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Modern Methods for Diagnosis of *Gardnerella* Infection

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Comparison of morphological, bacteriological, serological, and genetic methods for diagnosis of *Gardnerella* infection in vaginosis showed that the first three methods are preferable, while the genetic method (polymerase chain reaction) is more efficient for identification of *Gardnerella vaginalis* in culture.

Key Words: *bacterial vaginosis; Gardnerella vaginalis; polymerase chain reaction; immunofluorescent test*

After investigations of H. L. Gardner and C. D. Dukes, who validated the etiological role of *Gardnerella vaginalis* in bacterial vaginitis, this nosological entity was named gardnerellosis. Further studies revealed the role of anaerobic bacteria in the development of this disease. At present, the disease called bacterial vaginosis (BV) is regarded as an infectious noninflammatory syndrome associated with vaginal biotope dysbiosis, characterized by extremely high concentration of obligate anaerobic microorganisms and a drop in the content of lactobacilli or their complete absence in vaginal secretion. *G. vaginalis* can be isolated from the vagina of about 50% patients with BV [1,12], and some authors isolated it from female rectum [9-11]. The presence of *G. vaginalis* in vaginal smears is not always associated with clinically apparent disease [3], and therefore R. Amsel *et al.* [5] proposed clinical and laboratory criteria for the diagnosis of BV. One of the main tests is morphological analysis of vaginal smears, detecting decreased lactoflora, other criteria are the absence of inflammation elements and presence of so-called key cells.

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This approach differs from the standpoint of H. L. Gardner and C. D. Dukes, as it takes no account of the etiology of BV. The role of *G. vaginalis* remains unclear in such a method of diagnosis of BV. Isolation and identification of this bacterial culture is very difficult. At present new methods of identification of *G. vaginalis* and studies of this microorganism in BV are tested. The development of methods for agent detection by indirect signs (DNA, antigens, or metabolites) is now in progress. The main requirement to such diagnostic systems is high sensitivity and specificity. Laboratory trials of these systems are sometimes very effective, but their application for the analysis of clinical material often provides doubtful results.

We compared the diagnostic value of polymerase chain reaction (PCR), immunofluorescent test (IFT), morphological, and bacteriological methods in *Gardnerella* infection. Our purpose was to define optimal conditions for isolation and identification of pure bacterial culture by cultural, tinctorial, antigenic, and genetic properties in comparison with the reference strain ATCC-14018 *G. vaginalis*, analysis of clinical material, and comparison of the findings with laboratory data.

MATERIAL AND METHODS

G. vaginalis isolated by H. L. Gardner and C. D. Dukes deposited in American National Collection (ATCC-

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14018) was used as the reference strain. Reference *Lactobacillus* cultures were obtained from the Laboratory of Biomedical Technologies, I. M. Sechenov Moscow Medical Academy. The set of reference cultures included *L. fermentum* B-7 59 TA-4, 90 TS-4 (21), 90 TS-4 (10); *L. plantarum* B_6 77TA-2, 8RA-3, ATCC-1024; *L. brevis* B-1309; *L. casei* NCDO-152; *L. bulgaricus* NYZO-3501; *L. acidophilus* Zh-1 87 M-A6. Isolates from patients identified as *Streptococcus hemolyticus, Micrococcus sp., Enterococcus sp., Proteus vulgaris, Escherichia coli* (lactose-positive and lactose-negative), *Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans,* and nonidentified anaerobic gram-positive hemolytic and nonhemolytic cocci were collected at Central Institute of Skin and Sexually Transmitted Diseases and Moscow State Medical Stomatological University. Vaginal secretion (10 µl) was collected using a Volkmann spoon in 27 patients, in 14 of whom BV was confirmed by the criteria proposed by R. Amsel *et al.* [5]. The samples were resuspended in 2 ml normal saline and inoculated after Gold [4] to Colombian blood agar with 5% donor blood and antibiotics (nalidixic acid, gentamicin, and amphotericin B). The dishes were cultured under anaerobic conditions using GasPak CO_2 System BBL No. 43 70308 (Becton Dickinson).

Indirect IFT after Coombs was performed using commercial Vagi Slide preparation, including immune serum containing antibodies to *G. vaginalis* and FITC-labeled antiglobulin serum.

In addition, immune rabbit serum to ATCC-14018 strain was prepared by 4 intravenous immunizations with bacterial suspension was used in serological tests (including IFT).

For bacterial agglutination test (AT), isolated cultures were washed with normal saline, centrifuged twice at 4000 rpm for 15 min, and incubated with 5% trypsin in phosphate buffer (pH 8.0) at 37°C for 1 h. Then the culture was washed with normal saline, resuspended, and AT with sera to the reference strain was performed.

All reference bacterial cultures and clinical samples were analyzed by PCR, in which specific DNA fragment of *G. vaginalis* was amplified by cyclic elongation of *G. vaginalis* DNA-specific primers with thermostable DNA polymerase. Primers flanking the 5'-terminal fragment for 16S rDNA (401 n.p.) were selected. The test system consisted of three kits: for isolation of *G. vaginalis* DNA from clinical material, for PCR with primers specific for *G. vaginalis* DNA, and for detection of PCR products. DiaGen-Gardnerella PCR procedure detects at least 10^3 *G. vaginalis* cells per ml.

RESULTS

Immune serum to ATCC-14018 strain was tested by AT with homologous and heterologous cultures. Serum titer for homologous culture was 1:3200. The same serum did not agglutinate heterologous cultures except some lactobacilli strains (Table 1).

The maximum titer with heterologous culture was no more than 1:80, and hence, strains isolated from clinical material and agglutinating with this serum in titer above 1:160 could be identified as *G. vaginalis.*

In IFT the reference strain fluoresced at a maximum immune serum dilution of 1:320 and at a heterologous culture dilution of 1:20.

All cultures identified as *G. vaginalis* were positive in PCR (Table 2). Heterologous cultures, including strains isolated from patients and identified to the species level, were negative in this test. The only exception was the reference strain BMT *L. casei* NCDO-152 which gave a doubtful reaction and *S. aureus* from the collection of the Institute of Skin and Sexually Transmitted Diseases (weak positive reaction).

At present the only variant of nucleotide sequences of *G. vaginalis* ribosomal RNA genes was repor-

TABLE 1. AT with Serum to Strain ATCC-14018

Reference strains	Titer	Isolated cultures	Titer
L. fermentum B-7 59 TA-4	0	*Streptococcus hemolyticus*	0
L. fermentum 90 TS-4 (21)	1:80	*Micrococcus sp.*	0
L. fermentum 90 TS-4 (10)	0	*Enterococcus sp.*	0
L. plantarum B6 77 TA-2	1:80	*Proteus vulgaris*	0
L. plantarum 8RA-3	0	*Staphylococcus aureus*	0
L. plantarum ATCC-1024	1:40	b^+, b^- (polymorphic hemolytic)	0
L. brevis B-1309	1:40	c^+	0
L. casei NCDO-152	0	c^+ (hemolytic)	0
L. bulgaricus NYZO-3501	0	c^+ (hemolytic)	0
L. acidophilus	0	c^+	0

Note. c^+) Unidentified gram-positive cocci; b^+, b^-) unidentified gram-positive and gram-negative bacilli, respectively.

TABLE 2. Identification of Pure Cultures with DiaGen-Gardnerella Test System (*M±m*)

Parameter	*G. vaginalis*		Other representatives of vaginal microbiocenosis (*n*=25)	
	PCR positive	PCR negative	PCR positive	PCR negative
Number of cultures, abs.	9	0	2	23
%	100	0	8±1	92±1

ted without special reference to its serovariant. *G. vaginalis* are highly homologous by 16S rDNA sequences with bifidobacteria, lactobacilli [6], actinomyces [8], but not with *S. aureus*. The homology of the primed region for bifidobacteria, lactobacilli, and actinomyces is 81.5-87.7, 70-72, and 74.3-82.5%, respectively, while for *S. aureus* only 67-68%. Three additional *S. aureus* strains were tested and gave negative reaction, which means that weak positive reaction with the reference strains from the Central Institute of Skin and Sexually Transmitted Diseases is an artefact. The studies demonstrated high specificity of the test system.

Gram-staining detected key cells in 52% clinical samples from patients with BV, while the use of specific immune sera detected these cells in 46% samples. The IFT data coincided with the results of Gram staining in 88% cases. In two cases, no microorganisms reacting with the serum were found on the detected key cells and in one case microorganisms specifically reacting with the serum were located extracellularly. Twenty-two cultures were isolated from clinical samples on selective medium, and *G. vaginalis* DNA was detected by PCR in 59% samples. The frequency of *G. vaginalis* detection by AT and IFT was 47 and 40%, respectively; the results of these tests coincided with PCR data in 88 and 80% cases, respectively, which implies that PCR is more sensitive than immunological methods. PCR was always positive with seropositive samples. Cultures genetically identical to *G. vaginalis* did not react with the serum to the reference strain in 12-25% cases. This may be due to relatively low (in comparison with PCR) sensitivity of serological methods or due to the presence of other *G. vaginalis* serotypes in patients. Seven serogroups of *G. vaginalis* are known [7]. The possibility of circulation of different serovariants of *G. vaginalis* was confirmed in further experiments. Two clinical isolates and ACTT-14018 were studied in IFT with two immune sera. Serum from Vagi Slide kit reacted with all three cultures, while the serum to the reference strain specifically fluoresced with the homologous culture and the second isolate, but not with the first isolate. Therefore, the Vagi Slide serum is more universal immunochemical reagent detecting *G. vaginalis* of different serotypes, while the serum prepared by us is highly specific and does not react with *G. vaginalis* serotype differing from ATCC-14018 strain.

Microscopy of vaginal smears confirms clinical diagnosis of BV, but it is not always caused by *G. vaginalis*, which is proven by the absence of specific fluorescence of microorganisms located on the key cells with immune serum and isolation of cultures not identified by genetic signs as *G. vaginalis* from the same material. On the other hand, *G. vaginalis* can be isolated in cases when the key cells are absent in the vaginal secretion, *i. e.* the agent does not adhere to the epithelium and is located extracellularly. Immunological tests can be regarded as supplementary to the morphological method; they help more accurately identify the agent in pathological material.

Hence, BV can be regarded as dysbacteriosis in which *G. vaginalis* plays an important role, but in many cases other opportunistic microorganisms can predominate in the vaginal microflora, without increase in the *G. vaginalis* population. Additional tests will help to identify the etiology of BV in such cases: isolation of pure culture and its identification by immunological and genetic methods. PCR is a highly sensitive and specific method for identification of *G. vaginalis* culture, but direct detection of *G. vaginalis* DNA in vaginal secretion can lead to overdiagnosis of BV [2].

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