ORIGINAL ARTICLE

ANTAGONISTIC ACTIVITY OF REPRESENTATIVES OF ORAL BIOCENOSIS

DOI: 10.36740/WLek202106138

Natalya V. Kotelevska, Olha N. Boychenko, Yuliia V. Sidash, Andrii V. Zaitsev, Ivan Yu. Popovych, Anatolii K. Nikolyshyn
POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: Calculate the antagonistic activity of representatives of the oral biotope.

Materials and methods: The mathematical techniques used in biotechnology and dentistry. The calculations were carried out on the basis of the research data obtained by the investigators from Ukrainian Medical Stomatological Academy (UMSA) (Poltava).

Results: The paper shows that the oral biocenosis is a group of microorganisms that have similar characteristics within the group, but different outside. This may indicate to the relationships between the bacteria, existing within such groups. A graphical demonstration of the associates of the oral biotope with different DMF represents their growth phases depending on the intensity of dental caries. The different slope and phases of the logistic curve of their growth may indicate certain relationships arising between groups of microorganisms.

Conclusions: The symbiotic relationships within the oral biocenosis can be manifested by the antagonism, as indicated by the index of antagonistic activity, which in most cases can be calculated. Antagonistic activity can be determined more precisely, for which special techniques have been developed. Antagonistic activity of associates of biocenosis can serve as an indicator of the "conditionally pathogenic - pathogenic" transformation. Data on antagonistic activity can be used in oral hygiene activities and treatment of dental caries to impact the oral microbiocenosis. Data on antagonistic activity may have a predictive value.

KEY WORDS: oral cavity, dental caries, biotope, biocenosis, antagonistic activity

Wiad Lek. 2021;74(6):1493-1498

INTRODUCTION

The study of microbial organizations on the body surfaces and cavities has been studied in medicine since L. Pasteur suggested that human and animal diseases, similar to rotting and fermentation, are caused by certain types of microorganisms, and R. Koch discovered and studied the causative agents of serious human infectious diseases and laid the foundation of the new science of microbiology, medicine has paid great attention to.

In dentistry, it is scientifically proven that microorganisms of dental plaque are the cause of origination and development of dental caries [1, 2]. Dental caries has been studied worldwide. The Ukrainian Medical Stomatological Academy (UMSA, Poltava) is no exception [3 - 7]. Microflora representatives, causing dental caries, isreferred to the normal flora of the oral cavity [8]. Regarding the dysbiosis, a paradigm is currently being developed that this pathology arises due to disruption of the dynamic equilibrium between the forces of opposing biological objects, namely, macro- and microorganisms [9]. Recent researches are devoted to the problem of symbiosis of microorganisms in biological objects in the form of biofilms. Depending on the tasks set byinvestigators the attitude towards microorganisms varies from negative to directly opposite [10]. This situation makes it rational to study the relationship between the representatives of oral microflora. Understanding the correlations between them can be helpful in choosing a therapeutic effect with optimal parameters (type, method, effect, duration).

THE AIM

The paper was aimed at presentation of correlations between the associates of oral biotope using mathematical analysis.

MATERIALS AND METHODS

Studies made at the Department of Therapeutic Dentistry and the Department of Microbiology, Virology and Immunology of the Ukrainian Medical Stomatological Academy (UMSA), as well as publications related to the study of microflora have been used during the research. Bibliosemantic analysis, interference and extrapolation of the resulting data on the correlations in oral biocenosis, mathematical methods, online calculator have been utilized.

RESULTS

Graphical representation of the microflora of the oral biocenosis at various values of the DMF index, based on data

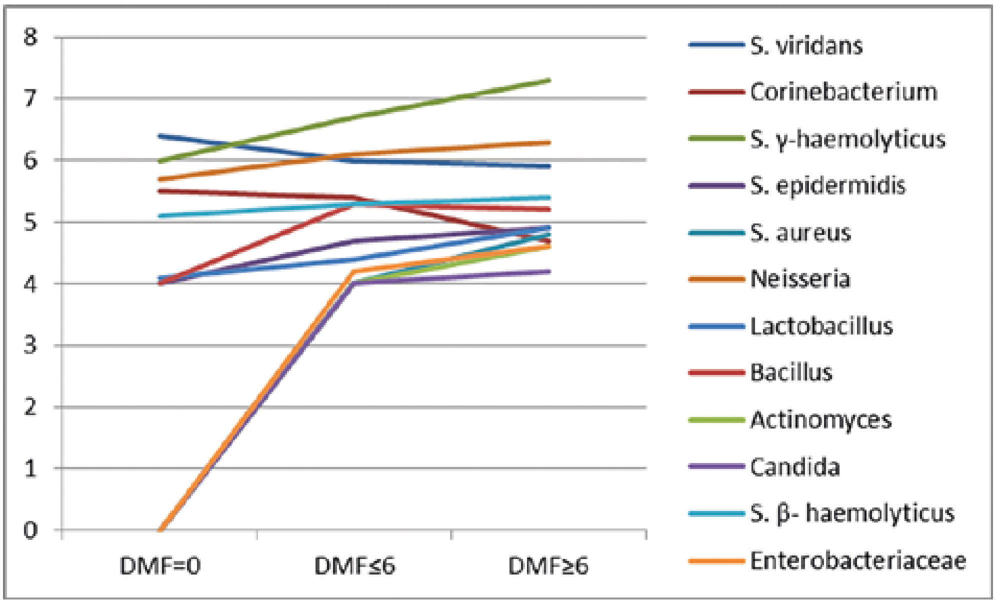

Fig. 1. Species microbiota content of oral fluid in individuals with varying intensity of dental caries, lg CFU/ml.

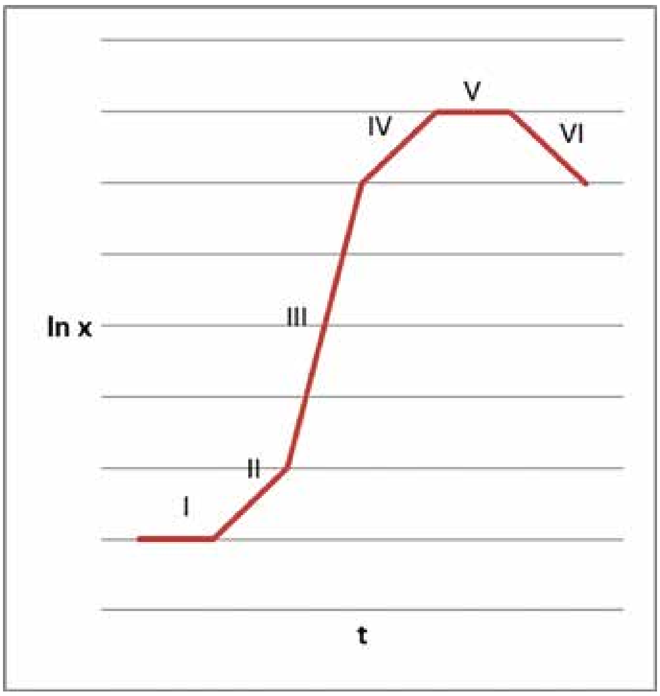

Fig. 2. The growth curve of biological organisms. I – the lag-phase; II – phase of growth acceleration; III – phase of exponential growth; IV – phase of growth retardation; V – stationary phase; VI – phase of cultureextinct.

from a study conducted by the UMSA investigators, illustrates fragments of the growth of biological objects corresponding to the logistic growth curve. The growth dynamics diagrams of the representatives of the oral microbiota in individuals with different DMF values have different slope, similar to the different growth phases of microorganisms.

This fact indicates certain intermicrobial relationships between the associates of the oral biocenosis. Apparently, antagonism is a type of symbiotic relationships.

The antagonistic activity of the representatives of the oral biotope was determined at different intensity of the carious process.

Determination of the antagonistic activity of the representatives of the studied microflora of the oral biotope showed that in most cases this is possible.

The erroneous result in determining the AA of the representative of the fungal microflora, as well as other representatives with an exponential growth site of the logistic curve, can be explained by the incompleteness of data about it, or by the actual absence of *Candida* in the biocenosis in DMF = 0. It can also be associated with the need to introduce additional coefficients into the formula, or to adapt it more correctly.

Noteworthy, the colonies of *S. γ-haemolyticus* increase insignificantly with increasing intensity of dental caries that is more cariogenic compared to fungi.

In 2013, the faculty members of the UMSA (Department of Therapeutic Dentistry; Department of Microbiology, Virology and Immunology; T.A. Petrushanko, V.V. Chereda, G.A. Loban) conducted a study of the oral microbiocenosis in people aged 19-25 years with different intensity of dental caries. The study was devoted tothe analysis of the percentage and quantitative ratio of the microflora of the oral biotope in individuals with different DMF indices (the WHO's recommendations, 1963). The findings showed that the increase in the intensity of dental caries promoted disbalance in the composition of theoral microflora. Noteworthy, in all examined individuals of this age, regardless the DMF value, the PMA index (Parma modification, 1960) was zero [11].

Graphical demonstration of the findings of the study of oral microbiocenosis in individuals with different caries intensity enabled visualization of the developmental and state indicators of the studied microflora. The presentation of the material (Fig. 1) demonstrates the assignment of the representatives of the oral biotope into several groups according to their amount in the saliva of the subjects with different DMF.

Graphic image demonstrates a group of microorganisms, which, in a certain way, reduces colony formation with an

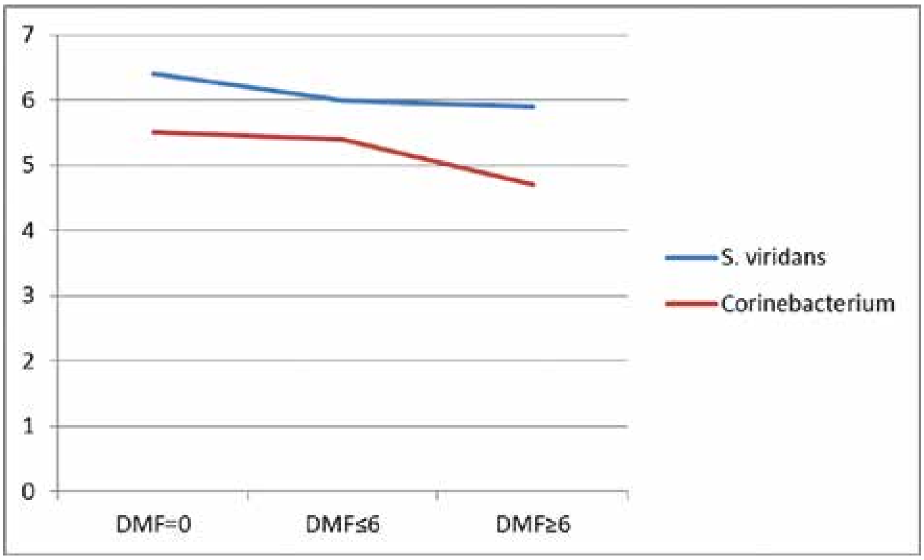

Fig. 3. The dependence of oral microbiota representatives at Phase V and VI of the logistic curve on the intensity of dental caries, lg CFU/ml.

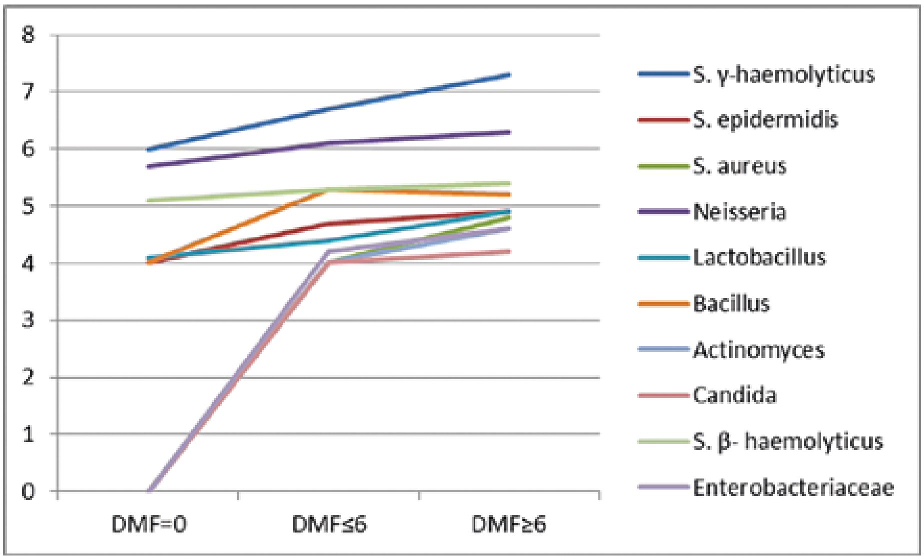

Fig. 4. The dependence of oral microbiota representatives at Phase III and IV of the logistic curve on the intensity of dental caries, lg CFU/ml.

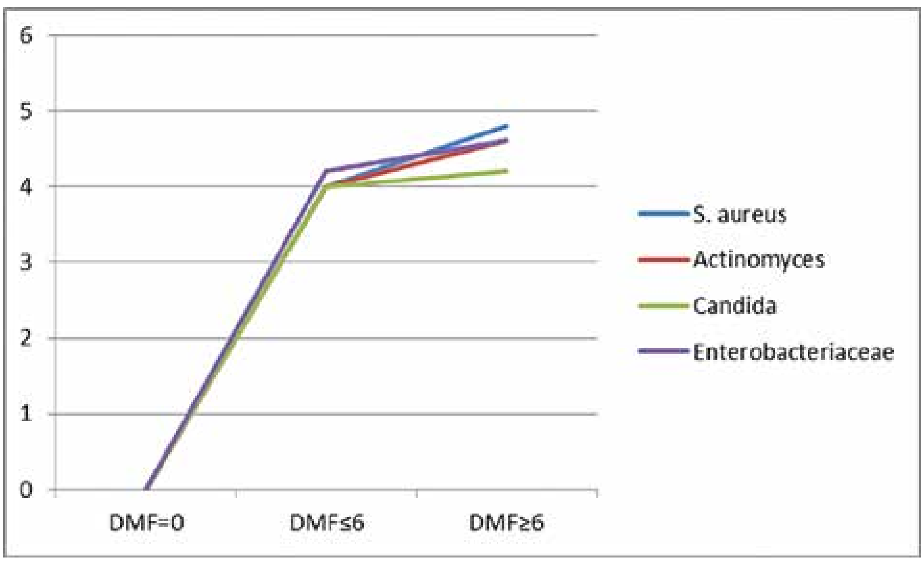

Fig. 5. The dependence of oral microbiota representatives at Phase III and IV of the logistic curve with a pronounced exponential growth site from the intensity of dental caries, lg CFU/ml.

increase of DMF (Fig. 3), and a group of microorganisms, which increases colony formation with the rise of DMF (Fig. 4).

Within the group of microorganisms that increase the number of colonies with an increase in DMF, a group with a pronounced exponential area of growth can be distinguished (Fig. 5).

Populations of any species existing on the Earth, being in conditions favorable for life, increase their numbers according to a certain pattern (Fig. 2) [12].

The curves of the presented type are called S-shaped curves. For the first time, the Belgian mathematician Pierre Verhulst introduced the equation for such a curve to describe the population size, and called the curve logistic. A great contribution to the development of the approach was made by Raymond Pearl, who used it to describe changes in the number of populations of organisms and population size. Biologists call the similar graphics the Pearl curve [13].

The populations of bacteria, plants, animals and any living speciesexisting in favorable conditions increase in size exponentially. After some time, due to excessive population size, the habitat is weakened and degraded. Ecological crisis comes, during which the population size rapidly reduces to a level lower than the degraded environmental capacity. Collapse comes. If during the collapse the environment is gradually restored, then, subsequently, the population size also increases. It enters the stabilization phase, and its size will vary at the level specified by the capacity of the environment. If not, then the population becomes extinct [14].

Curves of changes in the number of representatives of the oral biocenosis at different values of the DMF index in a study conducted by the UMSAinvestigators visualize the fragments of biological objects'growth corresponding to the logistic growth curve. The diagrams of changes in the number of thestudied microorganisms in individuals with different DMF values have a different slope, similar to the different stages of organisms'growth.

To achieve the objectives of the research,mathematical analysis is commonly used in the studies,related to microflora analysis. The faculty members of Sumy State University and State University "L.V. Gromashevskiy Institute of Epidemiology and Infectious Diseases" of the NAMS of Ukraine (Kiev) have analyzed the incidence of morbidity and etiological structure of the acute intestinal infections and investigated the intermicrobial relationships of the dominant pathogens. In the study, antagonistic index was calculated by the formula:

$$A = (K / K + T) \times 100\% \quad (1)$$

where:K – thenumberofcoloniesoftheinvestigatedculture; T – the number of colonies of test-strain [15].

Another study relates to microbiology and can be used to study the mechanism of intermicrobial interactions, in particular the role of individual cellular components of microorganisms in the regulation of antagonistic relationships in microbial communities. The CFU and antagonistic activity (AA) is calculated in the experimental and control samples according to the formulas:

$$AA_{control} = (A - B_1) / A \times 100\% \quad (2)$$

$$AA_{experience} = (A - B_2) / A \times 100\% \quad (3)$$

where:AA - antagonistic activity of microorganisms; A - growth control of indicator strain, CFU t_8 / CFU t_0 (control 1); B_1 - control of antagonistic activity, CFU t_8 / CFU t_0 (control 2); B_2 - the degree of growth of the indicator strain, CFU t_8/CFU t_0 (test); t_0 - the beginning of the growth of microorganisms; t_8 - the number of microorganisms within 8 hours. The change in antagonistic activity is calculated by the formula $_{\Delta}AA = AA_{control} - AA_{experience}$.[17].

The third work relates to the field of microbiology and biotechnology and represents the production of the new bacterial strain *Lactobacillus paracasei* 1, which can be used in the microbiological industry and in the production of probiotics. The adhesive properties of lactobacilli against human and porcine erythrocytes were assessed by the formula:

$$PA = (D_{c1} + D_{c2} - D_{exper}) / D_{c1} \times 100\% \quad (4)$$

where: PA - an indicator of adhesion; D_{c1} - optical density of the supernatant in the 1st control sample; D_{c2} - optical density of the supernatant in the 2nd control sample; D_{exper} - the optical density of the supernatant in the experimental sample [23].

Mathematical expressions used in the above works are used in dentistry to determine the prevalence of pathological processes. In particular, the calculation of the prevalence of dental caries occurs in the same way, which is carried out according to the formula [24]:

$$J_к = (N_к / N_{exam}) \times 100\% \quad (5)$$

where:$J_к$ - prevalenceofdentalcaries; $N_к$ - number findi viduals with decayed teeth; N_{exam} - number of examined individuals.

In determining inflammatory periodontal diseases, a similar technique is used in assessing the severity and prevalence of gingivitis in a patient:

PMA = sum of points / 3 x number of teeth x 100% (6)

This is papillo-marginal-alveolar (PMA) index of inflammation in the periodontitis [14].

Generally, similar mathematical formulas can be used inprocessing dynamic statistical data. The formula of the adhesion index of *Lactobacillus paracasei* 1 is most adaptable to the objectives of our research. We replace the ПА with antagonistic activity, $D_{c1}$with the number of microorganisms with a lower DMF, $D_{c2}$with the number of microorganisms with a higher DMF, $D_{exper}$with the number of microorganisms with the minimal DMF, possible for the reference value. In this case, we obtain an expression that can provide accurateassessment of the antagonistic activity of the associative organisms of the oral biotope studied by the UMSA investigators. The formula can be calculated using online math calculators [18].

To calculate the antagonistic activity (AA) of the representatives of the oral biotope, we take several microorganisms with different growth phases. Calculate AA for corynebacteria (*Corynebacterium*is a representative of normal flora, which decreases its number with increasing DMF). Adapt Formula 4 to our calculations:

$$AA = (N_1 + N_2 - N_n) / N_1) \times 100\%, \quad (7)$$

where: AA - the antagonistic activity of microorganisms; N_1 - the number of colonies of microorganisms with low DMF; N_2-the number of colonies of microorganisms with high DMF; N_n - number of colonies of microorganisms in the physiological norm. In this case, in DMF = 0, we take the number of microorganisms equal to the physiological norm (see Fig. 3).

Substitute the numerical values for corynebacteriain DMF ≤ 6 in the adapted formula 7: AA = ((5.5 + 5.4 - 5.5) / 5.5) x 100%. Perform a calculation by online calculator and get the value: AA = 98.18%. Calculate AA for DMF ≥ 6: AA = ((5.4 + 4.7 - 5.5) / 5.4) x 100%. The value of AA is 85.18%.

Make calculations for *Candida* fungi: in DMF ≤ 6 AA = ((0 + 4 -0) / 0) x 100%. The calculation on the online calculator gives the value of AA = error, i.e. calculation error. InDMF ≥ 6: AA = 205%.

Make calculations for *S. γ-haemolyticus*, including *S. mutans* [21]: in DMF ≤ 6 AA = ((6 + 6,7- 6) / 6) x 100%. The calculation on the online calculator gives the value of AA = 111,67%. In DMF ≥ 6: AA = 119,40%.

DISCUSSION

On the Earth, bacteria do not live in isolation, therefore their properties and functions in the existence of other microorganisms are not identical to those in monocultures. This fact dictates the need to study the intermicrobial interactions of the associates of biocenoses. Numerous researches have shown that one of the types of symbiotic relationships is the antagonism [20]. The properties manifested by bacteria in its process are one of the mechanisms for the formation and functioning of microbial communities. Human microbiocenoses are no exception [21]. The above publications reported that the study of intermicrobial correlations between the associates made it possible to distinguish several of their varieties: indifferent, stimulating, inhibiting, and inverting [22].

Contemporary dentistry put forward some representatives of the normal microflora of the oral biotope, namely, oral streptococci, lactobacilli and some actinomycetes as a pathogenic onset of dental caries. These microorganisms ferment carbohydrates with the formation of acids in the process of vital activity, contributing to the demineralization of hard tooth tissues [23]. A number of publications contain reference on the fact that carious pathology is manifested by the increase in the number of fungi of the genus *Candida* in the oral cavity [24, 25]. These data put more emphasis on the problem of considering correlations within the microbiocenoses of the human body.

CONCLUSIONS

The study shows that the oral biocenosis is a group of microorganisms that have similar characteristics within the group, but different outside. This may indicate existence of relationships between the components of bacteria within these groups. And different slope of the logistic curve between groups of microorganisms may indicate certain relationships arising between these groups.

A graphic representation of the associates of the oral biotope in different DMF represents the phases of the logistic curve depending on the intensity of carious pathology.

The symbiotic relationships within the oral biocenosis can be manifested by the antagonism, as indicated by the index of antagonistic activity, which in most cases can be calculated. Antagonistic activity can be determined more precisely, requiring application of special techniques.

Antagonistic activity of associates of a biocenosis can be the indicator of the "conditionally pathogenic-pathogenic" transformation. A slight increase in the *S. γ-haemolyticus* colonies with an increase in the intensity of dental caries highlights the need to study the cariogenic representative of this group of microorganisms.

Data on antagonistic activity can be used in oral hygiene activitiesand treatment of dental caries to impact the oral microbiocenosis. Data on antagonistic activity may have a predictive value.

REFERENCES

1. Hofunh Ye.M. Osnovy odontolohiyi (propedevtyka) [Fundamentals of odontology (propaedeutics)]. Kharkiv, Kyiv: Medvydav; 1931, 282 p. (in Ukrainian).
2. Black G.V. Konservierende zahnheilkunde. Berlin: Verlag von Hermann Meusser; 1914, 343 p.
3. Zaytsev A.V. Likuvannia neuskladnenoho kariiesu zubiv u khvorykh starshykh vikovykh hrupp zobmezhenoiu rukhomistiu oporno-rukhovoho apparatu z vykorystanniam atravmatychnoi vidnovnoi tekhniky [Treatment of uncomplicated dental caries in patients of older age groups with limited mobility of the musculoskeletal system using atraumatic restorative techniques] [dissertation]. Poltava; 2012. 140 p. (in Ukrainian).
4. Pavlenkova O.S. Osoblyvosti klinichnoho perebihu i profilaktyky kariiesu zubiv u ditei, yaki chasto khvoriiut na hostri respiratorno-virusni infektsii [Features of the clinical course and prevention of dental caries in children who often suffer from acute respiratory viral infections] [dissertation]. Poltava; 2016. 164 p. (in Ukrainian).
5. Dubinin S.I., Zaytsev A.V., Vatsenko A.V. et al. Mezhmikrobnyye vzaimodeystviya oral'nogo biotopa [Intermicrobial interactions of the oral biotope]. Georgian medical news. 2020; 299:131-137. (in Russian).
6. Zaytsev A.V., Boychenko O.N., Sidash Y.V. et al. Qualitative analysis of oral lactobacilli parameters at different stages of human life cycle and different caries indices. Wiad Lek. 2020;73(6):1207-1210.
7. Sidash Yu.V., Boychenko O.N., Popovich I.Yu. et al. Effect of ultrasound on S. epidermidis museum culture. Wiadomości Lekarskie. 2020;73(4):786-788.
8. Krendelev M.S. Normal'naya mikroflora rotovoy polosti cheloveka [Normal microflora of the human oral cavity]. Modern problems of science and education [Internet]. 2015;5. https://www.science-education.ru/ru/article/view?id=21628 (in Russian).
9. Okushko V.R. Osnovy fiziologii zuba [Fundamentals of Tooth Physiology]. Tiraspol': Izd-vo Pridnestr. un-ta; 2005. 240 p. (in Russian).
10. Boychenko O.N., Kotelevskaya N.V., Nykolyshyn A.K. et al. Analyz predstavlenyy o zubnykh otlozhenyyakh [Analysis of ideas about dental plaque]. Bulletin of problems in biology and medicine. 2017;1(3):19-24. (in Russian).
11. Petrushanko T.O., Chereda V.V., Loban H.A. Yakisnyi sklad mikrobiotsenozu porozhnyny rota osib molodoho viku z riznoiu intensyvnistiu kariiesu [Qualitative composition of the oral microbiocenosis of young people with different caries intensity]. The world of medicine and biology. 2013;(1):57-59. (in Ukrainian).
12. Pishak V.P., Bazhora Yu.I. Medychna biolohiia [Medical biology]: pidruchnyk. Vinnytsia: Nova knyha; 2004. 656 p. (in Ukrainian).
13. Molchanov A.V. Razvitiye teorii S.P. Kapitsy. Naseleniye Zemli kak rastushchaya iyerarkhicheskaya set'. Sankt-Peterburg; 2006. Chapter 9.5, Glavnyy zakon rosta chislennosti izolirovannoy populyatsii [The main law of growth in the size of an isolated population]. 2021. http://avmol51.narod.ru/Glavnyj_zakon_rosta.html. (in Russian).

14. Riznichenko Yu. Modeli vzaimodeystviya vidov [Models of Species Interaction]. DOCPLAYER. 2008. https://docplayer.ru/52697094-Modeli-vzaimodeystviya-vidov.html. (in Russian).
15. Malysh N.G., Kholodilo Ye.V., Chemich N.D. et al. Etiologicheskaya struktura i osobennosti mezhmikrobnykh otnosheniy dominiruyushchikh vozbuditeley ostrykh kishechnykh infektsiy v severo-vostochnom regione Ukrainy [Etiological structure and peculiarities of intermicrobial relations of the dominant causative agents of acute intestinal infections in the northeastern region of Ukraine]. Epidemiologiya i Vaktsinoprofilaktika. 2015;(4):41-48. (in Russian).
16. Bukharin O.V., Semenov A.V., Cherkasov S.V., Sgibnev A.V. Institut kletochnogo i vnutrikletochnogo simbioza UrO RAN, patentoobladatel'. Sposob opredeleniya sposobnosti mikroorganizmov regulirovat' antagonisticheskuyu aktivnost' bakteríy [A method for determining the ability of microorganisms to regulate the antagonistic activity of bacteria] [Internet]. Patent Rossii № 2376381C2. https://patents.google.com/patent/RU2376381C2/ru . (in Russian).
17. Marakulin I.V., Darmov I.V., Pozolotina N.V., Orlova A.Yu. Federal'noye gosudarstvennoye byudzhetnoye obrazovatel'noye uchrezhdeniye vysshego obrazovaniya «Vyatskiy gosudarstvennyy universitet», patentoobladatel'. Shtamm bakteriy lactobacillus paracasei 1, ispol'zuyemyy dlya prigotovleniya probioticheskogo preparata [The bacterial strain lactobacillus paracasei 1 used for the preparation of a probiotic preparation]. Patent Rossii № 2608871. http://www.findpatent.ru/patent/260/2608871.html/ . (in Russian).
18. Borisenko A.V. Kariyes zubov [Tooth decay]. Kiev: Kniga plyus; 2005. 416 p. (in Russian).
19. Onlayn-kal'kulyator. Primery resheniya zadach po matematike [Online calculator. Examples of solving problems in mathematics]. https://math.semestr.ru/example.php. (in Russian).
20. Semenov A.V. Antagonizm kak rezul'tat mezhmikrobnykh vzaimodeystviy [Antagonism as a result cross-species interaction between micro-organisms]. Bulletin of the Orenburg Scientific Center of the Ural Branch of the Russian Academy of Sciences [Internet]. 2013;1-8. http://elmag.uran.ru:9673/magazine/Numbers/2013-1/Articles/SemenovAV(2013-1).pdf. (in Russian).
21. Bukharin O.V., Valyshev A.V., Gilmutdinova F.G. et al. Ekologiya mikroorganizmov cheloveka [Ecology of human microorganisms]. Yekaterinburg: UrO RAN; 2006. 546 p. (in Russian).
22. Cherkasov S.V., Semenov A.V. Mikrobnaya regulyatsiya antagonisticheskoy aktivnosti laktobakteriy [Microbial regulation of the antagonistic activity of lactobacilli]. Siberian Medical Journal. 2012;(2):78-82. (in Russian).
23. Nikolishyn A.K. Terapevtychna stomatolohiia [Therapeutic dentistry]: pidruch. dlia studentiv stom. fak. vysh. med. navch. zakl. IV rivnia akredytatsii. 2-he vyd. Vinnytsia: Nova Knyha; 2012. 680 p. (in Ukrainian).
24. Shabashova N.V., Novikova V.P., Kuzmina D.A. et al. Candida spp. i mikrobiotsinoz polosti rta u detey s dekompensirovannoy formoy kariyesa [Candida spp. and microbiocinosis of the oral cavity in children with decompensated caries]. Problems of medical mycology. 2011;(1):23-27. (in Russian).
25. Ghasempour M., Sefidgar S.A., Eyzadian H. et al. Prevalence of candida albicans in dental plaque and caries lesion of early childhood caries (ECC) according to sampling site. Caspian J Intern Med. 2011;2(4):304-308.

The paper has been written within the research work made at the Department of Therapeutic Dentistry of the Ukrainian Medical Stomatological Academy, entitled "Development of pathogenetic prevention of pathological changes in the oral cavity of persons with internal diseases" (state registration number 0121U108263, deadlines 2021-2025).

ORCID and contributionship:

Natalya V. Kotelevska: 000000027095653X A, F
Olha N. Boychenko: 0000000310795719 B, D
Yuliia V. Sidash: 000000018955754X B,C,D
Andrii V. Zaytsev: 0000000331235681 B, C, D
Ivan Yu. Popovych: 0000-0003-1720-095X B, C, D
Anatolii K. Nikolyshyn: 0000000243957828 E

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Yuliia V. Sidash
Poltava State Medical University
23 Shevchenka st., 36011 Poltava, Ukraine
tel: +380509809865
e-mail: u.v.sidash@ukr.net

Received: 04.11.2020
Accepted: 12.04.2021

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article