PREVALENCE OF CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE CO-INFECTIONS AMONG PATIENTS WITH NEWLY DIAGNOSED SYPHILIS: A SINGLE-CENTRE, CROSS-SECTIONAL STUDY

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SUMMARY

Objectives: The aim of the study was to determine the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* co-infections among patients with newly diagnosed syphilis.

Methods: In patients with any stage of newly diagnosed syphilis swabs were performed from urethra, rectum, pharynx and cervix according to the gender and type of sexual intercourse. From these smears standard validated nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections were done.

Results: From 548 (488 men, 60 women) screened patients co-infection was detected in 15.9% of the cases. The majority of the co-infections (86.2%) were asymptomatic. The overall prevalence of chlamydial infection was 11.1% and 8.8% for gonococcal infections. In men who have sex with men (MSM) the prevalence of co-infections was significantly higher (20.0%) than in heterosexual men and women (4.2%) ($p < 0.001$). In MSM patients the presence of co-infection was significantly associated with HIV infection ($p < 0.001$). Among MSM 9.6% of the tests detected infection in anorectal site, while prevalence in urethral (2.8%) and pharyngeal (2.4%) localization was significantly lower. In heterosexual patients prevalence was less than 2.0% in all anatomic sites.

Conclusions: The implementation of screening tests in case of sexually transmitted infections in patients with newly diagnosed syphilis is an important part in the management of this disease. These results suggest that screening of asymptomatic heterosexual patients leads to detection of minimum co-infections, but in MSM (especially HIV positive) should always be performed at least in anorectal site, where asymptomatic co-infections are common.

Key words: syphilis, gonorrhea, chlamydia, MSM, screening

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INTRODUCTION

Even in developed countries syphilis still remains an important medical problem with an increasing incidence, especially in specific sub-populations such as men who have sex with men (MSM) (1). These patients are also at an increased risk of acquiring other sexually transmitted infections (STIs). Therefore, it is not surprising that all current guidelines dealing with syphilis management recommend testing of other STIs in all newly diagnosed patients (2–5). The question is how and to what extent should the investigation be performed to be sufficient while at the same time not to be too burdensome on the healthcare system. However, studies dealing with the prevalence of the most common co-infections and effectiveness of screening in patients with syphilis are scarce especially in the Eastern and Central European regions. *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and *Chlamydia trachomatis* (*C. trachomatis*) are common co-infections in syphilis patients (1). A large proportion of these infections, especially in the pharyngeal and rectal localization, may be asymptomatic (6). If these co-infections are missed during the syphilis investigation and treatment, patients can further spread these co-infections as the drug of choice for syphilis treatment is penicillin which is not

effective against chlamydial and a large proportion of gonococcal infections (7). Moreover, the increasing antibiotic resistance of *N. gonorrhoeae* is a rising problem and it is necessary to minimize the number of infected patients (8).

Several studies tried to determine the most effective screening strategy for gonococcal and chlamydial infections in different populations in Western Europe, Australia and the United States (9, 10). Some of these studies attempted to suggest an appropriate strategy for screening of these infections among pregnant women (11–13), MSM (14–16) or HIV positive patients (17), but none of them was performed in the Central or Eastern European regions or was focused on patients with syphilis. From previously published studies we know that the risk factors for acquiring gonococcal and chlamydial infections are MSM, age between 15–24 years, a higher number of sexual partners, and a previous history of STI and HIV infection (9, 10, 14, 16, 17). A large proportion of patients with syphilis meet these risk factors hence testing for these infections in patients diagnosed with syphilis is necessary.

Prevalence studies especially in regions where chlamydial and gonococcal control activities are missing or are based only on case management are a valuable source of information for physicians and local authorities. Therefore, we performed a study to determine the prevalence of chlamydial and gonococcal co-infections among patients diagnosed with syphilis and treated at the Dermatovenerology Department of Na Bulovce Hospital, Prague, Czech Republic.

MATERIALS AND METHODS

Design and Population

A prospective cross-sectional study in patients who attended the Dermatovenereology Department of Na Bulovce Hospital, Prague, Czech Republic, was carried out between December 2010 and December 2015. The department is a specialized tertiary care centre and annually examines around 2,500 patients for STIs (of these, about a quarter are HIV positive MSM). Screening tests for *C. trachomatis* and *N. gonorrhoeae* infections were proposed to all patients with newly diagnosed syphilis. Screening was done in patients with primary infection or re-infection in all stages of syphilis (primary, secondary, early latent, late latent, and neurosyphilis) immediately after disease confirmation. All cases of syphilis were confirmed by the National Reference Laboratory for Diagnostics of Syphilis, Prague, Czech Republic. In patients with unknown HIV status at the time of the diagnosis, an HIV test was performed and repeated 3 months later. The patients in whom syphilis was diagnosed during the investigation for symptoms of other STI were not included in the study. Approximately 700 cases of syphilis are reported in the Czech Republic annually. This study during a five-year period screened over 15% of patients with newly diagnosed syphilis (18).

Clinical Investigation and Laboratory Methods

Swabs according to the type of sexual intercourse were performed in all patients who agreed with screening. Patients did not have to fulfill any special informed consent as the nucleic acid amplification tests (NAATs) are standard medical procedures. During the examination patients were asked about their age, sexual orientation and types of sexual intercourse. Among women practicing vaginal intercourse swabs from the cervix and the urethra were conducted. In women with a history of oral sexual intercourse, rectal or pharyngeal smear was performed as well. In male patients a urethral smear was taken and in the case of practicing receptive oral or anal intercourse, a swab from the pharynx and rectum was also performed. Samples were immediately transported to the laboratory.

There are currently several diagnostic methods for both infections. However, these methods differ in their sensitivity and specificity according to the type of material and tested locations. For the purpose of our study we chose NAATs as they are the most sensitive tests for chlamydial and gonococcal infections in all anatomic sites (urethra, cervix, rectum, pharynx). All previous studies have shown high sensitivity over 85% and specificity 97% of the NAATs in all types of specimens for both chlamydial and gonococcal infections (19–21). NAATs sample processing was done in the Microbiology laboratory of Na Bulovce Hospital using standardized methods. *N. gonorrhoeae* and *C. trachomatis* were detected by real-time PCR. DNA was extracted using "QIAamp DNA Blood Mini Kit" (Quiagen 51104) according to the manufacturer's protocol. The real-time PCR diagnostic kits used were "GeneProof Neisseria gonorrhoeae PCR Kit" (NG/ISIN/025) and "GeneProof Chlamydia trachomatis PCR Kit" (CHT/ISIN/025). The thermal cycler "CFX Connect™ Real-Time PCR Detection System" was used. Real-time PCR for *N. gonorrhoeae* detected two fragments: a conservative fragment of porA pseudogene and 16S r RNA. The second increased detection sensitivity by including strains with mutated porA gene. Real-time PCR for *C. trachomatis* detected specific 16S RNA sequence and multicopy sequence of cryptic plasmid. The lymphogranuloma venereum (LGV) genotype was identified by PCR amplification of a 262 bp fragment of the target DNA using dual-priming oligonucleotide (DPO) Seeplex® STI Master Panel 5 test developed by Seegene Inc. (Korea). This method targets the *pmp-H* gene and enables the simultaneous detection of LGV-serovars and the differentiation of L1-3 from other serovars (22).

Statistical Analyses

For statistical analyses patients were stratified according to their sexual preference to either MSM or heterosexual groups as they are very different populations. Heterosexual men and women are presented together, because in our study they did not differ in prevalence and demographic factors. For the distribution of co-infections in different anatomic sites, patients were divided into three categories – chlamydial, gonococcal and mixed (gonococcal + chlamydial) infections. In anorectal site all positive tests for *C. trachomatis* were further tested for chlamydia biovar. Hence, we divided the chlamydial infections into two sub-groups – LGV (caused by *C. trachomatis* L1-L3) and non-LGV chlamydial infections (*C. trachomatis* D-K). Statistical data processing was performed with Statistical Package for the Social Sciences (SPSS) software version 24.0. Logistic regression analyses were performed to assess determinants associated with the presence of co-infections. Determinants associated in the univariable analysis ($p < 0.1$) were counted in multivariable analysis. Pearson chi square test with Yates correction or Fisher's exact test was used

to calculate prevalence in different anatomic sites. All tests were two sided and the significance level was $\alpha = 0.05$.

RESULTS

During five years of the study, 548 (86.6%) out of 633 cases of newly diagnosed syphilis underwent the screening. These included 488 men and 60 women; 334 patients (331 men, 3 women) were HIV positive, 212 patients were HIV negative at the time of the screening and in the repeated HIV test 3 months later. In total 2,802 NAATs were performed: 1,401 for *C. trachomatis* and 1,401 for *N. gonorrhoeae*. Overall 120 tests were positive in 87 (15.9%) patients. From this overall number, asymptomatic infections were revealed in 77 (88.5%) and only 10 (11.5%) patients reported any symptoms during screening (5 patients with mild urethral discharge − all cases were chlamydial urethritis and 5 patients with anal discomfort or mucus in the stool – diagnosed as 4 chlamydial and 1 gonococcal proctitis). Overall prevalence of *C. trachomatis* co-infections of 11.1% was higher than for *N. gonorrhoeae* (8.8%). Only 85 (13.4%) patients, who were diagnosed for syphilis during the study, were not included. These were patients in whom asymptomatic syphilis was revealed because of investigation for other STI (37 patients with urethral discharge, 14 patients with proctitis) and patients who did not attend screening tests (34 patients).

The prevalence of co-infections differed significantly according to the sexual preferences of the patients. Among patients with heterosexual orientation only 6 (4.2%) had positive screening while 81 (20.0%) among MSM patients (OR = 5.77, 95% CI = 2.46–13.53, $p < 0.001$). In heterosexual males only 2 (2.4%) had positive screening (both positive for *N. gonorrhoeae* in urethra) and any infection reported 4 female patients (6.7%) (two with positive cervical test for *C. trachomatis*, one with gonococcal and another one with chlamydial urethral infection). Due to very low prevalence of co-infections in heterosexual patients further statistical analyses could not be performed for this group.

In MSM group prevalence of co-infections was significantly associated with the HIV infection (adjusted OR = 4.86, 95% CI = 2.08–11.33, $p < 0.001$). Positive screening had 7.8% HIV-negative and 23.6% HIV-positive MSM individuals. Highest prevalence of co-infections in MSM was observed in the age cohort 25–34 years, where 25.9% of patients had at least one co-infection ($p < 0.05$). We did not observe a difference in prevalence rates between different stages of the diseases or in comparison of the first infection versus syphilis reinfection (Table 1). In MSM cohort prevalence of *C. trachomatis* co-infections was even higher in all demographical and disease stage parameters compared to *N. gonorrhoeae* cases. For both co-infections prevalence was significantly associated with HIV infection ($p < 0.05$) (Table 2).

More than two thirds (70.1%) of patients with positive screening had rectal co-infection. Urethral and pharyngeal co-infections were present in 25.3% and 18.4% of patients, respectively. A cervical test was positive only in two (1.8%) patients. Further analysis revealed that 16.1% of patients with co-infections had multi-localized infection with urethra and anus as the most frequent combination (Fig. 1). The multi-localized infections were detected only among MSM.

In the study 44.8% of the detected infections were chlamydial infections, 29.9% gonococcal and 25.3% of patients with positive screening demonstrated both infections. In anorectal site almost the same representation of non-LGV chlamydial (29.5%), gonococcal

Table 1. *Patients with newly diagnosed syphilis and positive screening test for C. trachomatis and/or N. gonorrhoeae stratified by sexual preference, demographic characteristic and disease stage (N = 548)*

Characteristic	MSM (n = 404)						Heterosexual men and women (n = 144)			
	n	Positive screening	OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	n	Positive screening	OR (95% CI)	p-value
Age										
≤ 24	33	5 (15.2%)	0.87 (0.31–2.38)	0.78	1.06 (0.37–3.06)	0.91	13	2 (15.4%)	–	–
25–34	143	37 (25.9%)	1.69 (1.02–2.81)	< 0.05	1.72 (1.01–2.91)	< 0.05	52	4 (7.7%)	–	–
≥ 34	228	39 (17.1%)	1		1		79	0 (0%)	–	
HIV status										
Positive	314	74 (23.6%)	3.66 (1.62–8.25)	0.002	4.86 (2.08–11.33)	< 0.001	20	0 (0%)	–	–
Negative	90	7 (7.8%)	1		1		124	6 (4.8%)	–	
Syphilis stage										
Primary	63	11 (17.5%)	0.71 (0.34–1.49)	0.36	0.67 (0.31–1.43)[a]	0.30	12	0 (0%)	–	–
Secondary	155	30 (19.4%)	0.80 (0.47–1.37)	0.42	0.71 (0.41–1.23)[a]	0.22	21	0 (0%)	–	–
Latent	174	40 (23.0%)	1		1		106	6 (5.7%)	–	
Neurosyphilis	12	0 (0%)	–		–		5	0 (0%)	–	
Syphilis reinfection										
Yes	175	32	0.82 (0.50–1.35)	0.44	0.62 (0.37–1.05)[a]	0.08	6	0 (0%)	–	–
No	229	49	1		1		138	6 (4.3%)	–	
Overall	404	81 (20.0%)					144	6 (4.2%)		

[a]OR are adjusted for age and HIV status

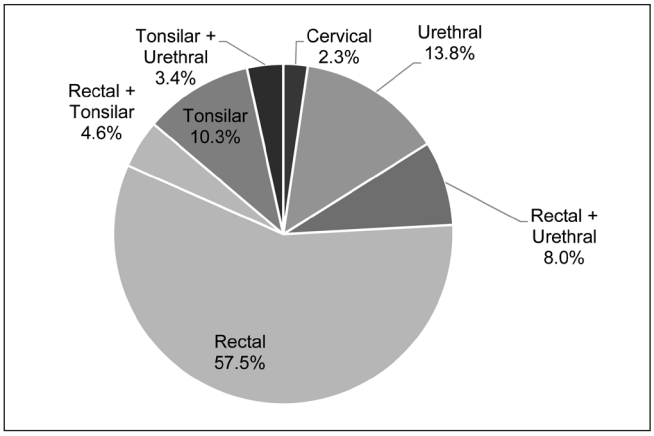

Fig. 1. *Distribution of detected co-infections in patients by anatomic site.*

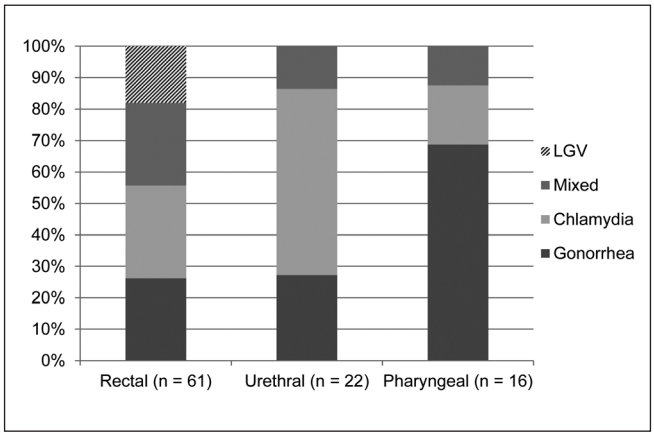

Fig. 2. *Representation of co-infections in patients by anatomic site.*

(26.2%), mixed (26.2%), and LGV infection (18.0%) was found. In the urethra, more than half (59.1%) of infections were caused by *C. trachomatis*. In the pharynx, more than three quarters (68.8%) of the co-infections were represented by *N. gonorrhoeae* (Fig. 2).

Overall 2,802 NAATs were carried out. Of this total number 120 (4.3%) samples were positive for one of the investigated co-infections. Prevalence of both co-infections was higher in all anatomic sites among MSM compared to heterosexuals. In MSM rectal swabs proved the highest efficiency compared to the other anatomic sites, out of 806 swabs tested 75 (9.3%) were positive ($p < 0.001$). The proportion of positive tests from the urethra (2.8%) and pharynx (2.4%) was significantly lower. The prevalence of chlamydial infection among MSM was higher in urethral and anorectal site, while in pharynx gonococcal infections were more common. Among heterosexuals the prevalence in all localizations was lower than 2% (Table 3).

DISCUSSION

In this cross-sectional study we assessed the prevalence of *N. gonorrhoeae* and *Chlamydia trachomatis* co-infections among patients with recently diagnosed syphilis in the Czech Republic. Generally, the prevalence of gonococcal and chlamydial co-infections in our study was moderate. At least one of the screened co-infections was found in less than one sixth of the patients (15.9%). Prevalence of co-infections varied significantly according to the sexual orientation, HIV status, age and localization.

Surveillance reports are an important source of information for physicians and healthcare providers. Without this data it is difficult to set up screening strategies for mainly asymptomatic co-infections to be sufficient but also cost-effective. Despite ef-

Table 2. *Prevalence of C. trachomatis and N. gonorrhoeae co-infections among MSM with newly diagnosed syphilis by demographic characteristic and disease stage (N = 404)*

Characteristic	*C. trachomatis*				*N. gonorrhoeae*		
	n	Positive patients	Adjusted OR (95% CI)	p-value	Positive patients	Adjusted OR (95% CI)	p-value
Age							
≤ 24	33	3 (9.1%)	0.82 (0.23–2.97)	0.76	3 (9.1%)	1.26 (0.34–4.63)	0.73
25–34	143	24 (16.8%)	1.33 (0.73–2.41)	0.36	22 (15.4%)	1.91 (0.99–3.69)	0.05
≥ 34	228	31 (13.6%)	1		20 (8.8%)	1	
HIV status							
Positive	314	54 (17.2%)	5.42 (1.85–15.84)	0.002	40 (12.7%)	2.85 (1.05–7.73)	< 0.05
Negative	90	4 (4.4%)	1		5 (5.6%)	1	
Syphilis stage[a]							
Primary	63	7 (11.1%)	0.55 (0.22–1.34)	0.19	6 (9.5%)	0.71 (0.27–1.88)	0.50
Secondary	155	20 (12.3%)	0.62 (0.33–1.15)	0.13	18 (11.6%)	0.86 (0.44–1.70)	0.67
Latent	174	31 (17.8%)	1		21 (12.1%)	1	
Neurosyphilis	12	0 (0%)	–	–	0 (0%)	–	–
Syphilis reinfection[a]							
Yes	175	25 (14.4%)	0.74 (0.41–1.34)	0.32	20 (11.4%)	0.90 (0.47–1.74)	0.76
No	229	33 (9.9%)	1		25 (10.9%)	1	
Overall	404	58 (14.4%)			45 (11.1%)		

[a]OR are adjusted for age and HIV status

Table 3. *Prevalence of N. gonorrhoeae and C. trachomatis co-infections among patients with newly diagnosed syphilis by anatomic site (N = 548)*

Site	MSM (n = 404)					Heterosexual men and women (n = 144)				
	C. trachomatis	*N. gonorrhoeae*	Overall	OR (95% CI)	p-value	*C. trachomatis*	*N. gonorrhoeae*	Overall	OR (95% CI)	p-value
Urethra	15/400 (3.8%)	7/400 (1.8%)	22/800 (2.8%)	1		1/125 (0.8%)	2/125 (1.6%)	3/250 (1.2%)	1	
Rectum	44/389 (11.3%)	31/389 (8.0%)	75/778 (9.6%)	3.77 (2.32–6.13)	< 0.001	0/14 (0%)	0/14 (0%)	0/28 (0%)	–	–
Pharynx	5/377 (1.3%)	12/377 (3.2%)	18/754 (2.4%)	0.86 (0.46–1.63)	0.86	0/40 (0%)	1/40 (2.5%)	1/80 (1.3%)	1.04 (0.11–10.16)	–
Cervix	–	–	–	–	–	2/56 (3.6%)	0/56 (0%)	2/112 (1.8%)	1.50 (0.25–9.09)	–
Overall	64/1166 (5.5%)	50/1166 (4.3%)	114/2332 (4.9%)			3/235 (1.3%)	3/235 (1.3%)	6/470 (1.3%)		

Data are number of detected infections/number of performed tests (%).

forts of the European Centre for Disease Prevention and Control (ECDC) gonorrhea and especially chlamydia testing and reporting in the Eastern and Central European regions remain insufficient (23). Moreover, in many countries across Western Europe *C. trachomatis* control activities like opportunistic testing for specific high-risk groups or organized screening tests are available but in the Eastern and Central European regions such programmes are not implemented or infrequently done (24, 25). Therefore, in regions where surveillance data are limited or missing, data obtained from a prevalence study with a sufficient number of patients may be essential source of information for specific implementation of prevention and screening strategies.

The prevalence of observed co-infections was higher among men than women. This difference was caused by MSM group, since the detection of co-infections among the heterosexual men and women was low and did not differ from that reported in the general population (26). Our results suggest that similarly as in the general population the prevalence of gonococcal and chlamydial infections among patients with recently diagnosed syphilis is strongly associated with sexual preference (9).

The prevalence of co-infections in MSM in our study was higher than was reported in the MSM population (15). Such result in a high-risk population of patients with already diagnosed STI (syphilis) is not surprising but is also caused by a high proportion of HIV-positive patients among MSM patients in our study, because the prevalence of co-infections in the HIV-positive MSM group was more than threefold higher than in HIV-negative MSM. This was similarly observed in other studies performed in MSM patients (6, 15). Detection of STIs (which are often asymptomatic) in this group is also important in the prevention of spreading HIV because it is known that STIs facilitate infection by the HIV virus (27). In patients who are already HIV positive, it is also necessary to diagnose STIs as soon as possible because every ongoing infection can cause progression of HIV (28).

Although it can be assumed that patients with syphilis reinfection will be more prone to risky sexual behaviour and therefore may have a higher prevalence of other STIs, in our study we did not observe any statistically significant differences in the co-infections rates in patients with first infection compared to patients with syphilis re-infection. There was also no difference in the prevalence according to the stage of the disease, with exception of lower prevalence in the patients with neurosyphilis. This may be due to a relatively small number of patients with this stage of diseases in our study. Another cause may be explained by higher probability of antibiotic treatment in these patients since they had acquired syphilis and possible STIs co-infections.

It is not surprising that most co-infections were detected in anorectal site whereas asymptomatic infections were mainly detected by the screening. Rectal infections were predominantly detected in HIV positive MSM individuals practising receptive anal sex. In these patients also multi-localized infections were the most common as in other published studies (6). The spectrum of co-infections was different by anatomic site. As stated before, the vast majority of co-infections were asymptomatic therefore it seems logical that in the urethra there were more prevalent chlamydial infections, as gonococcal urethral infections are usually symptomatic in males (29). In anorectal site gonococcal, LGV, non-LGV chlamydial and mixed infections were almost equally represented. It is important to note that 8 out of 11 (72.7%)

confirmed LGV infections were asymptomatic and 3 patients had only minimal symptoms. Screening of the most high-risk groups of patients with syphilis can also help to capture asymptomatic carriers of LGV infection.

The efficiency of performed complex NAATs screening was low, as only 4.3% of swabs were positive for at least one of the tested co-infections. Only rectal swabs detected gonococcal or chlamydial co-infection in almost every tenth test. A small proportion of detected co-infections in the urethra in both MSM and heterosexuals is probably due to the fact that most of chlamydial and gonococcal infections in this localization are symptomatic and patients with urethral discharge and dysuria may rather check a medical examination. The efficiency in cervical location was similar, but significantly less tests were performed, so the result can be distorted.

The main limitation of this study is that it presents data collected from a single centre and the prevalence of STIs can vary in different regions. Nevertheless, we assume that the study results are reproducible because in a recently published study from our centre, we have demonstrated that the characteristics of our patients are similar to those described in other European regions (30). We were also not able to further statistically analyze groups of heterosexual males and females because of the very low prevalence of co-infections in this group. Due to the voluntary nature of testing, a small part of patients diagnosed with syphilis in our department did not attend the screening and this could lead to a distortion of the results. Also we cannot exclude the possibility of both false positive and false negative results, as none of the NAATs has neither 100% sensitivity nor specificity. However, in all cases when another diagnostic test was also performed (gonococcal cultivation) the results of NAATs were identical.

CONCLUSIONS

In conclusion, our study confirmed that STI screening is an essential part of syphilis management. The prevalence of gonococcal and chlamydial co-infections among patients with newly diagnosed syphilis in the Czech Republic varies considerably according to the sexual orientation and HIV status in MSM. Presence of both co-infections in patients in our region was associated with similar risk factors as observed in Western Europe and in the United States. It is crucial to emphasize that the vast majority of diagnosed co-infections were asymptomatic and if the screening would not have been performed, these patients would have continued to spread it even after syphilis treatment with penicillin. The efficiency of sensitive but expensive NAAT testing in asymptomatic heterosexual HIV-negative individuals is low and leads to diagnosis of minimal chlamydial and gonococcal infections. Conversely in MSM especially in HIV-positive patients it should be always performed at least in anorectal site, where asymptomatic co-infections are common.

Conflict of Interests
None declared

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