UDC: 579.264:616.321:616.12-053.2

ANTAGONISM OF A. VIRIDANS TO CONDITIONALLY - PATHOGENIC MICROFLORA OF THE NOSE AND OROPHARYNX OF CHILDREN WITH CARDIAC PATOLOGY

Stepansky D.O.

State Establishment "Dnipropetrovsk Medical Academy", Dnipropetrovsk

Background

Search for harmless and simultaneously effective probiotics - bacterial biological preparations, which should be successfully used for the treatment and prevention of infectious deseases is currently important [7]. Aerococcus viridans № 167- museum aerococcuses strain, upon which the drug with probiotic action is manufactured – 'A-bacterin' [2,4]. *A. viridans i*s of particular interest, as it is representative of the normal microflora of human with broad spectrum of antibacterial action [2]. The use of this microorganism has a number of advantages: the absence of side effects on the body; high adhesive abilities; resistance to lysozyme in saliva; the ability of use in patients, sensitized to antibiotics and chemotherapeutic drugs; immunostimulant effects on the human body [1]. First, we had to examine the state of conditionally - pathogenic microflora (CPM) of nose and oropharynx of patients with infant cardiac patology. Our particular interest was represented by *S. pyogenes*, which - by scientific data - is among the essential microorganisms that cause heart diseases [3,6]. The purpose of the study was to investigate the antagonism of *A. viridans № 167* and autostrains of aerococcuses, isolated at patients, to conditionally - pathogenic microflora of the nose and oropharynx of children with cardiac patology. Thus, we wanted to get data about the applicability of aerococcuses correction of fauces and nasopharynx microbiocenosis and in complex health care measures aimed on cardiac pathology.

Materials and methods

At the first stage of the study the microflora of the nose and oropharynx of 2 investigated categories was examined:

The first category - 40 children 4-14 years with cardiac pathology who were treated in cardiological department of the City Children's Hospital № 2 m. Dnipropetrovsk.

The second category - 40 healthy children 4-5 years attending kindergartens of Industrial district of Dnipropetrovsk (control group).

Inoculation of material and isolation of pure cultures of microorganisms were conducted with the use of well known methods by morphological, cultural and biochemical properties [5]. Mucus from the nose and mouth was the material for bacteriological study. The take of material was held by a laboratory assistant-bacteriologist. For collection of material sterile cotton swabs, 1 nasal tampon and 1 tampon for oropharynx, embedded in test tubes, for each child were used.

To isolate microorganisms dense growth medium was used: 5% blood agar (BA), Garro, Chistovich, Endo and Saburo medium. The study used culture media production company HayMedia (India).

Inoculated cups with BA, Garro and Endo medium were incubated 18-24 hours at 37 ° C, Chistovich medium - 24 hours at 37 ° C and 24 hours at 20-22 ° C, Saburo medium - 18-24 hours at 37 ° C and 4 days at 22 ° C.

As additional tests for streptococci, paper discs with optochine, bile and bacitracin 0.04 IU were used. Thus, for the study, the culture identified as S. pyogenes (on condition of sensitivity to bacitracin 0.04 IU) and S. pneumoniae (on condition optochine sensitivity and positive in deoxycholate test) were selected.

Strains of conditionally pathogenic microorganisms isolated from the first analyzed category: 11 strains - *S. aureus*, 7 strains - *S. pyogenes*, 1 strain - *P. mirabilis*, 6 strains - *C. albisans.*

Strains of conditionally pathogenic microorganisms isolated from the second analyzed category: 11 strains - *S. aureus*, 4 strains - *S. pyogenes*, 6 strains - *S. pneumoniae*, 2 strains – *K. pneumoniae*, 6 strains - *C. albisans*. Also in the first group were allocated 5 autostrains of aerococcuses, in the second - 11 autostrains of aerococcuses.

Studying of the the effect on the *A. viridans* strains was the second stage of work. The method of deferred antagonism was used for that. As a test-culture, strain of *A. viridans № 167*, taken from the museum of microbiology, virology, immunology and epidemiology department of SE "DMA" was taken.

Suspension of *A. viridans* overnight culture was streaked on beef-extract agar in isotonic sodium chloride solution ($2x10^8$ bacterial cells).

To determine the optical density of microbial suspension Densi-La-Meter device was used. The optical density by a Mc Farland - scale was 0.7 IU. Inoculation conducted on diameter of Petri plates.

Inoculates were incubated for 24 hours at 37 ° C. After incubation, suspension of overnight cultures of the studied strains were perpendicularily streaked to the *A. viridans* cultures, which grew up on the cup. The optical density was measured for each culture separately and was identical to the density of *A. viridans*.

Evaluation of the experiment was carried out by measuring the diameter of growth inhibition zone in mm. Results were processed statistically. For the statistical analysis application package "Statistica v6.1®" was used. Quantitative features are presented as average value and its standard deviation (M ± m). For comparison single-factor analysis of variance ANOVA (F), Student test (t) or Mann-Whitney test (for small samples) were used. A statistically significant difference was considered when $p < 0,05$.

Results and Discussion

40 children aged 4-14 years with cardiac pathology and 30 healthy children aged 4-5 years were examined. From the nose and oropharynx of these

children 54 strains of opportunistic microorganisms were identified: 22 strains of *S. aureus*, 11 strains of *S. pyogenes*, 6 strains of *S. pneumoniae*, two strains of *K. pneumoniae*, one strain of *P. mirabilis*, 12 strains of *C. albisans*. We studied the antagonism of *A. viridans № 167* and isolated autostrains of aerococcuses to all 54 isolated strains. Severity of antagonism is evidenced by the size of growth inhibition zone (mm).

Antagonism of *A. viridans № 167* to CPM of the children's oropharynx is presented in Table 1.

The table shows that *A. viridans* manifests the antagonism to all studied strains of gram-positive and gram-negative microorganisms, except *C. albisans. A. viridans* antagonistic activity to staphylococci (10 + 3 mm) and streptococci (10 + 2mm) is at the approximately same level.

Ratio of growth inhibition zones (mm) of staphylococci, streptococci and enterobacteria strain under the action of *A. viridans № 167* (series 1 - with infant cardiac patology; series 2 - healthy children) is displayed on Fig. 1.

The picture shows (in cultures from healthy children) that antagonism to *S. aureus* and *S. pyogenes* is on the same level, just below - to *S. pneumoniae* and *K. pneumoniae*. It is interesting to compare the antagonism of aerococcuses to clinical isolates of *S. pyogenes* and similar strains from carriers (healthy children category). Impact of aerococcuses on *P. mirabilis* strain appeared at the highest level.

Table 1 Antagonistic activity of A. viridans № 167 to opportunistic strains

Name of the culture	Number of studied strains		Average value of growth inhibition zone (mm). M ± m	
	Children with cardiac patology	Healthy Children (control)	Children with cardiac patology	Healthy Children (control)
S. aureus	11	11	8,0 ± 3,0	10 ± 3,0
S. pyogenes	7	4	7,5 ± 2,5	10 ± 2,0
S. pneumoniae	0	6	-	9 ± 1,0
K. pneumoniae	0	2	-	8 ± 1,0
P. mirabilis	1	0	12,0 ± 2	-
C. albicans	6	6	0	0

Note: p <0.001 compared with healthy children

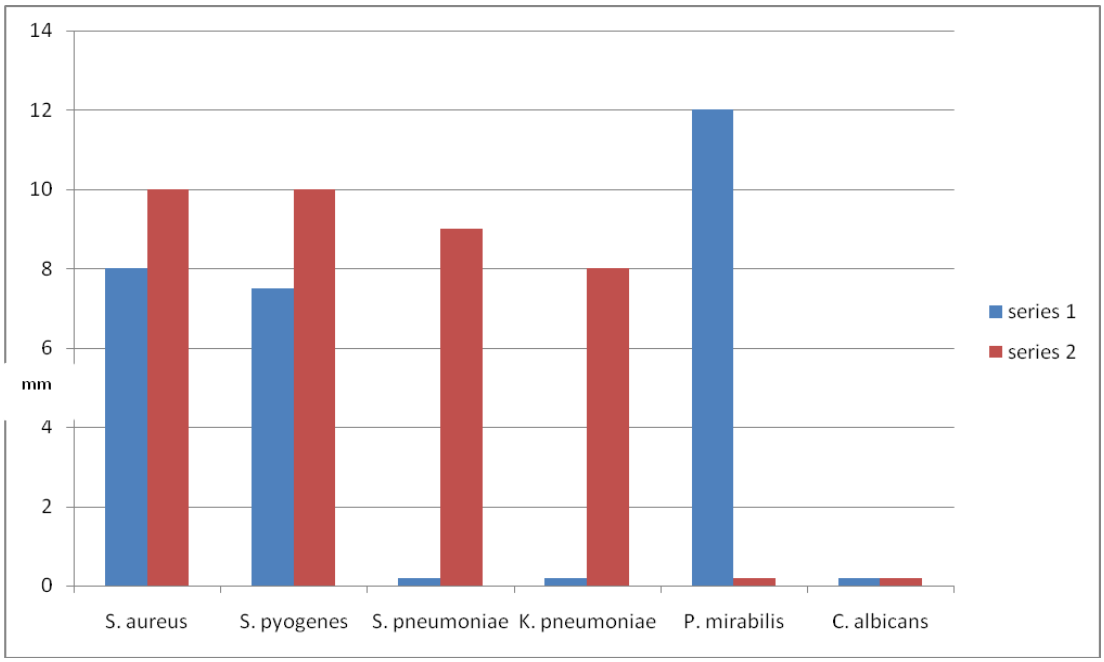

Fig. 1. Ratio of growth inhibition zones of aerococcuses' test strains

Table 2. Antagonistic activity of aerococcuses autostrains to opportunistic strains

Name of the culture	Number of studied strains		Average value of growth inhibition zone (mm). M ± m	
	Children with cardiac patology	Healthy Children (control)	Children with cardiac patology	Healthy Children (control)
S. aureus	11	11	9,5 ± 3,0	12,5 ± 3,0
S. pyogenes	7	4	7,5 ± 2,5	11,0 ± 2,0
S. pneumoniae	0	6	-	9,5 ± 1,5
K. pneumoniae	0	2	-	7,5 ± 1,0
P. mirabilis	1	0	9,0 ± 3,5	-
C. albicans	6	6	0	0

Note: p <0.001 compared with healthy children

Results in Table 2 show that autosimbionts of *A. viridans*, isolated from healthy children, are more antagonistic to CPM strains, isolated from these children, than autostrains of *A. viridans*, isolated from children with with cardiac patology, and higher than the museum strain of *A. viridans № 167* antagonism. Also they do not affect upon the growth of microorganisms of *Candida* kind.

Conclusions

Severe antagonistic activity of the museum strain and autostrains of *A. viridans* to isolated conditionally patogenetic strains was discovered: *S. pyogenes, S. pneumoniae, S. aureus, K. pneumoniae, P. mirabilis.*

Antagonism of *A. viridans № 167* to *C. albicans* was not found.

The recieved data can serve as a basis for further study of the strain of *A. viridans № 167* and aerococcuses' autostrains usage for correction of fauces and nasopharynx microbiocenosis and also for the complex treatment and preventive actions in case of cardiac pathology.

References

1. Autoprobiotics as means of infectious and inflammatory diseases prevention in humans in an artificial environment / V.K. Ilyin, A.N. Suvorov, N.V. Kirukhina [et al.] [Text] // Bulletin of Medical Sciences. – 2013. – Vol. – 2. - P. 56–62.
2. Kremenchutsky, G.N. Role of the human body microecology and the principles of its correction [Text] / G.N. Kremenchutsky, S.A. Rizhenko, S.I. Polishchuk. - Dnepropetrovsk, 2003. – P. 58–59.
3. Recommendations for the prevention, diagnosis and treatment of infective endocarditis [Text] // Ukr. cardiological magazine. – 2004. – Vol. – 4. – P. 115–121.
4. Ryzhenko, S.A. A-bacterin – new probiotic. – Dnepropetrovsk: Porohy, 2001. – 252 p.
5. Order of the USSR Ministry of Health number 535 of 04/22/85. "About the unification of microbiological research methods used in clinical diagnostic laboratories of health facilities".
6. Soboleva, M.K. Primary infectious endocarditis in children and adolescents [Text] / M.K. Soboleva, E.G. Sobolev, M.E. Skoblyakova // Pediatrics. – 2006. – Vol. 2. – P. – 11–17.
7. Shyrobokov V. P. New strategies in the development and clinical use of probiotics [Text] / V.P. Shyrobokov, D.S. Jankowski, G.S. Dyment // Bulletin of pharmacology and pharmacy. – 2010. – Vol. 2. – P. 18-30.

UDC: 579.264:616.321:616.12-053.2

ANTAGONISM OF A. VIRIDANS TO CONDITIONALLY - PATHOGENIC MICROFLORA OF THE NOSE AND OROPHARYNX OF CHILDREN WITH CARDIAC PATOLOGY

Stepansky D.O.

Introduction. Search for harmless and simultaneously effective probiotics, which could be successfully used for treatment and prevention of infectious deseases, is currently important. *A. viridans i*s of particular interest, as it is representative of the normal microflora of human with broad spectrum of antibacterial action. The use of this microorganism has a number of advantages: the absence of side effects on the body; high adhesive abilities; resistance to lysozyme in saliva; the ability of use in patients, sensitized to antibiotics and chemotherapeutic drugs; stimulation effects on the human immune system. **Material and methods.** The purpose of the study was to investigate the antagonism of *A. viridans № 167* and autostrains of aerococcuses, isolated at patients, to conditionally - pathogenic microflora of the nose and oropharynx of children with cardiac patology. At the first stage of the study the microflora of the of the nose and oropharynx of 2 investigated categories was examined – 40 children 4-14 years with cardiac patology and 40 healthy children 4-5 years old. The second stage of work was to study the effect of A. viridans on the explored strains. **Results and discussion.** *A. viridans* manifests the antagonism to all studied strains of gram-positive and gram-negative microorganisms, except *C. albisans. A. viridans* antagonistic activity to staphylococci (10 + 3 mm) and streptococci (10 + 2mm) is at the approximately same level. It is interesting to compare the

antagonism of aerococcuses to clinical isolates of *S. pyogenes* and similar strains from carriers (healthy children category). Impact of aerococcuses on *P. mirabilis* strain appeared at the highest level. Autosimbionts of *A. viridans*, isolated from healthy children, are more antagonistic to CPM strains, isolated from these children, than autostrains of *A. viridans*, isolated from children with with cardiac patology, and higher than the museum strain of *A. viridans № 167* antagonism, and do not affect the growth of microorganisms of *Candida* kind. Severe antagonistic activity of the *A. viridans № 167* strain and autostrains of *A. viridans* to isolated conditionally patogenetic strains was discovered: *S. pyogenes, S. pneumoniae, S. aureus, K. pneumoniae, P. mirabilis.* Antagonism of *A. viridans № 167* to *C. albicans* was not found. **Conclusion.** The recieved data can serve as a basis for further study of the strain of *A. viridans № 167* and aerococcuses' autostrains usage for correction of fauces and nasopharynx microbiocenosis and also for the complex treatment and preventive actions in case of cardiac pathology.
Key words*:* Aerococcus viridans, probiotics, autostrains of aerococcuses, children.