Correlation of serotypes, molecular and biological characteristics of *Chlamydia trachomatis* and clinical manifestations of *Chlamydia infection*

Związek serotypów oraz cech molekularnych i biologicznych *Chlamydia trachomatis* z kliniczną manifestacją zakażenia tym drobnoustrojem

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Abstract

Aim: This study aimed to establish the correlation between serotypes, molecular and biological characteristics of and various clinical manifestations of *Chlamydia* infection.

Material and methods: Quantitative evaluation of specific ompA fragments of a *Chlamydia trachomatis* gene as well as DNA synthesis were performed applying PCR in a real-time mode (Rotor-Gene 3000, Australia) with application of species-specific primers and TaqMan oligonucleotide tests. Standardization of the study was made against the NAGK one-copy human gene. Successions of primers, probes and conditions of amplification for quantitative evaluation of the NAGK human gene were adapted from the work by Gotoh et al. (2005). Serotyping of *C. trachomatis* was performed by PCR sequencing with application of couples of primers for detection of variable domains (VDI-VDIV) of the *omp1* gene.

Results: Predominant distribution of serotypes D (54.7%), K (35.7%) and (9.6%) of *C. trachomatis* has been revealed. In 94-95% of cases there is homology with standard D/B120 (X62918), D/B185 (X62919), K/UW31/Cx (AF063204) cultures. Nineteen isolates, isolated from the biological material and identified as serotype D, had 2 different nucleotides of D/B185 or D/B120 omp1 in positions 574 and 843, while 4 samples tended to have additional nucleotide change in position 1042. Fifteen isolates, identified as serovar K, had two different nucleotides of the K/UW31/Cx *omp1* gene in positions 503 and 628, while 4 isolates, identified as serovar C, had 4 different nucleotides of the C/TW-3/OT *omp1* gene in positions 569, 571, 972 or 1003.

Conclusions: Serotypes D and K of *C. trachomatis* turned out to be the most frequent cause of pathology of the urogenital tract. An associative correlation has been noted between C and K *C. trachomatis* serotypes. These two serovariants of *C. trachomatis* tend to more often possess resistance to antibiotics, which can serve as the main cause of development of arthropathic forms (Reiter's syndrome) and sterility in women.

Key words: clamidiosis, infectious process, serotypes, PCR.

Streszczenie

Cel: Celem pracy była analiza korelacji między serotypami, cechami biologicznymi i molekularnymi a różnorodnym obrazem klinicznym infekcji wywołanych przez chlamydie.

Materiał i metody: Przeprowadzono ilościową ocenę swoistych fragmentów ompA genomu *Chlamydia trachomatis*, jak również wykonano syntezę DNA metodą *real-time* PCR (Rotor-gene 3000, Australia) przy użyciu gatunkowo swoistych primerów i technologii TaqMan. Standaryzację badania oparto na pojedynczej kopii ludzkiego genu referencyjnego NAGK. Szeregi primerów, sond oraz warunki amplifikacji do ilościowej oceny ludzkiego genu *NAGK* oparto na doniesieniu Gotoh i wsp. (2005). Serotypowanie *C. trachomatis* wykonano za pomocą sekwencjonowania PCR z udziałem par primerów w celu wykrycia zmiennych obszarów (VDI-VDIV) genu *omp1*.

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Wyniki: Stwierdzono dominację serotypów D (54,7%), K (35,7%) i C (9,6%) *C. trachomatis*. W 94–95% przypadków wykryto homologię w stosunku do standardowych hodowli D/B120 (X62918), D/B185 (X62919), K/UW31/Cx (AF063204). Dziewiętnaście izolatów uzyskanych z materiału biologicznego i zidentyfikowanych jako serotyp D charakteryzowało się dwoma różnymi nukleotydami D/B185 lub D/B120 omp1 w pozycjach 574 i 843, podczas gdy w 4 próbkach występowała dodatkowa zmiana nukleotydów w pozycji 1042. Piętnaście izolatów zidentyfikowanych jako serowar C miało 4 różne nukleotydy genu *omp1* C/TW-3/OT w pozycjach 569, 571, 972 lub 1003.
Wnioski: Serotypy D i K *C. trachomatis* okazały się najczęstszą przyczyną patologii układu moczowo-płciowego. Stwierdzono istnienie korelacji między serotypami C i K drobnoustroju a obfitymi w powikłaniu odmianami zakażenia chlamydią. Wspomniane 2 serowarianty *C. trachomatis* wykazują częściej oporność w odniesieniu do antybiotyków, co może przyczynić się do rozwoju zmian stawowych (zespół Reitera) i bezpłodności u kobiet.

Słowa kluczowe: zakażenie chlamydiami, stan zapalny, serotypy, PCR.

Introduction

Causative agents of *Chlamydia* infections belong to the *Chlamydiaceae family*, *Chlamydia* genus. Within this genus are recognized four types of chlamydiae, i.e. *C. trachomatis, C. psittaci, C. pneumoniae* and *C. pecorum* [1].

Antigenic properties of chlamydiae are preconditioned by the interior membrane represented by liposaccharides. Outer membrane proteins (OMP) are integrated into it. Major outer membrane protein (MOMP) is known to comprise 60% of the general protein content. The rest of the antigen structure is represented by type 2 proteins of the outer membrane (OMP-2). The presence of a general bulk genus-specific antigen enables the *Chlamydia* infection to be diagnosed by means of the immune fluorescent method based on the level of specific antibodies in it. The genus-specific antigen is known to be different in all species of chlamydiae. It contains more than 18 different components. Its size in *C. trachomatis* is 155 kDa and it contains epitopes in protein of 40 kDa, the hsp-60 protein of thermal shock. The type-specific antigen is known to be different for serovars of *C. trachomatis*, and contains epitopes in 40 kDa, the MOMP protein, the 30 kDa protein in A and B serotypes [2, 3].

Eighteen antigenic variants (serotypes) of *C. trachomatis* have been differentiated and classified into three groups. Group I consists of causative agents of trachoma (serotypes A, B, Ba, C). Its carriers are known to be insects. The main way to get infected is to rub in the agent into the mucous coat of the eye. Group II (present only in man) comprises causative agents of urogenital clamidiosis (serotypes D, E, F, G, H, I, J, K). In cases of urogenital clamidiosis (UGC) the infection enters the body during sexual intercourse (sexual clamidiosis). Rare cases of this infection can occur when rubbing in the agent with dirty hands into the mucous membrane of the eye. Pneumonia of newborns caused by *C. trachomatis* occurs as the result of infection of the newborn with *Chlamydia* from the mother during birth.

Group III (serotypes L1, L2, L3) comprises causative agents of tropical venereal lymphogranuloma. Serotypes of venereal lymphogranulomatosis spread through the lymphatic system, infecting macrophages and epithelial cells.

The main ways to get infected with *C. trachomatis* are sexual, vertical and everyday regular contacts. Haematogenous way of spread of the infection is characteristic for serotypes D and K of *C. trachomatis,* associated with systemic rheumatoid diseases [1]. Besides, it has been established that H and L2/434/BU *C. trachomatis* types can infect the endothelial cells of human veins and stimulate expression of tissue factor that reaches its maximum 18 hours following infection.

From 60 to 80% of all strains belong to serotypes D, E and F, with minor variations in various parts of the world [4, 5]. The serotype is capable of defining the infectious nature of a causative agent, which can be measured by the number of inclusion-forming units (IFU) of *Chlamydia* in clinical samples. It has been proven that sex, age and race of patients as well as their infection can be connected with a definite type of *C. trachomatis* and associated with the number of IFU. Samples with strains of B group tended to have a larger number of IFU than samples with strains of group C [5]. In female organisms the IFU index is higher than in male organisms. Ageing causes a decrease of this index. It has been shown that persistence of the causative agent in the human organism is connected with serotype of group C [2].

Within the course of evaluation of dependence of the character of clinical pathology on the serotype of the causative agent it has been established that the rate of development of endocervicitis and bleeding in females with *Chlamydia* infection does not depend on the variant of serotype, while in males with a small number of polymorphonuclear leucocytes in urethral scrapings the probability of diagnosis of serotype F or G was rather high. Serotype variant F was found in women from San Francisco in the background of the presence of inflammation of pelvic organs [4]. In other clinical studies males with the symptoms of clamidiosis demonstrated a rather high probability of selection of serotype variant Da, in contrast to females, in whom serotype variant K had been isolated [4, 5].

Aim

This study aimed to establish the correlation between serotypes, molecular and biological characteristics of and various clinical manifestations of *Chlamydia* infection.

Material and methods

Forty two patients (18 females and 24 males), aged 17-44, who applied for medical aid at the Department for Prevention of Sexually Transmitted Infections attached to the Grodno Regional Dispensary for Skin and Venereal Diseases, Belarus, underwent complex clinical and microbiological examination. All the above patients were divided into several groups depending on the types of isolated serotypes of *C. trachomatis*, concentration of DNA of *C. trachomatis*, variant of the infection (monovalent/polyvalent infection), stability/sensibility to antibiotics and the prevailing clinical variant of *Chlamydia* infection (localization of inflammatory process, complications).

Verification of aetiological diagnosis

The diagnosis of *Chlamydia* infection was made on the basis of anamnesis, clinical signs, results of aetiological verification, i.e. search for IgA, IgG, IgM class antibodies to antigens of *C. trachomatis* by method of immune-enzyme analysis (IEA), and isolation of *Chlamydia* from scrapings obtained from the cervical canal and urethra by the method of direct reaction of immune fluorescence (DIF). In the case of positive IEA and DIF the material was studied by means of PCR (quantitative analysis). Patients with 100% positive PCR results were included in the study. Aetiological decoding of mixed infection was performed using routine methods, i.e. in cases of ureaplasmosis and mycoplasmosis we applied DIF and culture method (inoculation onto the IST medium with further evaluation of antibiotic resistance); in cases of candidosis and gardnerellosis by means of bacterioscopic analysis of Gram-stained smears; and in cases of trichomoniasis by means of bacterioscopic evaluation of smears stained with 1% solution of methylene blue.

Quantitative analysis of DNA of *Chlamydia trachomatis*

To quantitatively evaluate specific ompA fragments of a *C. trachomatis* gene, coding the main MOMP surface protein, we performed PCR in a real-time mode (Rotor-Gene 3000, Australia) with application of species-specific primers and TaqMan oligonucleotide tests. Standardization of the study was made against the NAGK one-copy human gene. Successions of primers, probes and conditions of amplification for quantitative evaluation of the NAGK human gene were adapted from the work by Gotoh et al. (2005) [6, 7].

Serotyping of *Chlamydia trachomatis*

In order to define serotypes of *C. trachomatis* 42 samples of DNA, isolated from 42 *C. trachomatis*-positive samples of biological material (scrapings of epithelial cells from the urogenital tract) amplified and purified in Centricon-100, underwent PCR sequencing with application of couples of primers for detection of variable domains (VDI-VDIV) of the *omp1* gene. Due to the existing differences in nucleotide sequences of variable domains of the *omp1* gene among these or those serovars the below pairs of primers were applied in genotyping of isolates of *C. trachomatis*:

C. trachomatis 1 – 5´ATGAAAAAACTCTTGAAATCGG–3´ (forward-primer P1);

C. trachomatis 2 – 5´ACTGTAACTGCGTATTTGTCTG–3´ (reverse-primer OMP2), enabling detection of the fragment of 1 *C. trachomatis* gene of ~1130 p.n. omp in length, containing all the four variable domains (VDI-VDIV);

C. trachomatis 1 – 5´ATGAAAAAACTCTTGAAATCGG–3´ (forward-primer P1);

C. trachomatis 2 – 5´CTTGKAYTTTAGGTTTAGATTGAGC–3´ (reverse-primer CT6R), enabling detection of the fragment of 1 *C. trachomatis* gene of ~675 p.n. omp in length, containing two variable domains (VDI, VDII);

C. trachomatis 1 – 5´GCTCAATCTAAACCTAAARTMCAAG–3´ (forward-primer CT6F);

C. trachomatis 2 – 5´ACTGTAACTGCGTATTTGTCTG–3´ (reverse-primer OMP2), enabling detection of the fragment of 1 *C. trachomatis* gene of ~481 p.n. omp in length, containing two variable domains (VDIII, VDIV);

C. trachomatis 1 – 5´TGGGATCGYTTTGATGTATT–3´ (forward-primer NL-F);

C. trachomatis 2 – 5´CCAATGTARGGAGTGAACAT–3´ (reverse-primer NL-R), enabling detection of the fragment of 1 *C. trachomatis* gene of ~481 p.n. omp in length, containing two variable domains (VDII, VDIII).

Out of *C. trachomatis*-positive samples all 42 samples were successfully amplified with application of P1-OMP2 primers, the interior pair of NL-F – NL-R primers and CT6F-OMP2 as well as P1-CT6R primers for semi-cluster PCR. For each reaction of amplification we added 10 P1-OMP2 of DNA matrix to the PCR mixture (the final volume of the reaction comprised 50 µl), containing PCR-buffer (10 mM Tris-HCL, 50 mM KCl, pH 8,3, 2,0 mM $MgCl_2$), 200 mM $MgCl_2$ of each deoxynucleotide triphosphate, Taq polymerase.

Primers were added in the following amounts (Table 1). The amplification programme was designed in the following way:

94°C – 10 min (initiation of reaction),
94°C – 30 s (melting),
50°C – 30 s (curing),
72°C – 2 min (elongation), } 40 cycles
72°C – 7 min (additional time of elongation of elongation at the end of the reaction).

The amplified fragments were visualized by electrophoresis in 1% agarose gel containing ethidium bromide. Electrophoregram of each 42 samples had 4 specific fragments, i.e. P1/OMP2, P1/CT6R, CT6F/OMP2 and NL-F/NL-R. The length of the P1/OMP2 fragment was represented by 1124 pairs of nucleotides (p.n.) in 28 samples and by 1114 p.n. in 14 samples; the length of the P1/CT6R fragment by 670 p.n. in 28 samples as well as by 679 p.n. in 14 samples; the length of the CT6F/OMP2 fragment in 28 samples by 479 p.n., in 14 samples by 480 p.n.; the length of the NL-F/NL-R fragment in all 42 samples was represented by 482 p.n.

PCR amplicons were purified using the BigDye XTerminator Purification kit and underwent sequencing (with further electrophoresis using the ABI Prism 310 genetic analyzer) using pairs of P1/OMP2, P1/CT6R, CT6F/OMP2, NL-F/NL-R primers and the following mode of operation:

96°C – 20 s – 30 cycles (melting),
50°C – 20 s (annealing),
60°C – 4 min (elongation),
4°C (incubation).
} 40 cycles

To increase the liability of the reaction the amplicons were sequenced in two directions. The information on all the nucleotide sequences was combined into one sequence with about 863 pairs of nucleotides, comprising 4 omp1 variable domains. This sequence comprised 73% of the total length of the *C. trachomatis omp1* gene.

Data on the nucleotide sequences of samples were considered with application of the (www.ncbi.nlm.nlm.nih.gov/blast/bl2seq/bl2.html) nucleotide-nucleotide BLAST search engine for identification of attribution of this or that sequence to a definite serotype.

Nucleotide sequences omp1 of genes of standard samples of all 15 serotypes (A-L3) were obtained from the GenBank database, i.e. A/Har-13 (J03813), A/Sa1/OT (M58938), B/Alpha-95 (U80075), B-Jali-20 (M33636), B/TW-5/OT (M17342), BaApache-2 (AF063194), C/TW3 (AF202455), C/TW3/OT (M17343), C/TW-3/OT (AF352789), D/B120 (X62918), D/B185 (X62919), D/IC-Cal8 (X62920), E/Bour-1990 (X52557), E/Bour-1997 (U78763), F/IC-Cal (X52080), G/UW57/Cx (AF063199), H/Wash (X16007), I/UW-12 (AF063200), J/UW36/Cx (AF063202), K/UW31/Cx (AF063204), L1/440-Bu (M36533), L2/434-Bu (M14738), L3/404-Bu (X55700), MoPn (M64171).

The sequencing analysis software module was applied to analyze the data on sequencing of DNA. The software processed the obtained data with application of multi-component analysis, by means of deducting the base line and scaling. Having processed the data the software could detect the peaks and define the sequence of the bases. Software for evaluation of data on sequencing was connected with the DataUtility software which could perform the following two functions: creation of matrices for their further application in computer software for accumulation and evaluation of the data as well as noise level control. The use of the program package enabled us to show the results of the experiment on the computer display (i.e. electrophoregrams, data processed in the form of tables and diagrams or a combination of electrophoretic data and corresponding diagrams).

Results

Sequence analysis of the region comprising VDI-VDIV showed that 15 samples of patients under study belonged to serotype K with 95% compliance with the sequence of K/UW31/Cx gene variant, and 23 samples were attributed to serotype D. In the case of 9 samples compliance with the sequence of D/B185 (X62919) gene variant comprised 95% while in 3 samples compliance with the sequence of the D/B185 (X62919) gene variant equalled 94%; 11 samples demonstrated 95% compliance with the sequence of the D/B120 (X62918) gene variant and 4 samples complied with serotype C with 95% compliance with the sequence of the C/TW-3/OT (AF352789) gene variant. Studies of sequences of nucleotides of 42 PCR-positive samples, conducted with application of the BLAST computer software, demonstrated the predominant prevalence of *C. trachomatis* genotype of serotype D (n=23, 54.8%) over serotype K (n=15, 37.7%) and C (n=4, 9.5%). In this case various nucleotide changes in the fragment of omp1 gene under study could be observed (Table 2).

It should be mentioned that 19 isolates, isolated from the biological material and identified as serotype D, had 2 different nucleotides of D/B185 or D/B120 omp1 in positions 574 and 843, while 4 samples tended to have an additional nucleotide change in position 1042. At the same time, 15 isolates, identified as serovar K, had two different nucleotides of K/UW31/Cx *omp1* gene in positions 503 and 628, while 4 isolates, identified as serovar C, had

Table 1. Amounts of primers used for the procedure

P1/OMP2		P1/CT6R		CT6F/OMP2		NL-F/NL-R	
P1	3.5 µl	P1	3.5 µl	CT6F	7.3 µl	NL-F	2.8 µl
OMP2	3.5 µl	CT6R	3.8 µl	OMP2	3.5 µl	NL-R	4.5 µl
Water	21 µl	Water	20 µl	Water	17 µl	Water	20 µl

4 different nucleotides of C/TW-3/OT *omp1* gene in positions 569, 571, 972 or 1003.

Thus, within the territory of the Republic of Belarus under study we observed the prevalent occurrence of serotypes D, K and C of *C. trachomatis* in the biological material of patients with chronic inflammatory diseases of the urogenital tract. Variant D (54.7%) serotype turned out to be the prevailing one; variant K (35.7%) is a less frequent phenomenon, while variant C (9.6%) turned out to be the least frequent one. At the same time we registered a 94-95% homology with standard cultures of D/B120 (X62918), D/B 185 (X62919), K/UW31/Cx (AF063204).

Below a comparison of the character of clinical manifestations of *Chlamydia* infection and topical diagnosis in patients with different serotypes of the causative agent is shown. Table 3 shows the distribution of patients under study depending on serotype and prevailing pathology.

Table 3 confirms that the group of patients with the diagnosed serotype C of *C. trachomatis* presents the greatest deal of interest. In spite of the small number of patients in this study group both females and males tended to have complications of the *Chlamydia* infection in the form of arthropathic kind of chlamidiosis (Reiter's syndrome) or sterility. It is very important to take this fact into account in the prognostic aspect of patients' condition. There were fewer such patients in the study group of subjects with D and serotypes of *C. trachomatis*. On the other hand not a single patient with serotype D of *C. trachomatis* had an arthropathic form of infection (Reiter' syndrome), which is also very important to take

Table 2. Nucleotide changes in structure of *omp1* gene of *C. trachomatis*

Serovar				Nucleotide	
type	%[a]	N[b]	number of changes	changes[c]	omp1 position[d]
C	9.5	4	4	G→A G→T	972 (VDIV) 1003 (VDIV)
D	54.8	19	2	T→G	843 (VDIII)
		4	3	T→G A→G	843 (VDIII) 1042 (VDIV)
K	35.7	15	2	C→T	503 (VDII)

[a]*Stands for percentage of PCR C. trachomatis of amplified samples for which serotype has been identified*
[b]*Stands for number of C. trachomatis samples containing the above changes in omp1 sequence*
[c]*Stands for the nucleotide differences from the most frequently occurring phylogenetic cultures* (*i.e. K/UW31/Cx, D/B120, D/B185, C/TW3, C/TW-3/OT*)
[d]*Stands for the position of a nucleotide from the starting omp1 C. trachomatis gene in which the given change occurs*

Table 3. Distribution of patients under study with chronic *Chlamydia* infection subject to *C. trachomatis* serotype, nature of pathology and complications

Sex	*C. trachomatis* serotypes of patients	Topical diagnosis (n)	Complications
Males, n=24	C/TW-3/OT (AF352789), n=2	Urethritis (1) Prostatitis (1)	Reiter's syndrome (2)
	D/B120 (X62918), n=5	Urethritis (5)	None
	D/B185 (X62919), n=5	Urethritis (5) Prostatitis (4)	None
	K/UW31/Cx (AF063204), n=12	Urethritis (12) Prostatitis (3)	Reiter's syndrome (3)
Females, n=18	C/TW-3/OT (AF352789), n=2	Vulvovaginitis (2)	Sterility (2)
	D/B120 (X62918), n=6	Vulvovaginitis + + Cervical erosion (2) Adnexitis (2) Vulvovaginitis (1) Endocervicitis (1)	Sterility (3)
	D/B185 (X62919), n=7	Vulvovaginitis (3) Endocervicitis (2) Adnexitis (1) Cervical erosion (1)	Sterility (3)
	K/UW31/Cx (AF063204), n=3	Vulvovaginitis (2) Cervical erosion (1)	Sterility (2)

into account when making the prognosis of the development of potential complications. The incidence of such complications as sterility in the groups under comparison was not equal, i.e. it ranged from 13.3% in patients with serotype K of *C. trachomatis*, and 26.0% with serotype D of *C. trachomatis*, up to 50% in patients with serotype C of *C. trachomatis*.

Also, the average history of the disease in patients with serotype C of *C. trachomatis* tended to be much higher than in patients with serotypes K and D of *C. trachomatis.*

The clinical presentation was represented by various kinds of pathology. Male patients with three serotypes of *C. trachomatis* tended to predominantly have urethritis and prostatitis. The incidence of this pathology in patients with serotype K of *C. trachomatis* turned out to be much higher than in other study groups. Female patients with serotype D of *C. trachomatis* tended to suffer from endocervicitis more frequently than patients with other serotypes. Sterility was diagnosed in females of all study groups but the ones with serotype C of *C. trachomatis* displayed a 100% incidence rate; in patients with serotype K, 2 of 3 patients suffered from this pathology; in subjects with serotype D (D/B120 (X62918) 3 out of 6 patients had this problem.

We have established before that patients with *Chlamydia* infection had a wide range of fluctuation in *C. trachomatis* DNA concentration. That fluctuation tended to depend upon various factors, i.e. from the conditionally low level (up to 1.0×10^4 copies/ml) to the medium (1.1×10^4 to 2.0×10^5) and the high one (more than 2.1×10^5 copies/ml). At the same time the average concentration of DNA *C. trachomatis* (without taking into account the nature of the pathology and localization of the inflammatory process) comprised $1.5 \pm 0.2 \times 10^5$ copies/ml in males and $4.3 \pm 0.5 \times 10^4$ copies/ml in females [2]. In this respect we have compared the data on DNA concentrations depending on serotypes of *C. trachomatis.* The data obtained are shown in Table 4.

Table 4 shows that the lowest concentrations of DNA in both female and male patients were observed in subjects with genotype C, which correlated with the previously obtained data on the low level of DNA in patients with complicated forms of chronic infection [1, 2].

The highest concentrations were registered in males with serotype D (higher than in females). Also, a higher level of DNA was observed in males with serotype K; this index was twice as high as in females.

Isolation of other sexually-transmitted causative agents (mixed infections) in patients with *Chlamydia* infection served as the reason for studies of dependence of the variant of the infection (mono-, mixed) on the kind of serotype. The results obtained are shown in Table 5.

Chlamydia as the only aetiological agent was revealed in only 10 patients (23.8%) under study. Mixed infection agents were *Ureaplasma* (28.6%), *Candida* (16.7%), *Mycoplasma* (14.3%), other fungi (11.9%), *Gardnerella* (4.8%).

Table 5 shows that irrespective of serotype and its variants no predominance of *Chlamydia* infection in the form of mono-infection (23.8%) was revealed. All patients under study with serotype C had *Chlamydia* accompanied by fungi of the *Candida* family. This causative agent rarely occurred in patients with serotype D and was not found in patients with serotype K. Female patients with serotype D tended to predominantly have mycosis. Ureaplasmas were more frequently observed in males and females with serotype K as compared to the subjects in other study groups.

A high rate of *C. trachomatis* resistance to antibiotics (poly-resistance) in chronic *Chlamydia* infection has been registered before [2]. The basic initial medicines prescribed in such cases are tetracyclines and macrolides. We studied the rate of *C. trachomatis* resistance to antibiotics in cases of administration of the above drugs depending on the variant of a serotype of a causative agent. These data are shown in Table 6.

Table 4. Findings of DNA concentration in patients with *Chlamydia* infection subject to *C. trachomatis* serotype

Sex	*C. trachomatis* serotypes of patients	DNA concentration [copies/ml]
Males,	C/TW-3/OT (AF352789)	$3.3 \pm 0.5 \times 10^3$
n=24	D/B120 (X62918)	$7.1 \pm 1.3 \times 10^4$
	D/B185 (X62919)	$8.6 \pm 0.4 \times 10^4$
	K/UW31/Cx (AF063204)	$4.1 \pm 1.0 \times 10^4$
Females,	C/TW-3/OT (AF352789)	$5.9 \pm 0.2 \times 10^3$
n=18	D/B120 (X62918)	$6.8 \pm 2.3 \times 10^4$
	D/B185 (X62919)	$5.9 \pm 1.9 \times 10^4$
	K/UW31/Cx (AF063204)	$2.2 \pm 0.3 \times 10^4$

Table 5. Presence of monovalent and polyvalent infection in patients with clamidiosis subject to *C. trachomatis* serotype

Sex	*C. trachomatis* serotypes of patients	Type of infection	Number of patients
Males, n=24	C/TW-3/OT (AF352789)	Monoinfection	1
		+ *Candidae*	1
	D/B120 (X62918)	Monoinfection	2
		+ *Ureaplasma*	1
		+ *Mycoplasma*	1
		+ *Candidae*	1
	D/B185 (X62919)	Monoinfection	1
		+ *Ureaplasma*	4
	K/UW31/Cx (AF063204)	Monoinfection	5
		+ *Candidae*	3
		+ *Ureaplasma*	3
		+ *Mycoplasma*	1
Females, n=18	C/TW-3/OT (AF352789)	Monoinfection	1
		+ *Candidae*	1
	D/B120 (X62918)	+ *Trichomonadae*	3
		+ *Mycoplasma*	2
		+ *Gardnerellae*	1
	D/B185 (X62919)	+ *Trichomonadae*	2
		+ *Mycoplasma*	2
		+ *Gardnerellae*	1
		+ *Ureaplasma*	1
		+ *Candidae*	1
	K/UW31/Cx (AF063204)	+ *Ureaplasma*	2
		+ *Mycoplasma*	1

Table 6. Stability of *C. trachomatis* to antibiotics depending on the kind of serotype (n=42)

Sex	*C. trachomatis* serotypes of patients	Tetracyclines	Tetracyclines and macrolides
Males, n=24	C/TW-3/OT (AF352789)	–/–	2/0
	D/B120 (X62918)	4/0	1/0
	D/B185 (X62919)	3/1	1/1
	K/UW31/Cx (AF063204)	6/0	6/0
Females, n=18	C/TW-3/OT (AF352789)	0/0	2/0
	D/B120 (X62918)	4/1	1/1
	D/B185 (X62919)	2/1	4/1
	K/UW31/Cx (AF063204)	2/0	1/0
Males + females, n=42	C/TW-3/OT (AF352789)	0/0	4/0
	D/B120 (X62918)	8/1	2/1
	D/B185 (X62919)	5/2	5/2
	K/UW31/Cx (AF063204)	8/0	7/0

Table 6 shows that *C. trachomatis* in all patients with serotype *C* demonstrated a 100% simultaneous resistance to both tetracyclines and macrolides. Keeping in mind that all of those patients had complicated forms of the infection, one can come up with an explanation of this fact.

A similar situation was observed in patients with serotype K, in whom all the isolated cultures of *C. trachomatis* demonstrated resistance to the antibiotics under study. By analogy with the group of patients with serotype C this group comprised patients with aggravated forms (arthropathic variant and sterility) that correlated with the already existing data on the predominant persistence of the causative agent connected with serotype C [2].

Conclusions

Based on the conducted studies the prevailing spread of serotypes D (54.7%), K (35.7%) and C (9.6%) has been determined in the biological material of the patients with chronic inflammatory diseases of the urogenital tract.

At the same time in 94-95% of cases homology with standard D/B120 (X62918), D/B185 (X62919), K/UW31/Cx (AF063204) cultures has been observed. It has also been determined that 19 isolates, identified as serotype D, tended to display a difference in two nucleotides of D/B185 or D/B120 *omp1* gene in position 574 and 843, while 4 samples tended to have an additional nucleotide change in position 1042. As for serotype K, nucleotide changes of K/UW31/Cx *omp1* gene in positions 503 and 628 have been observed. However, the largest number of nucleotide substitutions in this study was characteristic for serotype C, which displayed a difference in C/TW-3/OT *omp1* gene concerning 4 nucleotides in positions 569, 571, 972 and 1003.

Comparative analysis of serologic variants of the causative agent and clinical manifestations showed that the most frequent causes of inflammatory processes of the urogenital tract are associated with serotypes D and K of *C. trachomatis,* which cause development of urethritis, adnexitis, endocervicitis and other kinds of pathology. One can observe an associative connection between development of complicated forms of *Chlamydia* infection in the form of arthropathic forms and sterility and C and K C. trachomatis serotypes. These two sero-variants of *C. trachomatis* tend to have a higher degree of resistance to antibiotics, which can serve as one of the reasons for development of complications of *Chlamydia* infection in the form of arthropathic pathology (Reiter's syndrome) and sterility in women.

References

1. Semionov VM. Khlamidiynaya infektsiya (Chlamydia infection)/Semionov VM, Semionov DM, Khvorik DF, et al. Vitebskiy Gosudartstvenniy Meditsinskiy Universitet. Vitebsk 2005; 206.
2. Dean D. Recombination in the genome of Chlamydia trachomatis involving the polymorphic membrane protein C gene relative to omp A and evidence for horizontal gene transfer. J Bacteriol 2004; 186: 4295-306.
3. Frost SD. Using sexual affiliation networks to describe the sexual structure of a population. Sex Transm Infect 2007; 83 Suppl 1: 137-42.
4. Morre SA, Lyons JM. Murine models of Chlamydia trachomatis genital tract infection: use of mouse pneumonitis strain versus human strains. Infect Immun 2000; 68: 7209-11.
5. Morre SA, Moes RI, van Valkengoed JP, et al. Genotyping of Chlamydia trachomatis in urine specimens will facilitate large epidemiological studies. J Clin Microbiol 1998; 36: 3077-8.
6. Kostiuk SA, Kulaga OK, Poluyan OS. Kliniko-laboratornanya harakteristika vospalitelnykh processov reproduktivnykh organov, obuslovlennykh assotsiatsiyey vozbuditeley (Clinical and laboratory characteristics of inflammatory processes of reproductive organs preconditioned by association of causative agents)/Kostiuk SA. Virusniye infektsii: epidemiologiya, klinika, laboratornaya diagnostika i profilaktika. Mat. Mezhd. Konf. 29–30 noyabria 2007. Red. Prof. L.P. Titov. Minsk 2007; 180-183.
7. Kostiuk SA. Noviye aspekty klinicheskogo primeneniya polimeraznoy reaktsii (New aspects of clinical application of polymerase chain reaction)/Kostiuk SA, Kulaga OK, Khvorik DF. Meditsinskiye Novosti 2006; 5: 14-18.
8. Persson K. The role of serology, antibiotic susceptibility testing and serovar determination in genital chlamydial infections. Best Pract Res Clin Obstet Gynaecol 2002; 16: 801-14.