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HYDROGEN PEROXIDE PRODUCTION ACTIVITY AND ADHESIVE PROPERTIES OF AEROCOCCI, ISOLATED IN WOMEN

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Introduction

Antagonistic activity of probiotic microorganisms against other species of bacteria is an important mechanism of their ecology and it is widely used in practice. This activity is inherent in many heme-deficient bacteria, which include aerococci, and can be composed of several components: the production of organic acids, antibiotics, lysozyme, hydrogen peroxide and others. [1].

Ability to produce hydrogen peroxide under aerobic conditions and in a state of relative anaerobiosis was established in aerococci. They were divided into strong and weak producers, depending on the amount of peroxides [2].

Lack of data about peroxide-productive ability of aerococci, isolated from the lower genital tract of women, as well as a proven mechanism of hydrogen peroxide excretion in the oxidation of lactic acid [3], led to need in studying the aerococci hydrogen peroxide production level, to create autobacterial drugs, based on aerococci symbiont strains for sanitation of birth canal.

Colonization resistance of the vaginal mucous and normal microflora value depends largely on the degree of adhesion of microbial cells to the mucosal surface.

Along with numerous studies of lactobacilli adhesive properties to the vaginal epithelium [4, 5, 6], there are no data on the adsorption capacity of aerococci to the vaginal epithelial cells.

Material and methods

Presence and quantity of autosymbiont aerococci content in different parts of the birth tract (cervical canal, vagina, external genitalia skin (EGS) and perineum) was studied in 44 healthy women. Isolation and identification of aerococci from the women body was conducted by the method [7], taking into account growth on selective indicator medium, growth and biochemical activity in environments with selenium and tellurium salts, lactate oxidase and superoxide dismutase activity [3]. Hydrogen peroxide was determined by iodometric method [8]. Hydrogen peroxide production in 19 strains of aerococci (museum strain - Aerococcus viridans 167 and 18 strains of autosymbiont aerococci) was investigated. The level of hydrogen peroxide production was compared with aerococci adhesive activity to cells of vaginal epithelium.

Autosymbiont strains were used to evaluate the aerococci adhesion on vaginal epitheliocytes.

Epithelial cells were washed twice with chilled phosphate buffered saline (PBS) (pH 7,2) by centrifugation at 800 rpm. for 10 min. After determining the concentration of cell suspension with the camera Goryaeva under a light microscope it was diluted to 1.5 - 2 x 10^6 cells / ml. Further strain suspension was prepared with a concentration of 2 x 10^9 microbial cells / ml, 0.5 ml of epithelial cells suspension was mixed with 0.5 ml of aerococci culture suspension. Cell-bacterial mixture was incubated in the thermostat at (37 ± 1) ° C for 30 min., occasionally flicking. Then the mixture was washed from microbes that did not adhere with PBS three times at 600 rpm for 10 min. All manipulations were carried out in the cold. 1-2 drops of PBS was added to the sediment and smears on the glass were prepared. Preparations were fixed with 96 ° alcohol and stained by Romanowsky-Giemsa.

The average number of bacteria adherent to 25 epithelial cells was counted under a light microscope.

The study was repeated at least 3 times when evaluating the adhesion of each strain of microorganism. The average adhesion (AA) and the number of bacteria in one cell - microbe / cell in 10 fields of view were calculated, taking into account the results of all studies.

The level of bacterial adhesion was conditionally differentiated into four stages:

- not adhesive (AA = 0);
- weakly adhesive (AA = 1 - 5);
- medium adhesive (AA = 5 - 10);
- highly adhesive (AA above 10).

18 aerococci resident strains and 1 museum strain were explored in total.

Data are presented as the arithmetic mean (M), the standard error of the mean (± m). Statistical significance of averages differences were evaluated by Dennett's criterion with multiple comparisons, relative - by Pearson's chi-square criterion ($\chi 2$) at 5% significance level ($p < 0,05$).

Results and discussion

Comparison of hydrogen peroxide production levels between the museum and symbiotic Aerococcus viridans strains showed a lower level (from $p < 0,05$ to $p < 0,001$) in strains isolated from vaginal microbiocenosis, cervix and lower genital skin (Table. 1).

Table 1. The average amount of hydrogen peroxide formed by aerococci at the end of 24-hour incubation

№	Number of strain	Source of isolation	Average number of aerococci cells in 1 ml of nutrient broth (lg SUN/ml), M ± m	Average amount of hydrogen peroxide (mg/ml), M ± m
1	167	Museum strain	9,0±0,1	0,79±0,03
2	3C	Cervical canal	9,1±0,2	0,16±0,01 *
3	4C	-"- -"-	9,1±0,2	0,19±0,01 *
4	2V	Vagina	9,1±0,1	0,42±0,02 *
5	3V	-"- -"-	9,0±0,1	0,13±0,02 *
6	4V	-"- -"-	8,9±0,2	0,19±0,04 *

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7	9V	-"- -"-	8,9±0,1	0,34±0,03 *
8	10V	-"- -"-	9,0±0,1	0,44±0,01 *
9	15V	-"- -"-	9,1±0,1	0,14±0,03 *
10	16V	-"- -"-	9,1±0,1	0,13±0,02 *
11	25V	-"- -"-	9,0±0,1	0,07±0,005 *
12	28V	-"- -"-	9,0±0,2	0,09±0,005 *
13	34V	-"- -"-	9,1±0,2	0,24±0,03 *
14	41V	-"- -"-	9,0±0,1	0,11±0,02 *
15	4S	Skin of EGS	9,1±0,1	0,49±0,07 #
16	13S	-"- -"-	9,1±0,1	0,37±0,04 *
17	18S	-"- -"-	9,0±0,1	0,38±0,04 *
18	23S	-"- -"-	9,1±0,2	0,10±0,01 *
19	39S	-"- -"-	9,1±0,2	0,62±0,03 #

*Note: refers to strains of resident in the museum aerococci, isolated from the cervical canal (C), vagina (V), EGS and perineal skin (S); * - p <0,001; # - p <0,05 compared with the museum strain.*

Despite approximately the same number of multiplied cells, the amount of hydrogen peroxide produced by aerococci varied in a fairly large range. Thus, 8 Aerococcus viridans strains (167, 2V, 9V, 10V, 4S, 13S, 18S, 39 S) during reproduction in the number lg 8,9 -9,1 SUN / ml, extracted considerable amount of hydrogen peroxide in the range 0, 34-0,79 mg / ml. However, 11 autosymbiont aerococci strains, with identical number of multiplied cells produced hydrogen peroxide in much smaller amounts - within 0,07-0,24 mg / ml.

The foregoing results allowed to divide aerococci cultures into two groups: strong and weak peroxide producers.

Comparative evaluation of aerococci strains isolated from the birth canal activity, showed the predominance of weak producers of hydrogen peroxide (7 - 61.1% of cases) under strong (11 - 38.9%). Aerococci strains isolated from microbiocenosis of EGS skin showed the maximal activity compared with strains isolated from other parts of the birth path - 4 out of 5 cases (80.0%) compared with 3 of 13 (23.1%) cases (p <0,05).

Considering the possibility of aerococci autoprobiotic drugs development for sanitation and normalization of birth canal biocenosis, as well as enhancing the efficiency of vaginally applied eubiotics, cyto adhesion of aerococci to the vaginal epithelium cells study was conducted.

As is evident from Table 2, the aerococcal ability to adhere varied in a wide range (AA from 3.25 to 10.24). 3 strains were isolated from all aerococcal strains with low adhesion (15.8%), with average degree - 12 (63.2%) and 4 highly adhesive aerococcal strains were identified (21.0%) (Table. 3).

Table 2. Indicators of aerococci cyto adhesion in museum and autosymbiont strains (M ± m)

№	Source of isolation	Source of isolation	AA
1	167	Museum strain	8,11±0,15
2	3C	Cervical canal	6,43±0,12
3	4C	-"- -"-	6,16±0,12
4	2V	Vagina	10,1±0,10
5	3V	-"- -"-	10,0±0,14
6	4V	-"- -"-	7,92±0,13
7	9V	-"- -"-	5,48±0,11
8	10V	-"- -"-	10,24±0,17
9	15V	-"- -"-	6,15±0,13
10	16V	-"- -"-	7,45±0,12
11	25V	-"- -"-	10,21±0,16
12	28V	-"- -"-	5,50±0,16
13	34V	-"- -"-	5,90±0,13
14	41V	-"- -"-	7,56±0,17
15	4S	Skin of EGS	6,12±0,17
16	13S	-"- -"-	4,56±0,14
17	18S	-"- -"-	3,25±0,13

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18	23S	-"- -"-	3,73±0,11
19	39S	-"- -"-	5,15±0,12

Analysis of the aerococci symbiont strains ability to adhere (tab. 2) showed that highly adhesive strains were observed only in the vagina, while autosymbionts isolated from the skin of the genital organs had usually low or medium level of adhesion to epithelial cells. The average index of adhesion (AA) for all strains isolated from the vagina, equaled 7,87 ± 0,12, for the cervical canal strains - 6,30 ± 0,09, for strains from the skin of EGS - 4,89 ± 0,11 (p <0,001 in all comparisons).

Table 3. The degree of adhesion activity and aerococci hydrogen peroxide production

Aerococci strains	Total (Abs./%)	Degree of adhesion activity						Hydrogen peroxide production activity			
		Low		Medium		High		weak producers		strong producers	
		Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
167	1/ 100%			1	100					1	100
Auto-symbionts	18/ 100%	3	16,7	11	61,1	4	22,2	11	61,1	7	38,9
Totally	19/ 100%	3	15,8	12	63,2	4	21,0	11	57,9	8	42,1

Conclusion

1. Aerococcus viridans autosymbionts are a component of normal flora of the female genital organs.
2. Aerococci autosymbiont cultures isolated from the birth canal, are mainly weak hydrogen peroxide producers (61.1%) and have medium adhesive (61.1%) and high adhesive (22.2%) properties on vaginal epithelium.
3. Strains isolated from vagina possessed higher adhesive ability (p <0,001).
4. Aerococcus viridans museum strain 167 was the strongest hydrogen peroxide producer (from p <0,05 to p <0,001).

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Introduction. Antagonistic activity of probiotic microorganisms against other species of bacteria is an important mechanism of their ecology and it is widely used in practice. This activity is inherent in many heme-deficient bacteria, which include aerococci, and can be composed of several components: the production of organic acids, antibiotics, lysozyme, hydrogen peroxide and others. Ability to produce hydrogen peroxide under aerobic conditions and in a state of relative anaerobiosis was established in aerococci. They were divided into strong and weak producers, depending on the amount of peroxides. Lack of data about peroxide-productive ability of aerococci, isolated from the lower genital tract of women, as well as a proven mechanism of hydrogen peroxide excretion in the oxidation of lactic acid, led to need in studying the aerococci hydrogen peroxide production level, to create autobacterial drugs, based on aerococci symbiont strains for sanitation of birth canal. Colonization resistance of the vaginal mucous and normal microflora value depends largely on the degree of adhesion of microbial cells to the mucosal surface. Along with numerous studies of lactobacilli adhesive properties to the vaginal epithelium, there are no data on the adsorption capacity of aerococci to the vaginal epithelial cells. **Material and methods.** 18 aerococci resident

strains and 1 museum strain were explored in total. Presence and quantity of autosymbiont aerococci content in different parts of the birth tract (cervical canal, vagina, external genitalia skin (EGS) and perineum) was studied in 44 healthy women. Isolation and identification of aerococci from the women body was conducted by the method, taking into account growth on selective indicator medium, growth and biochemical activity in environments with selenium and tellurium salts, lactate oxidase and superoxide dismutase activity. Hydrogen peroxide was determined by iodometric method. Hydrogen peroxide production in 19 strains of aerococci (museum strain - Aerococcus viridans 167 and 18 strains of autosymbiont aerococci) was investigated. The level of hydrogen peroxide production was compared with aerococci adhesive activity to cells of vaginal epithelium. Autosymbiont strains were used to evaluate the aerococci adhesion on vaginal epitheliocytes. 18 aerococci resident strains and 1 museum strain were explored in total.

Results and discussion. Comparison of hydrogen peroxide production levels between the museum and symbiotic Aerococcus viridans strains showed a lower level (from $p < 0,05$ to $p < 0,001$) in strains isolated from vaginal microbiocenosis, cervix and lower genital skin. Despite approximately the same number of multiplied cells, the amount of hydrogen peroxide produced by aerococci varied in a fairly large range. Thus, 8 Aerococcus viridans strains (167, 2V, 9V, 10V, 4S, 13S, 18S, 39 S) during reproduction in the number lg 8,9 -9,1 SUN / ml, extracted considerable amount of hydrogen peroxide in the range 0, 34-0,79 mg / ml. However, 11 autosymbiont aerococci strains, with identical number of multiplied cells produced hydrogen peroxide in much smaller amounts - within 0,07-0,24 mg / ml. The foregoing results allowed to divide aerococci cultures into two groups: strong and weak peroxide producers. Comparative evaluation of aerococci strains isolated from the birth canal activity, showed the predominance of weak producers of hydrogen peroxide (7 - 61.1% of cases) under strong (11 - 38.9%). Aerococci strains isolated from microbiocenosis of EGS skin showed the maximal activity compared with strains isolated from other parts of the birth path - 4 out of 5 cases (80.0%) compared with 3 of 13 (23.1%) cases ($p < 0,05$). The aerococcal ability to adhere varied in a wide range (AA from 3.25 to 10.24). 3 strains were isolated from all aerococcal strains with low adhesion (15.8%), with average degree - 12 (63.2%) and 4 highly adhesive aerococcal strains were identified (21.0%). Analysis of the aerococci symbiont strains ability to adhere (tab. 2) showed that highly adhesive strains were observed only in the vagina, while autosymbionts isolated from the skin of the genital organs had usually low or medium level of adhesion to epithelial cells. The average index of adhesion (AA) for all strains isolated from the vagina, equaled $7,87 \pm 0,12$, for the cervical canal strains - $6,30 \pm 0,09$, for strains from the skin of EGS - $4,89 \pm 0,11$ ($p < 0,001$ in all comparisons).

Conclusion. 1. Aerococcus viridans autosymbionts are a component of normal flora of the female genital organs. 2. Aerococci autosymbiont cultures isolated from the birth canal, are mainly weak hydrogen peroxide producers (61.1%) and have medium adhesive (61.1%) and high adhesive (22.2%) properties on vaginal epithelium. 3. Strains isolated from vagina possessed higher adhesive ability ($p < 0,001$). 4. Aerococcus viridans museum strain 167 was the strongest hydrogen peroxide producer (from $p < 0,05$ to $p < 0,001$).

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