria used to identify TV include following: the size dimension of the parasite, the shape, the mobility, persistence of the undulent membrane and its position.

Trichomonas culture has been used to preserve the viability of the trophozoites using Diamond’s medium.

**Statistical analysis**

Epidemiologic measures of disease occurrence were applied to calculate frequency of positive samples. Categorical variable are presented in a table in absolute and relative frequency. Chi-square test was used to examine statistical significance for categorical variables. Normality of quantitative data was tested by Shapiro-Wilk test. Continuous data were presented as median with interquartile range or mean ± standard deviation depending on the normality data distribution. Statistical significance was set at p<0.05. Statistical analysis were performed by SPSS software package (Version 17.0 for Windows).

**Results**

In the present study standard diagnostics for TV detection were analyzed. Prevalence of T. vaginalis among males and females in Canton Sarajevo in the period of June 2016 – March 2017 has been presented (Figure 1).

The period prevalence of TV among tested population in the period of June 2016 - March 2017 was 5.6% (269/4784).

Out of all positive swabs 1664 for all tested agents, in the Canton Sarajevo in the period of June 2016 – March 2017, proportion of positive TV was 16.2% (269/1664). According to the month, the highest recorded proportion of positive TV was in June 2016 with 34.3% (69/201) and the lowest in January 2017 with 2.1% (8/381) (Figure 2).

In the period from January-March 2017 in Canton Sarajevo total number of positive TV among males and females (urethral, vaginal and cervical) swabs (n=4784) in Canton Sarajevo in the period of June 2016 - March 2017

**Table 1. Demographic characteristics of recorded cases T. vaginalis positive swabs in Canton Sarajevo in the period of January-March 2017 (n=53)**

<table>
<thead>
<tr>
<th></th>
<th>Number (f)</th>
<th>Percent (%)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>56.6</td>
<td>p=0.336</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>100.0</td>
<td></td>
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<tr>
<td>Age group</td>
<td></td>
<td></td>
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<tr>
<td>19-35</td>
<td>38</td>
<td>71.7</td>
<td>p=0.000</td>
</tr>
<tr>
<td>36-49</td>
<td>9</td>
<td>17.0</td>
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<tr>
<td>&gt;50</td>
<td>6</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>100.0</td>
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and females was 53 cases. The median age was 29 year interquartile range (25.0-37.5). There were 30 males (56.6%) and 23 (44.4%) females respectively with no statistical significance (p>0.05). The highest recorded frequencies of positive swabs of TV 38 cases (71.7%) were in age group 19-35 year, with statistical significance (p<0.05) (Table 1).

Discussion

Trichomoniasis is an important source of reproductive morbidity and thus it is an important public health problem. While TV is the most common non-viral STI globally and it is mostly asymptomatic, it is not a reportable disease and screening programs generally do not exist.

It is important that the parasitologists make differences between TV which is sexually transmitted from the other similar parasite (Pentatrichomonas hominis) which is the part of the normal microflora of the intestinal tract. STI surveillance data in B&H for trichomoniasis are limited for a number of reasons, including that asymptomatic individuals may not seek healthcare, so extent there might be a pool of undiagnosed infection in the wider population is unknown.

In the present study different swabs (urethral, vaginal and cervical) were analyzed in order to evaluate the diagnosis of trichomoniasis of males and females in Canton Sarajevo, B&H. Microscopic examination and culture for TV detection were applied. Microscopic examination is traditionally performed for TV diagnosis as it is quick and inexpensive, but also depends on the subjectivity of microscopists, transport and processing of the sample (6). Culture for TV is feasible, it requires a longer period to result and has not become the standard of practice.

Our data demonstrates that a standardized protocol for the detection of TV infection in B&H included microscopic evaluation (wet mount) and culture as well (7).

In the study 4784 swabs were tested (urethral, vaginal and cervical) for TV infection from males and females at the Department of Microbiology, Institute for Public Health of Canton Sarajevo in the period from June 2016 – March 2017. Out of 4784 specimens 1664 were positive for all tested agents while TV prevalence was 5.6 %. According to month the highest recorded prevalence was in June 13.3% (69/201) and the lowest in January 2017 with 2.1% (8/381). In the second part of the study we showed all TV positives with demographic characteristics for the period of the first trimester of 2017. Out of 53 TV positive, 56.6% were from males vs. 44.4% from females respectively with no statistical significance. The highest recorded frequencies of TV positive were from age group 19-35 year (71.7%). Radonjic et al. (8) compared wet mount, Giemsa stain, acridine orange fluorescent stain, cultivation and PCR to established which method or combination of methods was most effective in the laboratory diagnostics of TV. Data demonstrated that combination of different methods such as culture 21 (10.5%) and acridine orange staining 16 (8.0%) TV positive had the potential for better diagnosis of TV infection. Stoner et al. (4) suggested that vaginal fluid specimens for TV diagnosis should be stored in saline rather than on microscope slides until they are examined having in mind the impact of sample storage at room temperature on trichomonad motility. These findings also suggested that clinical sites which cannot perform microscopy within 1 hour of sample collection should consider the use of other diagnostic tests.

The British Association for Sexual Health and HIV (BASHH) 2014 guidelines recommend testing females according to clinical signs and symptoms, and males with persistent urethritis an/or who are contacts of individuals diagnosed with TV (9,10).

Conclusion

T. vaginalis was identified from urethral, vaginal and cervical swabs with standard diagnostics in B&H in higher frequency from males. Our data suggest that wet mount examination of fresh samples contribute to increase the sensitivity in the diagnosis of trichomoniasis.

Declaration of Interest

The authors declare no conflicts of interest.
References


