Stepanskyi Dmytro Oleksandrovitch
SE "Dnepropetrovsk Medical Academy
of Ministry of Health of Ukraine",
associate manager by a Department Microbiology,
Virology, Immunology and Epidemiology
Koshova Irina Petrivna
SE "Dnepropetrovsk Medical Academy
of Ministry of Health of Ukraine",
teacher General and Clinical
Pharmacy Department
Kremenchutskyi Gennady Mykolayovitc,
SE "Dnepropetrovsk Medical Academy of Ministry
of Health of Ukraine" professor Department
Microbiology, Virology,
Immunology and Epidemiology
Xeniya Fedoryak
Medical University of Lodz, Poland
English division, 2nd year

BIOTIPES OF AEROCOCCUS VIRIDANS

SUMMARY

A. viridans microorganisms are widespread in nature, they are representatives of the macro-microbiocenosis. There is evidence in the literature on the allocation of A. viridans during various diseases without an analysis of the immune status and the study of other possible pathogens that are the main cause of the disease. The presented in the literature genetic analysis of A. viridans took from the pathological focus does not gives us an opportunity to associate it with certain characteristics aerococcus. We conducted the research of excretion A. viridans from healthy macroorganisms and from the environment to study their biological characteristics

(for antibacterial compounds, the ability to produce reactive oxygen species, antioxidant system activity and antagonist activity). Based on these characteristics A. viridans were divided into three biotypes with strict compliance scientists features.

Key words: aerococcus, biotypes, biochemical activity, antioxidant protection.

Formulation of the problem. Despite the fact that it is long time since the discovery and identification of aerococcus there is still no information about dissemination and characteristics of aerococcus takes part in the composition of microbial associations of endothermal animals. For the first time aerococcus was sorted out in 1953 [42, 43], by the inoculation of the air, the air from the hospitals [33], soil [34].

Analysis of recent research and publications. Right after it was reported that aerococcus found in pathological material in the infectious process. A. viridans were isolated from the blood of patients with endocarditis [25, 28], from the urine of patients with urinary tract infection [31], from the blood of patients with hypogranulocytosis [24, 40].

Aerococcus is prevalent in the animal microflora. The hemocultures of aerococcus were obtained from mice with bacterieamia [29]. It has been found that A. viridans is sensitive to penicilline, macrolide and chloramphenicol and resistant to aminoglycoside [26]. Were described the incidents of diseases in animals: pigs [36], cows' mastitis [39], amphibious – turtles [40].

The discovery of A. viridans in the focus of inflammation may not be indicative of their aetiological role without detailed bacteriological and virologic research of pathological material (disclosure or absence of Chlamydia or viruses). No study indicated the discovery of pathogenicity factors in aerococcus and attempts to simulate an experimental animal model of infection has not been successful [32].

At the same time it was shown the wide representation of aerococcus in microbiocenosis of macroorganisms [18].

Biological treatment and prevention drugs, developed on the basis of A. viridans 167 [2, 7, 11, 19, 20] were separated from the human milk by the professor

Gorbunova M. L. (1965) have demonstrated clinical efficacy in various pathological processes [21].

It has shown effectiveness for bacterial infection of the nasopharynx [4], increased immunoresponsiveness body [3, 15, 20] for preventing and treating salmonellosis [21], staphylococcal infection [13, 14, 16, 19] for control and normalization of the vaginal flora [18], the correction of dysbiosis and homeostasis of the organism [12], pulmonary tuberculosis [5], urologic complications of pathological processes [8], an antagonistic effect on the meningococcus [1].

Bold unsolved aspects of the problem. It has showed the intraspecific difference between aerococcus [17]. During studies at the molecular level in aerococcus (genetic characteristics, distribution of cell wall proteins during electrophoresis) have emerged a large number of aerococcus cultures with slight differences in characteristics assigned. This distribution is separated cultures do not allow to make the correlation between the molecular differences and their biological characteristics.

The aim of the study. The object of our work is to try to spread the culture divided into two fundamental characteristics of A. viridans (the ability to oxidize lactate production of hydrogen peroxide and selenium reduction of its salt).

Presenting main material. We studied gram-positive, catalase-negative cocci taken from biological material from healthy people, animals and birds, and delivered a comparative study to their taxonomic characteristics.

It has been studied 118 aerococcus crops; taken from the human body, 16 cultural objects taken from the environment (water, wash vegetables), 21 cultures taken from animals (mice - 10 aerococcus crops, pigs - 3 cultures, cows- 5 crops, chicken - 6 of Culture), a strain of sample preparation and bacterial-bacterine N and 4 links aerococcus cultures. As reference cultures were used strains A. viridans from the collection SSM: №1911 [28, 32, and 41].

Separation and identification of aerococcus cultures was held in the agreements methods offered by GN Kremenchutskiy et al (2009) [7].

For separation (gram+, gram-) cocci was used fluid from the oral cavity of healthy people and faeces of animals and birds as biological material. Intake and held crop material and identifying microbial culture were made in accordance with [10].

To distinguish aerococcus forms on biochemical activity was developed an indicated medium, that includes sodium selenite. The following composition: 1 l of distilled water: potassium iodide - 26 g, soluble starch - 10 g, sodium selenite - 0, 4 g, dry nutrient medium - 32 g. The incorporation into the potassium iodide and soluble starch in medium makes it possible to evaluate the activity of oxidase aerococcus resulting color of the colonies in the culture medium as deep blue color. Aerococcus colonies that reduce sodium selenite in the selenium initial stain appear red.

Sensitivity to antibiotics are evaluated by agar diffusion method using discs [10]. Aerococcus antagonistic action to the test cultures of microorganisms has been studied by the methods of deferred antagonism.

Glutathione peroxidase activity was determined by the method of [9]. The protein was identified using the method of [34]. Superoxide was determined by the method of [36]. The activity of superoxide was identified by the method of [6]. SOD activity was expressed in micromoles / min. mg protein.

During sowing crops aerococcus cultures separated on indicated media was noticed another color of the colonies and of the medium depending on the biochemical activity aerococcus. Culture, which oxidize potassium iodide sprouted colony with an intense dark color with dark coloring of the growth medium, the cultures were evaluated among 1 biotype aerococcus. Cultures that reduce selenium from sodium selenite colony sprouted, painted in red. These cultures were assessed as two biotypes aerococcus. Furthermore, it was observed that the growth of the colonies colored red with fade out within the area around the medium indicates the oxidation of potassium iodide and at the same time reducing selenium from sodium selenite about.

These cultures were rated as 3 biotype of aerococcus.(Pic.1) Distribution of separated cultures from different sources by biotypes is shown in Tab. 1

Pic. 1. Three biotypes Aerococcus viridans:

a) Black colonies – 1 biotype, red – 2 biotype of aerococcus;

b) Red colonies with black zones – 3 biotype.

Table 1

The distribution of crops on the forms of biochemical activity, depending on the emission source

Source of the release	Quantity of examined aerococcus cultures	Distrobution of cultures on forms of biochemical activity		
		Oxidation KJ (1 type)	Reduce of Na_2SeO_3 (2 type)	Both types of activity (3 type)
Reference Laborarory Reference Strains:	1911	+	-	-
	1914	-	+	-
	2439	+		
	2440,	-	+	-
	2452	+	-	-
human	Strain for production A-bacterine № 167	1	0	0
human	30	17	6	7
birds	23	22	3	10
cows	6	1	0	1
sheep	5	2	4	1
pigs	7	4	3	0
mice	10	6	2	2
air	5	4	2	1
water	23	19	2	2

A study of sensitivity of aerococcus strains to antibiotics of penicillin row and lysozyme was conducted on the samples, which aim is the cell walls of aerococcus. Details of these experiments in Table. 2

From the data of Table 2 can be seen the clear difference in the effect of antibiotics penicillin and lysozyme row, which shows the existence of the characteristic in the structure of cell walls 2, 1 and 3 biotypes.

Tab.2

Sensitivity of aerococcus strains to penicillin and lysozyme

Biotypes	Minimum abscopal concentration of antibiotics, microgram/ ml			
	penicillin	oxacillin	methicillin	lysozyme
1 biotype	60-125	120-500	1,92-3,84	15-61,44
2 biotype	0,06-0,12	0,06-0,12	0,06-0,12	2000-2500
3 biotype	62,5-125	62,5-125	0,06-0,12	1,92-3,84

Previously we have seen that aerococcus ROS production is a result of the oxidation of lactic acid, glycerol phosphate, glycine. To account for the production of ROS aerococcus antagonize as for pathogenic and opportunistic microorganisms. Table 3 shows the results of experiments on the detection of superoxide anion production and hydrogen peroxide bio. A. viridans.

Antioxidant protection of aerococcus cells comes true by functioning superoxide dismutase, glutathione peroxidase, also chemical reaction between hydrogen peroxide with pyruvic acid, that form lactic acid. In the tab. 4 are showed the characteristics of superoxide dismutase and GSH-peroxidase activity of biotypes A. viridans.

Antioxidant protection of aerococcus cells exist by functioning of superoxide dismutase, glutathione peroxidase, and a chemical reaction between the hydrogen

peroxide with pyruvate to produce lactic acid. Table. 4 shows the characteristics of SOD and GSH-peroxidase activity biotypes A. viridans.

Tab. 3

Production of superoxide anion and hydrogen peroxide in the course of oxidation by biotypes A. viridans 0,045 M sodium lactate

Biotypes	Specific activity of production superoxide (O^-_2) on 1 mg of protein FK by 1 min	Influence on the production O^-_2 Superoxide dismutase (1ЕД/1ml)	Accumulation of hydrogen peroxide in broth , mM
1 type	13,8 ± 0,97	1,7 ±0, 3	4,2+0,8
2 type	1,35±0,02	0,95±0,23	0,08+0,001
3 type	8,1± 0,9	5,6 ± 0,26	2,3,3+0,06

Tab.4

Superoxide dismutase and GSN-peroxide activity of biotypes A. viridans

Biotypes	Specific activity of superoxide dismutase ED 1 mg of protein for 1 min	Specific activity GSH-peroxide ED on 1 mg of protein for 1 min
1 type	5,45±0,7	0,44±0,04
2 type	8,35±1,02	0,95±0,23
3 type	12,1 ± 0,9	5,6± 0,26

Antagonistic activity of representatives of different biotopes A. viridans for catalase-negative strain of Vibrio NAG and catalase-positive E. coli with lactate oxidase activity and the accumulation of hydrogen peroxide at the time of growth medium were compared. (tab. 5)

These data show the production of biologically active substances of different biotypes A. viridans: hydrogen peroxide, superoxide, lactate oxidase, superoxide dismutase and GSH-peroxidase.

Tab. 5

Comparison of antagonistic and oxydase activity in different types of aerococcus

Biotypes	Biological activity of aerococcus strains			
	Diameter of zone of the suppression the growth of Vibrio NAG (mm)	Diameter of zone of the suppression the growth of *E. coli*, (mm)	Activity of LDG on 1 mg of prot.	Accumulation of hydrogen peroxide in broth (mM)
1 type	37,2±3,4	12,1±1,2	1112±87	4,2±0,8
2 type	18,2±5,1	5,0±1,2	231±211	0,08±0,001
3 type	47,5±5,6	15,7±4,3	714±15	2,3,3±0,06

Information in the tables indicate that biotypes 1 and 3 of A. viridans at the time of equimolar production of adenosine phosphosphoric acid have a stronger antagonistic effect than catalase-negative strains of Vibrio NAG and catalase-positive E.coli. The antagonistic effect of biotype 2 Aerococcus viridans for catalase-negative strain Vibrio AG and catalase-positive E.coli during decreased activity of lactate oxidase and virtually zero production of hydrogen peroxide can be explained by the additional substances such antagonistic microsin, produced by aerococcus,

Despite the significant concentration of adenosine phosphoric acid produced by aerococcus is grows on ordinary nutrient media, and they are ubiquitous representatives of the normal microflora and microorganisms. These facts suggest about the balanced intracellular redox regulation of aerococcus**.**

Conclusions and suggestions. Microorganisms of the genera Aerococcus, A.viridans species are widely distributed in biotype and healthy macroorganisms, in the environment, and can be divided into three biotypes whose members differ in their sensitivity to penicillin and lysozyme by their ability to oxidize the lactic acid production APA reduce selenium from selenite sodium. Activities of A.viridans antioxidant system (catalase, superoxide dismutase and glutathione peroxidase) biotypes differ depending on the intensity of production of APA.

There is a correlation between oxidative activity A. viridans and antagonistic activity of representatives of three biotypes for catalase-negative Vibrio NAB and catalase-positive E.coli.

Taking into account the antagonistic effect of A. viridans type 2 catalase positive E. coli it may be assumed that there is an additional mechanism for the production of antibacterial factor

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