

Urogenital *Chlamydia trachomatis* in the environment of soldiers from the Polish Special Forces

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Abstract

Introduction and objective. Urogenital *Chlamydia trachomatis* is one of the most common sexually transmitted bacterial pathogens. The aim of the study was to present the current occurrence of chlamydial infections among Polish soldiers, sexually-active men and women at reproductive age.

Materials and method. The research involved 253 active duty soldiers from the Polish Special Forces, 237 men and 16 women aged 26–57, stationed in Warsaw between October – November 2016. The study participants were asked to fill a socio-demographic questionnaire and then subjected to diagnostic tests. These included a urine test for the presence of *Chlamydia trachomatis* DNA using the Real-Time PCR assay with fluorescently labeled markers and probes, complementary to plasmid DNA of the bacteria (DNA isolated from urine samples was used as matrix).

Results. *Chlamydia trachomatis* infection was detected in two male soldiers, non-commissioned officers, at mean age 40.5 years (total: 38.0 years); reporting sexual contacts with 2–3 partners in the last 12 months (total: 141 soldiers – 1 partner, 66 – 2–3 partners, 46 – >4 partners), with no UTI symptoms.

Conclusions. Among all the study participants, of whom more than 40% reported sexual contacts with 2–4 or more partners within the last 12 months, only 0.8% were found to be infected. The low prevalence of *Chlamydia trachomatis* infection can be associated with a regular or frequent use of STI prevention measures during casual sex, or having a single sexual partner.

Key words

RT-PCR, *Chlamydia trachomatis*, Polish soldiers

INTRODUCTION

Urogenital *Chlamydia trachomatis* is an intracellular gram-negative bacteria transmitted by oral, vaginal or anal sex; it can also be transmitted vertically, from a mother to her newborn child during a vaginal delivery [1]. The risk of transmission from a single episode of sexual intercourse is estimated to be 10–20% [2, 3]. The most common risk factors for *Ch. trachomatis* infections are risky sexual behaviours (sex with prostitutes, casual sex with strangers, sex with multiple partners, homosexuality, sex with partners taking drug injections, sex with partners likely to have sexually-transmitted infections /STIs/ carriers, unprotected sex /condoms/) [4]. The infection is asymptomatic in nearly 90% of infected men and 70% of women [5].

The significant problem associated with *Ch. trachomatis* infection is its long incubation period and its frequent transition into a chronic form which may persist for many years. Transmitting the infection to other sexual partners can lead to delayed complications, including epididymitis, orchitis in men, and ectopic pregnancy and infertility in women [6]. Signs and symptoms of the infection in women include cervicitis (mucopurulent endocervical discharge, easily induced endocervical bleeding), sometimes urethritis (pyuria, dysuria, urinary frequency). The infection can spread

from the cervix to the upper reproductive tract (uterus, fallopian tubes) causing pelvic inflammatory disease [7]. Signs and symptoms in men include burning, frequent and painful urination, penile discharge, swollen and painful testicles [8].

Chlamydia trachomatis is a cosmopolitan infection with a global incidence estimated at 61.1 million cases in 2015 [9]. According to the World Health Organization (WHO) more than 50% of new cases are reported in South and South East Asia [4]. In the European Union, approx. 400,000 cases are diagnosed every year [5]. Chlamydia infection is the most common bacterial sexually-transmitted infection (STI) in the United States (1,526,658 cases were reported to the Centers for Disease Control and Prevention, and an estimated 2.86 million infections occur annually) [10]. A large number of cases are not reported because most people with *Ch. trachomatis* are asymptomatic and do not seek testing or medical assistance. The global prevalence of Chlamydia infections among women/men aged 15–49 years is estimated at 4.2% and 2.7%, respectively [11]. Two-thirds of the infections occur in people aged 15–24 [12]. Women are more often screened for Chlamydia than men, which is associated with the fact that they receive regular gynaecological and prenatal care, and they are more likely to develop a symptomatic urogenital infection [13]. The biological material used for *Ch. trachomatis* tests in adults is usually a cervical or a vaginal swab, and a urine sample in women, and a urethral swab or urine sample in men. The number of patients giving consent for Chlamydia screening tests, as well as the number of tests performed, largely

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increased when molecular diagnostics were introduced. Molecular biology testing, based on nucleic acid amplification tests (in particular non-invasive urine examination in men), have largely replaced culture or enzyme immunoassays owing to its high sensitivity [14]. In general, chlamydial infections are more often found among sexually-active individuals with multiple sexual partners. They are presently considered one of the most significant public health issues globally, also in the military environment [15].

The aim of the study was to present the current occurrence of *Chlamydia trachomatis* infections among the Polish Special Forces soldiers, sexually-active men and women at reproductive age.

MATERIALS AND METHOD

Ethics. The research project entitled “Prevalence of selected infections in the environment of soldiers from the Special Forces” was accepted by the Committee on Bioethics at the Military Institute of Medicine (Decision No 41/2016, dated 15 July 2016) on the basis of the Declaration of Helsinki (2013) and the rules elaborated by the European Union, “Good clinical practice for trials on medicinal products in the European Community. The rules governing medicinal products in the European Community” (1990), ratified by the Ethics Committee in Poland (March 1993).

Study population. The research involved 253 active duty soldiers from the Polish Special Forces stationed in Warsaw between October – November 2016; 237 men and 16 women aged 26–57. The study participants were asked to fill-in a socio-demographic questionnaire and then subjected to diagnostic tests. These included a urine test for the presence of *Chlamydia trachomatis* DNA using the Real-Time Polymerase Chain Reaction (PCR) assay with fluorescently labeled markers and probes, complementary to plasmid DNA of the bacteria (DNA isolated from urine samples was used as matrix). The tests were carried out using the Abbott m2000rt assay.

The following exclusion criteria were applied: patients using antibiotics or other antimicrobial agents (p.o./i.v./i.m.) within 7 days before the test; patients using vaginal preparations or medications applied to external genitals within 48 hours before the test; female patients during menstruation.

Preparation of patients and samples. Patients had to avoid sexual activity and limit their genital hygiene 24 hours before the test; patients collected 20–30 ml of first catch urine into a sterile container (they must not have urinated during the previous hour); the urine sample was delivered to a test site within 2 hours after sample collection. Up to 3 hours after collection, each urine sample (approx. 3 ml) was transferred to a tube containing a liquid transport medium (guanidine thiocyanate in Tris buffer) Abbott multi-Collect Specimen Collection Kit (Abbott, Germany). Up to 24 hours after collection, each urine sample was examined qualitatively by Real Time PCR assay.

Laboratory procedures. Real-Time PCR assays with fluorescently labeled markers and probes specific for plasmid DNA of *Chlamydia trachomatis* were used:

- sample preparation – a urine sample containing epithelial cells was treated with a detergent solution to release *Chlamydia trachomatis* DNA contained in the chlamydial reticulate bodies; a second detergent solution was then added to prepare the lysed specimen for amplification;
- PCR amplification – selection and amplification of the target sequence, internal control amplification, selective amplification;
- PCR product detection – the use of dual fluorescent dye-labeled probes allows for the real time detection of the PCR products by measuring the fluorescence intensity from the reporter dyes released during the amplification process. Probes consisted of oligonucleotides specific for *Chlamydia trachomatis* (CT) cryptic plasmid DNA, the CT *ompA* gene and the CT internal control, labeled with reporter and quencher dyes.

During the PCR reaction the probes are hybridized to the target sequence and then cleaved by 5' and 3' nuclease activity of the thermostable Z05 DNA polymerase; the amplification of the CT cryptic plasmid DNA and/or the target *ompA* gene DNA were measured independently and at a different wavelength than the CT internal control. The process was repeated for a specified number of cycles, each time with an effectively increased fluorescent intensity of the reporter dyes, permitting independent identification of the *Chlamydia trachomatis* DNA and the CT internal control.

Statistical analysis. Statistical analysis was performed using the statistical programme StatSoft. Inc. (2011), Statistica (data analysis software system), version 10.0. (SN JGNP3087539302AR-E), and Excel. The quantitative variables were characterized by the arithmetic mean of standard deviation or median or max/min (range). Statistical significance of differences between 2 groups (unpaired variables model) was processed with the t-Student test (or Welch test in the case of lack of homogeneity), or U Mann-Whitney test (in cases where conditions of performing the t-Student test were not satisfied or for variables measured by ordinal scale). Chi-squared tests for independence were used for qualitative variables. The qualitative variables were presented with the use of count and percentage. In all the calculations, the level of significance $p=0.05$ was used.

RESULTS

Chlamydia trachomatis infection was detected in 2 male soldiers, non-commissioned officers, at mean age 40.5 years, reporting sexual contacts with 2–3 partners in the last 12 months, using condoms in 50%, with no urinary tract infection (UTI) symptoms (Tab. 1).

There was no statistically significant difference regarding the age of the soldiers and the *Chlamydia trachomatis* infection distribution ($p=0.4594$). It was not possible to calculate the statistical significance of age between infected and non-infected patients, due to the small number of the infected individuals ($n=2$). There were no statistically significant differences between the marital status of soldiers examined ($p=0.7307$), the number of sexual partners in the last 12 months ($p=0.3247$), the use of condoms ($p=0.8762$) or the presence of symptoms ($p=0.9986$) and the presence of *Chlamydia trachomatis* infection.

Table 1. Sociodemographic variables of soldiers tested for *Chlamydia trachomatis* infection using RT-PCR (n=253)

Socio-demographic variables	Infected with <i>Chlamydia trachomatis</i> (n=2)	Non-infected with <i>Chlamydia trachomatis</i> (n=251)	P-value
Age			
Mean (SD)	40.5 (0.7)	38.0 (5.5)	----
Range	40.0–41.0	26.0–57.0	
Sex			
Women	0 (0.0%)	16 (6.4%)	0.2760
Men	2 (100.0%)	235 (93.6%)	
Military rank			
Officer	0 (0.0%)	70 (27.9%)	0.9325
Non-commissioned officer	2 (100.0%)	181 (72.1%)	
Marital status			
Single	1 (50.0%)	64 (25.5%)	0.7307
Married	1 (50.0%)	186 (74.1%)	
Partnership	0 (0.0%)	1 (0.4%)	
Number of sexual partners in the last 12 months			
1	0 (0.0%)	141 (56.2%)	0.3247
2–3	2 (100.0%)	64 (25.5%)	
> 4	0 (0.0%)	46 (18.3%)	
Use of condoms			
Yes, regularly	1 (50.0%)	105 (41.8%)	0.8762
No	0 (0.0%)	85 (33.9%)	
Sometimes	1 (50.0%)	50 (19.9%)	
Often	0 (0.0%)	11 (4.4%)	
Presence of symptoms			
No symptoms	2 (100.0%)	238 (94.8%)	0.9986
Urethral discharge, burning	0 (0.0%)	1 (0.4%)	
Frequent urination	0 (0.0%)	2 (0.8%)	
Urethral burning	0 (0.0%)	8 (3.2%)	
Urinary urgency	0 (0.0%)	2 (0.8%)	
History of <i>Ch. trachomatis</i> infection			
Yes	0 (0.0%)	0 (0.0%)	---
No	2 (100.0%)	251 (100.0%)	
History of other STIs			
Yes	0 (0.0%)	0 (0.0%)	---
No	2 (100.0%)	251 (100.0%)	
Receiving STI treatment			
Yes	0 (0.0%)	0 (0.0%)	---
No	2 (100.0%)	251 (100.0%)	
Use of antimicrobial agents within the last 7 days			
Yes	0 (0.0%)	0 (0.0%)	---
No	2 (100.0%)	251 (100.0%)	

DISCUSSION

This research presents the results of a study into the prevalence of *Chlamydia trachomatis* infections in the group of soldiers from the Polish Special Forces. The study participants were all sexually-active men and women, of whom 43.8% reported

sexual contacts with 2–4 or more partners in the last 12 months. 41.9% reported the regular use of condoms during sexual intercourse and 57.3% had a single sexual partner, which could potentially be the cause of the very low infection rates. The military environment has always been of special interest to the medical services with regard to the spread of infections and the routes of disease transmission, including sexual transmission. The military environment is an example of a closed community with professional activities very different from those experienced in the civilian environment. The soldiers are usually young, active and healthy men, frequently relocated to different military units across Poland or abroad, and reporting sexual contacts with both steady and casual sex partners. Chlamydia tests are routinely performed in military personnel, and especially among soldiers from the US Forces. Chlamydia infection has been the most commonly reported STD in the US Forces for many years [16]. Still in the early 1990s, the prevalence rate of *Ch. trachomatis* infections among the US soldiers was as high as 15.6%, especially among young recruits [17, 18]. A research study conducted several years later, between 2005–2010, involving 67,425 US Forces soldiers stationed in Fort Bragg (North Carolina, USA), one of the largest military bases in the USA, showed the overall prevalence of *Chlamydia trachomatis* infections of 3.3% in the study group (n=2,198). Such a drastic reduction in Chlamydia cases might have been attributable to a more regular use of STI prevention measures (condoms), mostly resulting from the fear of the global AIDS epidemic. Chlamydia was more often found in female soldiers, under 26 years of age, with lower rank, single, of non-white race [19]. American soldiers are a high-risk group as far as the incidence of STD's is concerned, as much as 40% of the US military personnel are under 25 years of age. Also, for this reason, the medical authorities of the US Army recommended screening for *Ch. trachomatis* in all young recruits joining the United States Armed Forces [20].

Screening tests for chlamydial infections are very rarely performed in soldiers from the Polish Armed Forces; therefore, the actual prevalence rates of *Chlamydia trachomatis* in the military environment in Poland is unknown; to-date, only 2 such studies have been conducted. Apart from the research project discussed in the presented article, Korzeniewski et al. conducted a similar study between March – April 2012. The study involved 66 professional soldiers, healthy, asymptomatic men, aged 27–44, serving in a military unit in Warsaw. Urine samples taken from patients were investigated using molecular methods (Cobas TaqMan, RT-PCR). Of all the study participants, 2 soldiers were found to be infected (aged 33 and 34-years-old) with *Ch. trachomatis* (3.0%). They both reported casual sex without STI prevention measures with >4 women in the last 12 months. In contrast, two-thirds of the examined group of soldiers reported the use of condoms during casual sexual contacts. According to the examined men, the regular use of STI prevention measures was associated with the fear of HIV infection [21].

The National Institute of Public Health – National Institute of Hygiene is the main governmental body responsible for monitoring the incidence rates of infectious diseases in Poland. Its first report on *Chlamydia trachomatis* prevalence in the general Polish population was issued in 2013. Between January – December 2013, sanitary and epidemiological stations and other medical facilities reported 309 Chlamydia cases across Poland (incidence 0.8/100,000 residents). The

report of 2016 showed 232 *Ch. trachomatis* infections (incidence 0.6/100,000 residents) [22]. It is difficult to assess whether such prevalence of chlamydial infections in a 38-million population is due to low detection rates (accidental diagnosis of symptomatic cases) or if it proves low incidence of chlamydial infections in the Polish population. All the previous studies carried out among professional soldiers, who are generally at a greater risk for an STI infection than other occupational groups, demonstrated low prevalence rates of *Chlamydia* infections, especially in the USA and the EU countries.

Limitations. The research task involved only those soldiers who gave informed consent to participate in the study, which limited the number of participants and the number of tests performed. The study group represented over 50% of the total manpower of the pre-selected military unit, and over 10% of the Polish Special Forces manpower.

CONCLUSIONS

Among all the study participants, of whom more than 40% reported sexual contacts with 2–4 or more partners within the last 12 months, only 0.8% were found to be infected. The low prevalence of *Chlamydia trachomatis* infection can be associated with the regular or frequent use of STI prevention measures during casual sex, or having a single sexual partner.

Conflict of interests

The author declares no conflicts of interests in relation to this article.

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