2. G. B. Kirillicheva, A. A. Kirillicheva, and M. A. Tumanyan, in: *Immunomodulators in Infectious Diseases* [in Russian], Moscow (1988), p. 55.
3. B. P. Lukashin, *Byull. Eksp. Biol. Med.*, **93**, No. 6, 112 (1982).
4. B. P. Lukashin and L. I. Nenarokova, *Ibid.*, **83**, No. 6, 736 (1977).
5. A. S. Mozzhukhin and F. Yu. Rachinskii, *Chemical Prevention of Radiation Injuries* [in Russian], Moscow (1979).
6. L. A. Tiunov, E. A. Zherbin, and B. N. Zherdin, *Radiation and Poisons* [in Russian], Moscow (1977).
7. G. N. Chernobaeva and L. D. Luk'yanova, in: *Pharmacological Correction of Hypoxic States* [in Russian], Moscow (1989), p. 160.
8. K. A. Chaubal and C. S. Godbole, *Radiat. Environ. Biophys.*, **22**, 281 (1983).
9. A. B. Schreiber, J. Kenney, W. J. Kowalski, *et al.*, *Proc. Natl. Acad. Sci. USA*, **82**, 6138 (1985).
10. O. Vos and W. S. D. Roos-Verney, *Radiat. Biol.*, **52**, 273 (1988).

Detection of *Chlamydia trachomatis, Mycoplasma hominis,* and *Ureaplasma urealyticum* by Polymerase Chain Reaction in Men and Women with Genital Diseases

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Polymerase chain reaction was clinically used to diagnose chlamydial and mycoplasma infection in the cervix and male urethra. Examinations of 2260 patients with disorders of reproductive function detected *C. trachomatis, M. hominis,* and *U. urealyticum* in 32.9, 9.7, and 25.7% of cases, respectively. A high incidence of mixed nongonococcal urogenital infections was observed, particularly in women. Seasonal fluctuations were shown in the detection rates of chlamydial and ureaplasmic infections.

Key Words: *polymerase chain reaction; C. trachomatis; M. hominis; U. urealyticum; mixed urogenital infections*

Independent clinical studies carried out in different countries have shown that in 50 to 90% of cases disorders of reproductive function are caused by mixed infections of the urogenital tract, the most common of which are nongonococcal infections, specifically, chlamydial and mycoplasmic [1,2,7-9,11]. The prolonged asymptomatic course and multiple clinical manifestations greatly impede the symptomatic diagnosis of these diseases. Traditional immunological methods of analysis are not always effective either, as these infections are usually latent. Isolation of the agent in a cell culture is expensive and laborious, making such a procedure difficult for routine laboratory diagnosis.

Advances in biotechnology have led to the development of highly sensitive and specific methods for the diagnosis of infections, based on unique properties of their agent's genome, one of these methods being polymerase chain reaction (PCR). PCR has been shown to be not inferior to the classical cell culture method in its basic parameters and

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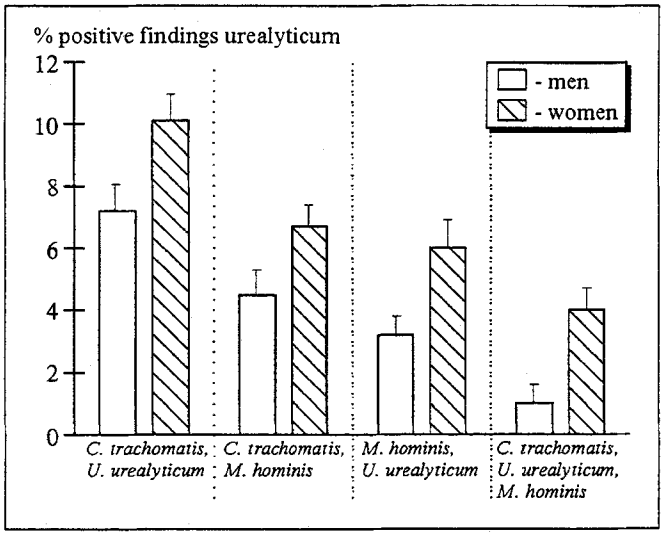

Fig. 1. Incidence of mixed infections in men and women with reproductive dysfunction.

is most effective for laboratory detection of *Chlamydia trachomatis* [10,13]. Published data indicate the efficacy of this method for detecting various representatives of *Mycoplasma* [4-6]. Moreover, PCR has permitted the detection of not only acute, but also latent infections, and is a reliable tool for epidemiological and statistical studies of the prevalence of various agents, their associations, seasonal fluctuations in disease course, and treatment efficacy.

This study deals with a clinical adaptation of the PCR method to the diagnosis of nongonococcal infections. We assessed the incidence of *C. trachomatis, M. hominis,* and *U. urealyticum* infections in men and women with various diseases of the reproductive system, as well as the incidence of mixed urogenital infections, and analyzed the rate of detection of these infections during one year. Diagnostic PCR system was used, permitting simultaneous detection of *C. trachomatis, M. hominis,* and *U. urealyticum.*

MATERIALS AND METHODS

A total of 2260 patients (791 men and 1469 women) with various disorders of reproductive function or inflammatory processes in the urogenital tract were examined starting in January, 1994. Each sample was simultaneously tested for three agents: *C. trachomatis, M. hominis,* and *U. urealyticum.*

Epithelial cells from the distal portion of the urethra of men and from the cervical canal of women were examined. The cells were scraped off using Medscand and Abbott probes. The samples were placed in normal saline and stored at -20°C for up to 1 month.

Clinical samples were prepared and the amplification reaction carried out as described previously [3]. The cells were lysed with a solution containing 6 M guanidine thiocyanate, and DNA was adsorbed on microporous glass, eluted after multiple washing, and analyzed by the PCR method. The reaction was carried out with 25 μl in 30 cycles using a programmed BIS incubator (Vektor Research and Manufacturing Unit) using primers amplifying the fragments of 16S genes of the agents' RNAs:
5'-CACGAGCTGACGACAACCATGCA-3', R1 — universal primer;
5'-CGTTTGCGACGCTTTTGGATG-3', R2 — primer for detecting *U. urealyticum*;
5'-GGTTAGCAATAACCTAGCCGCGA-3', R3 — primer for detecting *M. hominis*; and
5'-AAAGGGCGTGTAGGCGGAAAG-3', R4 — primer for detecting *C. trachomatis.*

In addition, 150 patients were examined using alternative pairs of primers:
5'-GATCGGTTTTCTCTTCGGTA-3'
5'-TCCATCGAGTTCTAGTTGCC-3', amplifies the fragment 507 nucleotide pairs (n.p.) in *C. trachomatis* plasmid pCHL1;
5'-GATGGTAAGTTAGTTGCTGAC-3'
5'-ACGACGTCCATAAGCAACT-3', amplifies the 456 n.p. fragment in the urease genome of *U. urealyticum*; and
5'-CTACCGCTATTTTGCCAGTTGCTAC-3'
5'-TATTGCTGGATAATGGCCGTATGAA-3', amplifies the 227 n.p. fragment in the P50 adhesin gene of *M. hominis.*

The reliability of differences in the detection rate and conjugation of the agents was assessed us-

TABLE 1. Incidence of Associations of *Chlamydia trachomatis, Mycoplasma hominis,* and *Ureaplasma urealyticum* in Patients with Reproductive Dysfunction

Infections	Incidence of associations, %	
	expected	actual
C. trachomatis and *M. hominis*	3.2±0.4	6.0±0.6
C. trachomatis and *U. urealyticum*	8.4±0.5	9.2±0.4
M. hominis and *U. urealyticum*	2.5±0.4	5.1±0.6
C. trachomatis, M. hominis, and *U. urealyticum*	0.8±0.2	2.9±0.5

Note. The results were obtained in examinations of 2260 patients.

ing Student's two-sample test and Statgraphics software for statistical data processing.

RESULTS

The versatility and high specificity of PCR permitted simultaneous diagnosis of *C. trachomatis*, *M. hominis*, and *U. urealyticum*. Chlamydias were detected in 32.9%, mycoplasmas in 9.7%, and ureaplasmas in 25.7% of the 2260 patients with various reproductive dysfunctions. In 49.5% of examinees none of these infections was detected. Additional examinations of 150 patients using other primer systems amplifying the fragments of other domains of the agents' genomes confirmed these results. For chlamydias the results coincided in 95%, for mycoplasmas in 96%, and for ureaplasmas in 99% of cases. The results are in line with published reports about a high prevalence of nongonococcal urogenital infections [1].

Analysis of the results of diagnosing urogenital infections showed a similar incidence of chlamydia (34.2 and 32.2%) and ureaplasma (24.9 and 26.3%) in men and women, respectively, whereas mycoplasmas were more frequently found in the cervical canal of women than in the male urethra: 11.2 and 7.2%, respectively. Evidently, the distal portion of the male urethra is not the optimal object for detecting *M. hominis*. Similar data were obtained by other scientists [12].

Associations of chlamydias, mycoplasmas, and ureaplasmas turned out to be much more common than we expected when their incidence was calculated as the product of their individual detection rates: 2.9% instead of the expected 0.8% (Table 1). Chlamydias and mycoplasmas, chlamydias and ureaplasmas, and mycoplasmas and ureaplasmas go hand in hand much more frequently than one would theoretically expect, so that we can speak of the conjugation of these infections.

Notable differences were observed in the incidence of mixed nongonococcal urogenital infections in men and women: *C. trachomatis* and *M. hominis* were concomitant in 4.6 and 6.7%, *U. urealyticum* and *M. hominis* in 3.2 and 6.0%, and *C. trachomatis* and *U. urealyticum* in 7.2 and 10.2% of cases, respectively (Fig. 1). All the listed associations of infections were detected in 3.9% of women and in 1.1% of men. It is possible that the low rate of detection of *M. hominis* in the male urethra in general was reflected in its detection rate as a component of mixed infections.

In addition, we assessed the time course of *C. trachomatis*, *M. hominis*, and *U. urealyticum* detection rates from January, 1994 to February, 1995

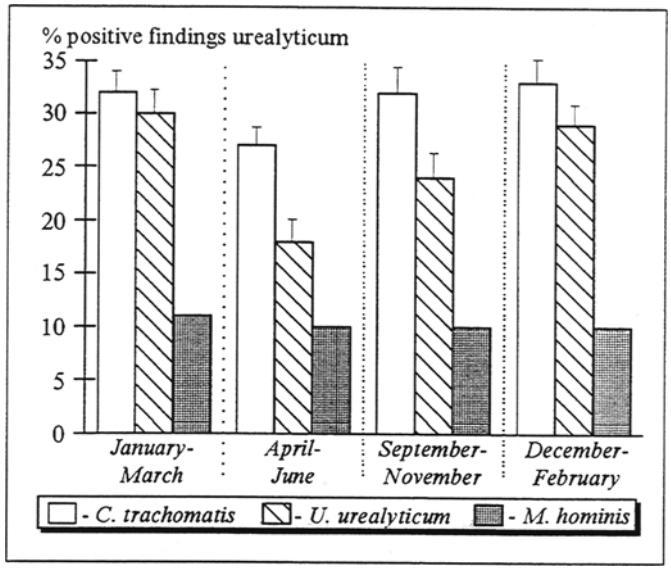

Fig. 2. Seasonal changes in the detection of *C. trachomatis*, *U. urealyticum*, and *M. hominis* in patients at the Research Center of Obstetrics, Gynecology, and Perinatology in January, 1994 – February, 1995. Monthly results are combined by seasons. No studies were carried out in July. The data for August are presented in the text.

(Fig. 2). The detection rates of chlamydias and ureaplasmas are higher in autumn and winter. This indicates that exacerbations of the inflammatory processes caused by these agents occur during the unfavorable part of the year. On the other hand, the incidence of mycoplasmas is virtually the same all year round. A surge of the relative number of positive diagnoses of all three infections was observed in August: 41.3, 15.5, and 31.1% for chlamydias, mycoplasmas, and ureaplasmas, respectively. This may be due to the fact that in summer, the vacation period, only patients with acute inflammations seek medical care. No studies were carried out in July.

Hence, the data on 2260 patients at the Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, indicate that nongonococcal urogenital infections are conjugated, since the actual incidence of various associations of chlamydias, ureaplasmas, and mycoplasmas is reliably higher than the expected value. Associations of chlamydia and ureaplasma are more common in women than in men. Seasonal fluctuations in the detection of *C. trachomatis* and *U. urealyticum* were observed over the period between January, 1994 and February, 1995: they were less frequent in spring and summer. The detection rate of *M. hominis* was virtually the same over the entire period of observation.

The high sensitivity of PCR and good reproducibility of its results permit us to recommend this diagnostic system for wide clinical use.

REFERENCES

1. V. I. Kozlova and A. P. Pukhner, in: *Viral, Chlamydial, and Mycoplasmatic Diseases of the Genitals* [in Russian], Moscow (1995), pp. 238-261.
2. I. I. Mavrov, *Vestn. Dermatol.*, No. 11, 11-13 (1991).
3. E. M. Khalilov, V. M. Govorun, M. Yu. Brodskii, *et al., Use of Polymerase Chain Reaction for Detecting Chlamydial, Mycoplasmatic, and Ureaplasmatic Infection in Practical Obstetrics and Gynecology (Informational Letter)* [in Russian], Moscow (1995).
4. S. Artiuchin, L. Stipcovits, and F. C. Minion, *Mol. Cell. Probes*, **7**, 381-385 (1993).
5. A. Blanchard, W. Hambrick, L. Duffy, *et al., Clin. Infect. Dis. ER*, **17**, S272-S279 (1993).
6. N. Cardeau, P. Lebel, and R. Brousseau, *J. Gen. Microbiol.*, **139**, 2431-2437 (1993).
7. E. E. Edet, *Brit. J. Clin. Pract.*, **47**, No. 1, 21-22 (1993).
8. M. A. Fisher, *W. V. Med. J.*, **89**, No. 8, 331-334 (1993).
9. K. M. Freund, *Hosp. Pract. [Off]*, **27**, No. 2, 175-186 (1992).
10. G. Jaschek, C. A. Gaidos, and L. E. Welsh, *J. Clin. Microbiol.*, **31**, No. 5, 1209-1212 (1993).
11. J. Paevonen, *J. Infect.*, **25**, Suppl. 1, 39-45 (1992).
12. D. Taylor-Robinson and W. M. McCormack, *The Mycoplasmas*, Vol. 2, New York (1979), pp. 307-366.
13. W. H. Vogels, P. C. van Voorst-Vaader, and F. P. Schroder, *J. Clin. Microbiol.*, **31**, No. 5, 1103-1107 (1993).

Effect of Minimal Bactericidal Doses of Chlorhexidine and Other Antiseptics on *Bacillus subtilis* 720 Ribosomes

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The bactericidal doses (BD_{99}) of antiseptics which reduce the number of viable *Bacillus subtilis* 720 by 2-3 orders of magnitude in 15 min have been determined. A new method for prelysis treatment of bacilli has been developed. Chlorhexidine is shown to be harmless for *B. subtilis* ribosomes and to act upon their cell wall and membrane. The studied antiseptics clearly fall into two groups in terms of their capacity to facilitate the lysis of bacilli.

Key Words: *Bacillus subtilis; chlorhexidine; antiseptics; ribosomes; degradation*

In contrast to the case with antibiotics, the mechanisms of the bactericidal effects of antiseptic agents, despite their long and broad history of use, are still little understood. One of the principal components of the cell is the ribosomal system, the destruction of which may lead to the loss of cell viability, and therefore, accumulation of data on its degradation will help unravel the subcellular underpinnings of the bactericidal action of antiseptics. This study was aimed at assessing the damage inflicted on ribosomes of a representative of the family Bacillaceae under the effect of BD_{99} (bactericidal dose) of chlorhexidine and some other antiseptics.

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MATERIALS AND METHODS

Bacillus subtilis 720 was cultured with aeration up to the exponential phase of growth (OD_{650}=1.0) in an original medium rich in yeast, pH 7.3, containing mineral salts from medium M-9 [3], 0.5% glucose, 0.2% yeast autolysate, and 0.2% casein hydrolysate. In order to assess the BD_{99}, 4 ml of bacterial culture were added to the mother liquor of antiseptic; after a 15-min incubation at 37°C the exposure was stopped by diluting the mixture with cold buffered normal saline, pH 7.0, and the cells were inoculated in meat-peptone agar. For detecting the damaged rRNA, the culture exposed to one or several BD_{99} of antiseptics was diluted 10-fold with normal saline at 0°C, sedimented by centrifugation, and washed once. The pellet was suspended in the same solution or TKM buffer (0.01 M Tris-

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