Original Article

Cross-sectional study of the vaginal microbiota in adolescents from the Turkestan Region: a clinical and ecological approach

Aigerim Kushkarova[1], Ugilzhan Tatykayeva[2], Aknur Yegizova[3], Shakhnoza Tatykayeva[4], Gulzhazira Tursynbayeva[5], Kenzhegul Ryskeldiyeva[6], Raushan Nurkhassimova[7], Zhansaya Torgaytova[8]

ABSTRACT

Background

Proper establishment of the vaginal microbiota is critical to adolescent reproductive health. Pubertal hormonal and physiological transitions, compounded by social and environmental stressors, can alter microbiome composition and structure. Given its ecological vulnerabilities, adolescents in Kazakhstan's Turkistan Region may face a higher risk of vaginal microbiota instability. The objective of this study is to examine the determinants and contributing factors of vaginal discharge in non-sexually active girls aged 13–19

Material and Method

This was a cross-sectional study. A total of 211 adolescent girls with no history of sexual activity were included. The cohort was divided into two groups: 98 participants in Group 1 and 113 participants in Group 2. Vaginal and urethral swabs were collected from all subjects, and vaginal discharge samples were analyzed using standard bacteriological techniques. Venous blood was drawn to assess seropositivity. In addition, lead concentrations were measured in hair samples, as well as in water and soil from the participants' residential environments.

Results

Vaginal discharge was reported significantly more often in association with dysmenorrhea, lower abdominal pain, malodor, itching, and acne vulgaris ($p < 0.05$). Bacteriological analysis identified Gardnerella vaginalis, Candida spp., Escherichia coli, and Ureaplasma urealyticum as the predominant pathogens. Elevated lead concentrations were significantly more common in participants with complaints of vaginal discharge. Among environmental biomarkers, hair lead levels demonstrated the highest sensitivity and specificity in predicting the presence of vaginal discharge compared with water and soil measurements. ***Conclusion:*** Vaginal discharge in non-sexually active adolescents was linked to both clinical manifestations and environmental lead exposure, highlighting hair lead levels as a valuable diagnostic marker.

Keywords

vaginal microbiota; vaginal discharge; adolescents; non-sexually active girls

INTRODUCTION

Adolescence represents a transitional stage from childhood to adulthood, characterized by significant physical, psychoemotional and social changes that can impact reproductive health. This period is critical for the development of reproductive potential, as it is during this period that the microbiological and immunological characteristics of the vagina, including the vaginal microbiota, are established. Recent research shows that the composition of the vaginal microbiota in adolescents can vary significantly under the influence of environmental, biological,

1. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, aigerim.kushkarova@ayu.edu.kz
2. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, ugilzhan.tatykayeva@ayu.edu.kz
3. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, aknur.egizova@ayu.edu.kz
4. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, shakhnoza.tatykayeva@ayu.edu.kz, ORCID ID MGT-6179-2025
5. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, tursynbaeva.gulzhazira@ayu.edu.kz
6. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, kenzhegul.ryskeldiyeva@ayu.edu.kz
7. MD, professor,Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, raushan.nurkhassimova@ayu.edu.kz
8. Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, zhansaya.torgaytova@ayu.edu.kz

Correspondence

Ugilzhan Tatykayeva, Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan, ugilzhan.tatykayeva@ayu.edu.kz

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and behavioral factors[1,2]. During this period, one of the most common gynecological complaints in children and adolescents is vaginal discharge, caused by a variety of etiological factors. In terms of frequency, this problem ranks second only to menstrual irregularities[3-5]. According to the World Health Organization (WHO), about 75% of women experience vaginal discharge at least once in their lifetime, with nearly half reporting recurrent episodes[6]. This issue is particularly pronounced during adolescence, as vaginal discharge in pubertal girls is among the most frequent reasons for medical consultations and often causes considerable anxiety for both the patients and their parents[7–9]. The importance of investigating this problem lies not only in the high prevalence of vaginal discharge complaints among adolescents but also in its diverse causes and potential implications for future reproductive health. Therefore, research into the factors influencing the frequency and nature of vaginal discharge in adolescent girls is an important area of modern medicine. The aim of this study is to investigate the causes and factors underlying vaginal discharge in girls aged 13–19 who are not sexually active.

METHODS AND MATERIALS

A cross-sectional study was conducted in the Turkestan region of the Republic of Kazakhstan. Prior to commencing the study, approval was obtained from the local ethics committee (Protocol No. 04/13 2021). The study included girls aged 13–19 who were sexually inactive, clinically healthy, and had not taken any medications in the past 7–10 days. Girls who refused to participate, had any medical conditions, or had sexual intercourse were excluded.

The patients were divided into two groups based on whether they complained of vaginal discharge:

- Group 1: No discharge (*n=98)*
- Group 2: Discharge present (*n=113)*

Demographic variables were recorded. Regardless of the presence of discharge, patients were asked about complaints of dysmenorrhea, abdominal pain, unpleasant odor, and itching. Vaginal and urethral swabs were taken from all subjects, and vaginal discharge was subjected to bacteriological examination. Venous blood was collected and seropositivity determined. Lead levels were also determined in hair, as well as in water and soil at the women's residence.

Statistical analysis

IBM SPSS Statistics v.24 was used for data analysis. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality of distribution. The parametric Student's t-test was used to assess differences between independent samples with a normal data distribution, and the nonparametric Mann-Whitney test was used for non-normal data distributions. Categorical variables were analyzed using the χ^2 test. The prognostic significance of lead content in biological and environmental matrices (hair, water, soil) was assessed using ROC analysis. Differences with a type I error probability of less than 5% ($p < 0.05$) were considered statistically significant.

Ethical clearence

This study was conducted in accordance with ethical standards. Ethical approval was obtained from the appropriate institutional review board, and informed consent was secured from all participants prior to data collection.

RESULTS

Tests for normal distribution (Kolmogorov-Smirnov and Shapiro-Wilk tests) showed that the hypothesis of normality was not supported for any group. Thus, the indicators of age, BMI, number of siblings, and lead levels in water, soil, and hair did not correspond to a normal distribution.

Table 1. Basic characteristics, complaints and lead levels in the surveyed groups

Characteristics	*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Age (years)	15.7±1.9 (13-19)	16.2±2 (13-19)	0.081[a]
Nationality (Kazakhs, %)	76.5	69	0.223[a]
Age at menarche (years)	13.0±1.1 (11-16)	13.2±1.2 (10-17)	0.236[a]
Cycle regularity (%)	72.4	61.9	0.106[a]
BMI (kg/m²)	20.6±2.5 (15.5-28.7)	20.9±3.3 (15.2-36.1)	0.506[a]
Number of siblings (n)	4±1.2 (1-7)	4.4±1.4 (1-9)	0.087[a]

Characteristics	*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Hirsutism (%)	15.3	19.5	0.428[b]
Dysmenorrhea (%)	35.7	57.8	0.002[b]
Abdominal pain (%)	1	31	0.000[b]
Itching (%)	1	93.8	0.000[b]
Malodor (%)	1	97.3	0.000[b]
Acne vulgaris (%)	32.7	48.7	0.018[b]
Lead in hair (mg/g)	0.5±0.3 (0.2-2)	2±1.0 (0.3-4.6)	0.000[a]
Lead in water (mg/dm³)	275.6±161.7 (185-1070)	448.9±287.6 (185-1020)	0.000[a]
Lead in soil (mg/kg)	12.6±8.8 (4.3-43.4)	20.2±13.8 (4.3-43.4)	0.000[a]

*Note: Comparisons between two results were performed using the Mann-Whitney ([a]) and χ^2 ([b]) tests. Values are expressed as mean ± standard deviation (minimum–maximum) and as percentages (%).

No differences in demographic characteristics were found between the groups, but lead levels in hair, water, and soil were statistically significantly different. Additionally, the group reporting discharge had significantly higher rates of dysmenorrhea, abdominal pain, itching, odor, and acne vulgaris compared to Group 1.

Higher BMI and age were not associated with increased discharge complaints (p = 0.506 and p = 0.084, respectively).

ROC analysis showed that the strongest correlation with the presence of excreta was observed for lead levels in hair (Fig. 1). For the lead level in hair, cut-off = 0.8910 (indicate the unit of measurement), the sensitivity was 88.5%, and the specificity was 89.8%. Despite the fact that a statistically significant correlation was found between the presence of excreta and the lead level in water (p = 0.000), as well as lead in soil (p = 0.000), the sensitivity values at a cut-off of 453.50 mg/dm³ for water and 14.6400 mg/kg for soil were only 40.7%, which is considered quite low.

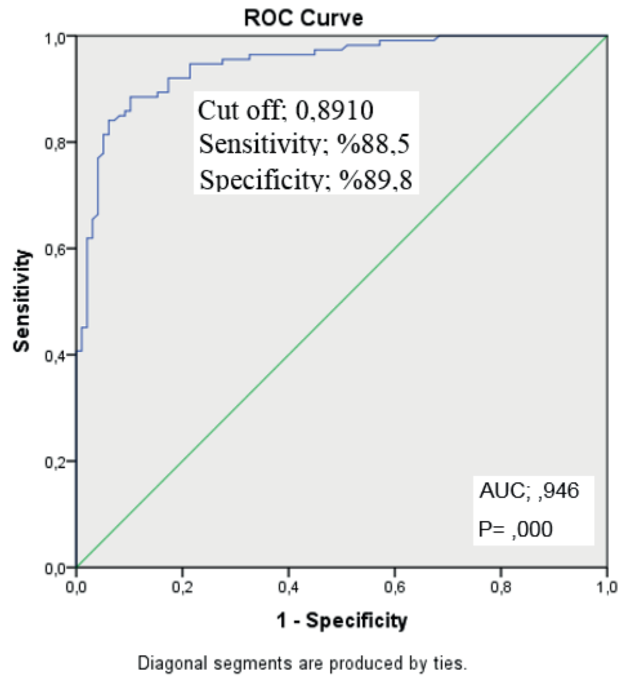

Figure 1. Relationship between excretion and hair lead levels

Table 2. Results of vaginal and urethral smears, and Femoflor-16 analysis

Parameters		*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Vaginal squamous epithelium	Few	95.9	4.4	0.000
	Moderate	3.1	52.2	
	Numerous	1	43.4	
Vaginal leukocytes	0-5	53.1	3.5	0.000
	6-10	44.9	24.8	
	>11	2	71	
Vaginal microbiota	Gardnerella			0.006
	Cocci	0	2.7	
	Bacilli	31	1.8	
	Bacilli + Candida	84,7	68.1	
	Bacilli + Cocci	1	13.3	
	Bacilli + Leptotrix	11.2	9.7	
	Bacilli + Candida +	0	0.9	
	Leptotrix	0	2.7	
	Bacilli + Cocci +	0	0.9	
	Candida			

Parameters		*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Urethral squamous epithelium	Few	96.9	9.7	0.000
	Moderate	2	48.7	
	Numerous	1	41.6	
Urethral leukocytes	0-5	84.7	10.6	0.000
	6-10	14.3	25.7	
	>11	1	63.7	
Urethral microbiota	Gardnerella	0	2.7	0.009
	Cocci	3.1	1.8	
	Bacilli	84.7	69.9	
	Bacilli. + Candida	1	12.4	
	Bacilli + Cocci	11.2	8.8	
	Bacilli + Leptotrix	0	0.9	
	Bacilli + Candida + Leptotrix	0	2.7	
	Bacilli + Cocci + Candida	0	9	
Femoflor-16	Normocenosis	78.6	4.4	0.000
	Conditional normocenosis	17.3	13.3	
	Moderate anaerobic dysbiosis	4.1	61.9	
	Severe anaerobic dysbiosis	0	20.4	

Note: Between-group categorical comparisons were performed using the χ^2 test. Values are presented as percentages (%).

When analyzing Table 2, it was found that in group 2, the epithelial and leukocyte indices in vaginal and urethral smears were statistically significantly higher than in group 1. The most frequently detected pathogen in both vaginal and urethral smears in group 2 was Candida spp., followed by Gardnerella vaginalis.

According to the Femoflor 16 study, among the microorganisms associated with severe anaerobic dysbiosis, in all cases the combination of Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp. and Megasphaera spp. + Veillonella spp. + Dialister spp. was noted. In addition, various combinations included Eubacterium spp., Peptostreptococcus spp., Sneathia spp. + Leptotrichia spp., Atopobium vaginae.

In the group of moderate anaerobic dysbiosis, according to Femoflor 16, among the increased microorganisms, the most frequently detected combination was Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp., Eubacterium spp. and Megasphaera spp. + Veillonella spp. + Dialister spp. — in group 1 in 3 patients (75%) and in group 2 in 62 patients (88.6%). Additionally, there were various combinations involving Enterobacteriaceae, Staphylococcus spp., Mycoplasma hominis, Peptostreptococcus spp., Sneathia spp. + Leptotrichia spp., Atopobium vaginae, Lachnobacterium spp. + Clostridium spp., Mobiluncus spp. + Corynebacterium spp.

The combination of Ureaplasma urealyticum + parvum was detected in group 1 in 1 patient (25%) and in group 2 in 42 patients (48.6%) as part of mixed associations, and also as a monoinfection - only in group 1 in 1 patient (25%) and in group 2 in 8 patients (11.4%).

In the conditional normocenosis group, according to Femoflor 16 data, the following variants were identified: in group 1 in 3 patients and in group 2 in 4 patients — only Candida spp. and Mycoplasma hominis (in group 2, in an additional 2 patients — only Candida spp.); in group 1 in 4 patients and in group 2 in 1 patient — only Lachnobacterium spp. + Clostridium spp. and Mycoplasma hominis; in group 1 in 6 patients and in group 2 in 2 patients — Ureaplasma urealyticum + parvum in combination with Eubacterium spp. or Staphylococcus spp.; in group 1 in 4 patients and in group 2 in 6 patients — Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp. in combination with Mycoplasma hominis and/or Ureaplasma urealyticum + parvum and/or Eubacterium spp. and/or Mobiluncus spp. + Corynebacterium spp. Trichomonas vaginalis and Neisseria gonorrhoeae were not detected in any of the groups.

Table 3. Seropositive results

Parameters		*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Chlamidya (%)	Ig A Ig G	0 2	0 0.9	1.000 0.598
Gardnerella vaginalis (%)	Ig G Ig M	13.3 0	76.1 0.9	0.000* 1.000
Human papillomavirus (%)	Ig G Ig M	78.6 0	72.6 1.8	0.313 0.500
Mycoplasma hominis (%)	Ig G Ig M	4.1 11.2	5.3 14.2	0.754 0.524

Parameters		*Group 1 (n=98)*	*Group 2 (n=113)*	*p-value*
Ureaplasma urealyticum (%)	Ig G Ig M	15.3 1	16.8 2.7	0.766 0.625
Cytomegalovirus (%)	Ig G Ig M	100 22.4	100 32.7	1.000 0.097

When analyzing the seropositivity rates in Table 3, it was found that the frequency of Gardnerella vaginalis infection in group 2 was significantly higher than in group 1, and this difference was statistically significant (76.1% and 13.3%, respectively; p = 0.000).

In the group without discharge, no microbial growth was observed in 29.6% of cases, whereas growth was observed in all patients with discharge. Culture results are presented as percentages and absolute values in Figure 2. Data analysis revealed that the detection rate of Escherichia coli was slightly higher in group 2 compared to group 1 (8.8% and 3.1%, respectively; p = 0.050). The level of Candida albicans was also higher in group 2, but no statistically significant differences were found between the groups (p = 0.081).

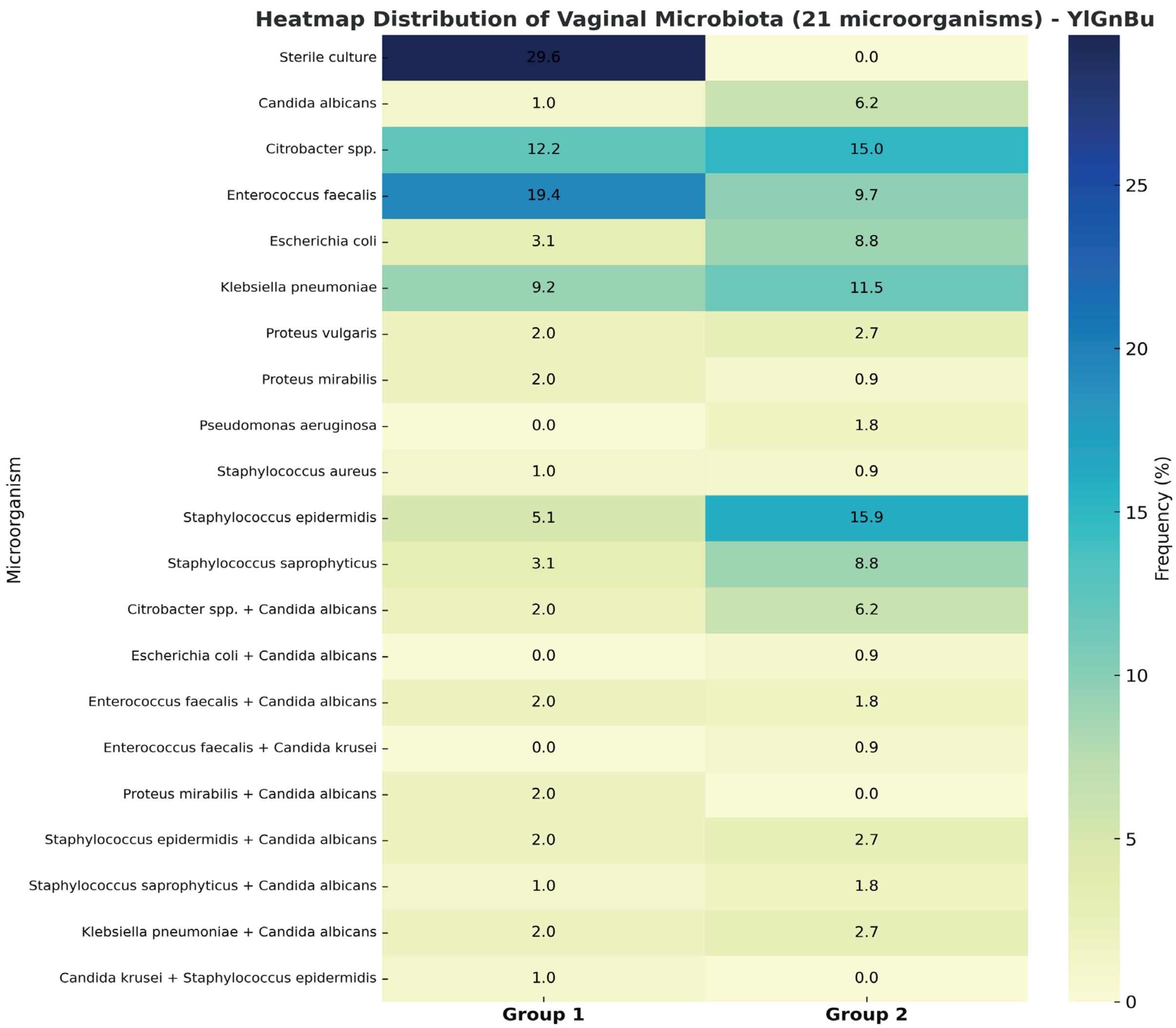

Figure 2. Heatmap of vaginal microbiota distribution

DISCUSSION

Vaginal discharge during puberty is a natural result of activation of the hypothalamic-pituitary-ovarian axis and increased estrogen secretion. Increased functional activity of the glands of the vaginal mucosa leads to increased secretion volume, which, in the absence of pathological symptoms such as itching, burning, and unpleasant odor, should be considered normal [(10-12)]. This study confirms that vaginal discharge is one of the most common gynecological complaints in sexually inexperienced girls and adolescents. Even in the absence of sexual activity, this symptom is relatively common and necessitates a thorough differential diagnosis. It is important to note that the presence of microorganisms in laboratory tests does not necessarily signify a clinically significant infection. In our study, the range of microorganisms detected largely aligns with the findings reported by Agana and colleagues. The most common pathogens in the group with clinical manifestations were Gardnerella vaginalis, Candida spp., and Escherichia coli, which is consistent with data from international studies[13]. However, it is noteworthy that in the group without significant discharge, opportunistic microorganisms, including intestinal bacteria, were also detected in some adolescents. This confirms the authors' conclusions that the presence of microorganisms does not always indicate a clinically significant infection but requires interpretation based on age, hormonal status, and the clinical presentation.

In a study by Baka S. et al., it was shown that among gram-positive Cocci and gram-negative Bacilli, representatives of the fecal microflora were most frequently encountered. A similar picture was observed in our study, particularly in the group of patients without clinical discharge[7]. Of particular interest are environmental factors that can increase the risk of abnormal vaginal discharge. In particular, chronic exposure to heavy metals such as lead is considered a significant determinant of reproductive health. Determining lead concentrations in adolescent hair is used as a valid biomarker of long-term exposure.

Lead has been shown to have endocrine-disrupting effects, alter the regulation of sex hormones, and reduce local immunity of the mucous membranes[14].

Therefore, the interpretation of vaginal discharge in adolescents should be based on a multifactorial analysis that includes not only microbiological and hormonal mechanisms but also environmental determinants. Further research should be aimed at exploring the relationship between the state of the vaginal microbiota, heavy metal exposure, and the clinical manifestations of reproductive disorders in adolescence.

Study limitations

This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design does not allow for establishing causal relationships between ecological factors, vaginal microbiota composition, and clinical symptoms in adolescents. Second, the sample size, although sufficient for preliminary analysis, may not fully represent the entire adolescent population of the Turkestan region, particularly rural subgroups. Third, the study relied on Femoflor-16 analysis, which, while widely used, does not capture the full diversity of the vaginal microbiota and may overlook less prevalent microbial species. Fourth, potential confounding factors such as diet, hygiene practices, and previous antibiotic use were not assessed in detail, which could have influenced the observed microbiological profiles. Finally, the absence of longitudinal follow-up limits the ability to evaluate dynamic changes in the vaginal microbiota over time.

CONCLUSION

It was found that in adolescent girls who are not sexually active, the presence of complaints of vaginal discharge is statistically significantly associated with symptoms such as dysmenorrhea, lower abdominal pain, odor, itching, and acne vulgaris. The most important pathogens causing vaginal discharge are Gardnerella vaginalis, Candida, Escherichia coli, and Ureaplasma urealyticum. Trichomonas and gonococci were not detected in any group. Elevated lead levels were statistically significantly more common in patients complaining of vaginal discharge, with the highest sensitivity and specificity observed when analyzing the relationship between hair lead levels and the presence of vaginal discharge.

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Authors's contribution:

Data gathering and idea owner of this study: U.

Tatykayeva, A. Yegizova,

Study design: S. Tatykayeva

Data gathering: G. Tursynbayeva, K. Ryskeldiyeva

Writing and submitting manuscript: R. Nurkhassimova, Z. Torgaytova

Editing and approval of final draft: A. Kushkarova

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