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Antibiotic properties of nisin in the context of its use as a food additive

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Background. *Scientific research has demonstrated that microbial pathogens could develop resistance to multiple antibacterial agents. Food additives, in particular preservatives, have also been shown to increase antimicrobial resistance of food-contaminating microorganisms. In this work the lantibiotic nisin was shown to exhibit: it contributes to the development of the antibiotic resistance of pathogenic and opportunistic microorganisms, reduces the immune status, the development of an imbalance of intestinal microbiocenosis, affects the body's metabolism through the regulation of DNA transcription.*
Purpose. *To assess the risks of nisin (E234) use taking into account its impact on the biological properties of microorganisms-food contaminants.*
Material and methods. *Calculation of nisin consumption with food under conditions of scenarios 1 and 2 was carried out taking into account the bodyweight of consumers of different age groups in the Russian population in the software Exel. The analysis of scientific data on the biological properties of nisin, including the ability to form to the resistance of microorganisms was provided.*
Results and conclusion. *For the first time, the calculated amounts of the food additive-preservative nisin (E234) in the intestinal contents were shown to exceed the minimum inhibitory concentrations of nisin for representatives of the normal flora of the human gastrointestinal tract in consumers of all ages by from 40 to 27064 times, depending on the consumption scenario (with minimum and maximum exposure levels). It has been argued that the safety of nisin used as a food additive needs to be re-assessed taking into account its considerable contribution to the antimicrobial resistance of food pathogens.*

K e y w o r d s : nisin; food additive; antimicrobial resistance; pathogenic and opportunistic microorganisms.

For citation: Bagryantseva O.V., Khotimchenko S.A., Petrenko A.S., Sheveleva S.A., Arnautov O.V., Elizarova E.V. Antibiotic properties of nisin in the context of its use as a food additive. *Gigiena i Sanitaria (Hygiene and Sanitation, Russian journal).* 2020; 99 (7): 704-711. DOI: https://doi.org/10.33029/0016-9900-2020-99-7-704-711 (In Russian)

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Gratitude. We Express our gratitude to Professor, a full member of the Russian Academy of Sciences, Victor Tutelyan for his most invaluable support and guidance of this work.
Conflict of interest. The authors declare no conflict of interest.
Acknowledgment. This research was funded by the federal budget grant issued to the Federal Research Center for Nutrition, Biotechnology and Food Safety, project No. 0529-2018-0113: "Formation of the methodological and regulatory framework to ensure modern requirements for the quality of food and development of technological evaluation of the effectiveness of specialized food products" Section: "Justification of hygienic regulations for the use of food additives for the formation of the legislative framework of the Eurasian Economic Union".
Contributions: Bagryantseva O.V. – research conceptualization and design, data collection and processing, statistical data processing, writing the text, editing. Khotimchenko S.A. – research conceptualization and design, data collection and processing, statistical data processing, writing the text, editing, writing the text, editing. Petrenko A.S. – research conceptualization and design, data collection and processing, statistical data processing, writing the text, editing. Sheveleva S.A. – research conceptualization and design. Arnautov O.V. – research conceptualization and design. Elizarova E.V. – research conceptualization and design, data collection and processing, statistical data processing. All co-authors – approval of the final version of the manuscript, responsibility for the integrity of all parts of the manuscript.
Received: March 19, 2020
Accepted: May 19, 2020
Published: August 28, 2020

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Антибиотические свойства низина в контексте его применения в качестве пищевой добавки

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Введение. *Известно, что микробные патогены могут вырабатывать резистентность к множественным антибактериальным препаратам. Пищевые добавки, в частности консерванты, также могут способствовать повышению устойчивости микроорганизмов – загрязнителей пищевых продуктов к противомикробным препаратам. В данной статье показано, что лантибиотик низин проявляет все свойства, характерные для антибиотиков, а именно: способствует развитию антибиотикорезистент-*

DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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ности патогенных и условно патогенных микроорганизмов, снижению иммунного статуса организма, развитию дисбаланса микробиоценоза кишечника, оказывает влияние на обмен веществ организма посредством регуляции транскрипции ДНК.

Цель *– оценить риски применения низина (Е234) с учётом его влияния на биологические свойства микроорганизмов – загрязнителей пищевой продукции.*

Материал и методы. *Расчёт потребления низина с пищей в соответствии с условиями сценариев 1 и 2 проводили с учётом массы тела потребителей различных возрастных групп населения России в программе Exel. Проведён анализ научных данных о биологических свойствах низина, в том числе о способности формировать к нему резистентность микроорганизмов.*

Результаты и заключение. *Впервые показано, что расчётные количества пищевой добавки-консерванта низина (Е234) в кишечном содержимом превышают его минимальные ингибирующие концентрации для представителей нормофлоры ЖКТ человека у населения всех возрастов от 40 до 27 064 раз, в зависимости от сценария потребления (при минимальном и максимальном уровнях воздействия). Доказано, что безопасность низина, используемого в качестве пищевой добавки, нуждается в переоценке с учётом его значительного вклада в устойчивость к противомикробным препаратам пищевых патогенов.*

Ключевые слова: низин; пищевая добавка; устойчивость к антимикробным препаратам; патогенные и условно патогенные микроорганизмы.

Для цитирования: Багрянцева О.В., Хотимченко С.А., Петренко А.С., Шевелева С.А., Арнаутов О.В., Елизарова Е.В. Антибиотические свойства низина в контексте его применения в качестве пищевой добавки. *Гигиена и санитария.* 2020; 99 (7): 704-711. DOI: https://doi.org/10.33029/0016-9900-2020-99-7-704-711

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Благодарность. Выражаем благодарность профессору, действительному члену Российской академии наук Виктору Тутельяну за его неоценимую поддержку и руководство этой работой.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Финансирование. Данное исследование финансировалось за счёт гранта федерального бюджета, выданного Федеральному научному центру питания, биотехнологий и безопасности пищевых продуктов, проект № 0529-2018-0113: «Формирование методической и нормативной базы обеспечения современных требований к качеству пищевых продуктов и разработка технологической оценки эффективности специализированных пищевых продуктов», раздел: «Обоснование гигиенических нормативов использования пищевых добавок для формирования законодательной базы Евразийского экономического союза».

Участие авторов: концепция и дизайн исследования – Багрянцева О.В., Хотимченко С.А., Шевелева С.А.; сбор и обработка материала, статистическая обработка данных – Багрянцева О.В., Хотимченко С.А., Петренко А.С., Арнаутов О.В., Елизарова Е.В.; написание текста, редактирование, утверждение окончательного варианта статьи, ответственность за целостность всех частей статьи – Багрянцева О.В., Хотимченко С.А., Петренко А.С., Арнаутов О.В.

Поступила 19.03.2020
Принята к печати 19.05.2020
Опубликована 28.08.2020

Introduction

Multi-resistant bacteria are currently considered as an emergent global threat and a major public health problem. Resistant microorganisms are often found in foods as a result of extensive use of antibiotics and other antibacterial agents (preservatives, processing aids, detergents, etc.) in the food supply chain [1, 2].

The excessive use of antibiotics has created antibiotic gradients which led to the presence of chemicals below minimum inhibiting concentrations (sub-MIC) in the natural habitats. Even though at sub-MICs, the microbial population remains intact, antibiotics still have important effects on microbial cells. Chemicals in sub-MICs promote resistance in future generations. They increase the microbial mutation rates, phenotypic and genotypic variabilities, and affect the biofilm formation. In addition, sub-MICs induce resistance in the horizontal gene transfer. Finally, these sub-MICs play an essential role in the multi-species populations where even small changes in species interaction can have cascading population-level effects. As a result, microorganism's populations contain mostly antibiotic-resistant strains [3–5].

Studies confirm the link to exist between the use of antibiotics in food supply and antimicrobial resistance (the AMR) of human pathogens [3, 6]. AMR microorganisms ingested by consumers with food present an immediate risk to public health (through the conjugation, the transformation and the transduction). Studies have shown that the AMR causes an increase in infectious and non-infectious alimentary-dependent diseases, and the mortality rate. The elevated virulence and the AMR often accompany each other [1, 7].

Nisin belongs to the class of lantibiotics (peptide antibiotic) [5]. The resistance to lantibiotics including the nisin has been previously observed in the food-borne gram-positive pathogens [8–11]. Several studies were recently published on the effect of lantibiotics at the sub-MIC [12–18].

At present, nisin (E 234) is authorized for use in foods. The Committee on Food Additives of the Codex Alimentarius Commission regularly approves nisin for use in more and more food categories. The objective of this article is to report results of the exposure assessment of nisin from the positions AMR problem used as a food additive in ready-to-eat foods based on different scenarios. The assessment is accompanied by a full-scale review of recent studies that demonstrate that nisin exhibits antibiotic properties. We argue that the use of nisin in foods needs to carefully examined with a broad risk assessment to take into account its impact on the biological properties of microbiological food contaminants.

Material and methods

The number of consumed food products containing nisin (E 234) was determined according to the data provided by the Russian Federal State Statistics Service [19].

Calculation of exposure was carried out according to the following formula:

$$C = \frac{F \cdot MU}{365}$$

where C is the consumption of nisin, F – the amount of all consumed foods for all households, in kg/year/person, and MU is the nisin content in foods consumed.

Calculation of nisin consumption under the established conditions of scenario 1 and scenario 2 was carried out taking into account the bodyweight of consumers of different age groups, as well as taking into account gender differences. Calculations were carried out using Excel.

Results

Antimicrobial activity of nisin. As mentioned in the introduction, nisin (E 234) is a lantibiotic (bacteriocin) - antimicrobial peptide of 34 amino acids produced by non-genetically modified strains of *Lactococcus lactis subsp. Lactis* [20]. Various nisin preparations are currently used as preservatives in processed food. Nisin could be used in canned meat products to reduce the viability of the spores of *Cl.botulinum*, significantly increasing the sensitivity

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DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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Table 1

Regulations for the use of food additive – nisin (E 234) (TR TS 029/2012)

Food additive	Food categories	Maximum use level, mg/kg
Nisin (E 234)	Semolina or tapioca puddings and similar products	3.0
	Mature and processed cheeses	12.5
	Curd cheeses and cream cheeses (mascarpone type)	10.0
	Pasteurized liquid egg products (white, yolk, whole egg)	6.25

of the bacterial spores to heat [21]. However, evidence of the effectiveness of the method remains questionable. In countries of the Eurasian Economic Union, nisin can also be used in the food industry as a processing aid [22].

Both the FAO/WHO's Joint Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) evaluated the safety of nisin and established an acceptable daily intake (ADI) of 33,000 units of nisin per kilogram of body weight [23, 24]. The re-evaluation of nisin in 2017 by EFSA concluded that the proposed extension of the use of nisin as a food additive should not be of safety concern. At the same time, EFSA recommended that the risk of inducing antimicrobial resistance to nisin in pathogenic bacteria through its use as a food additive should be evaluated separately [25].

It is known that nisin A has a relatively narrow range of the antimicrobial activity and shows bacteriostatic activity against gram-positive microorganisms – *Listeria spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *Clostridium spp.*, mycobacteria and another [8–11, 26–28]. However, there are many of reports on resistance of gram-positive food-borne gram-positive microorganisms to the nisin [8–11, 28]. It was also shown that after one treatment of the sub-culturing medium with nisin in concentration 100 ME/ml increased the AMR of S.agalactiae by the factor of 40 [21]. Exposure of a penicillin-susceptible strain of S.pneumoniae to nisin (1 mg/L) in liquid culture resulted in the rapid appearance of stable nisin-resistant mutants in which the MIC increased from 0.4 to 6.4 mg/L and the resistance trait was transferable by genetic transformation [26]. At the same time, gram-negative microorganisms (*Salmonella spp.*, *Shigella spp.*, *E.coli*, and other microorganisms of Enterobacteriaceae family, *Campylobacter spp.*) which are the most critical contaminants of ready-to-eat foods, commonly causing food poisoning and acute enteric infections are not sensitive to nisin. Due to nisin's relatively narrow antibacterial spectrum, low solubility, and instability at physiological pH, it acts as a poor antibacterial when used alone in vivo [29, 30]. Nisin A does not restrain the growth of spoiling microorganisms – *Proteus spp.*, *Ps.aeruginosa*, and many species of lactic-acid-producing bacterium, yeasts, and molds [31]. Cytotoxicity of nisin was observed to concerning red blood cells, vaginal epithelium, and rabbit spermatozoa at a concentration of 5–640 μg/ml. At the concentration of 50 μg/ml nisin caused hemolysis of erythrocytes [23].

Some studies consider the action of antimicrobial peptides on enzymes, gene transcription and DNA synthesis [32]. Nisin increases apoptosis and reduces cell proliferation in neck squamous cell carcinoma (HNSCC) cells in concentrations 5, 10, 20, 40, and 80 μg/mL[33] and Human Asterocytoma Cell Line (SW1088) – brain tumor [34].

Nisin was also shown to promote the formation of biofilms [3, 18]. It was demonstrated that amino acids which are essential components of meat protein (D-cysteine, D- or L-aspartic acid, and D- or L-glutamic acid) significantly improve the antibacterial activity of nisin against S. mutans and at a concentration of 40 mM can prevent its growth. Suggested that the salt stress at low temperature, low pH could provide cross-protection against nisin and activated of the cell envelope stress response controlled by gene LiaR [15]. Suggested that the salt stress at low temperature, low pH could provide cross-protection against nisin and activated of the cell envelope stress response controlled by gene LiaR [5, 10, 11, 35, 36].

Table 2

Maximum use levels for E 234 according to the General Standard for Food Additives (CAC 192-1995)

Food additive	Food categories	Maximum use level, mg/kg
Nisin (E234)	Cereal and starch based desserts (e.g. rice pudding, tapioca pudding)	3
	Cheese analogues	12
	Clotted cream (plain)	10
	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	12
	Edible casings (e.g. sausage casings)	7
	Fine bakery wares (sweet, salty, savoury) and mixes	6
	Flavoured fluid milk drinks	12
	Heat-treated processed comminuted meat, poultry, and game products	25
	Heat-treated processed meat, poultry, and game products in whole pieces or cuts	25
	Liquid egg products	6
	Processed cheese	12
	Ready-to-eat soups and broths, including canned, bottled, and frozen	5
	Ripened cheese	12
	Unripened cheese	12
	Whey protein cheese	12

The safety evaluation of nisin, conducted by JECFA [23], was based essentially on its chemical properties and did not fully address the fact that this substance exhibits a considerable biological activity in the human body. Besides, the evaluation did not consider nisin produced by genetically modified strains which in turn have not been evaluated for their safety. The evaluation conducted by EFSA in 2017 indicated the exposure of all studied populations to nisin was lower than 1 mg/kg body weight. However, the EFSA recommended evaluating separately the risk of inducing AMR to nisin in pathogenic bacteria through its use as a food additive [25]. This should be assessed with consideration of the potential use of nisin (E 234) as an antimicrobial drug in humans and domestic animals. The data indicate that nisin is an antibiotic and could promote the AMR problem. At the same time, the AMR problem has become a worldwide problem [7, 37, 38].

Exposure assessment for nisin used as a food additive. In our work, we carried out the risk assessment of the consumption of a food additive - nisin A - by various social and age groups of the population of the Russian Federation from the positions AMR problem. The risk assessment of the food additive was carried out based on calculations of its consumption by the population of the Russian Federation following two scenarios as per recommended intakes established in the Eurasian Economic Union (the EAEU –Belarus, Kazakhstan, Armenia, Russia, and Kyrgyzstan) and those recommended by WHO and Codex Alimentarius Commission [39].

Scenario 1 (minimum consumption) – included the analysis of possible nisin consumption by the population of the Russian Federation with foods for which the use of nisin was established under the Technical Regulations of the Customs Union "Safety Requirements for Food Additives, Flavors and Processing Aids" (TR TS 029/2012) [22] (Table 1).

Scenario 2 (maximum consumption) – included the analysis of possible consumption of nisin by the population with food products for which the use of nisin was permitted by the GSFA adopted in 2018 year [40] (Table 2).

DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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Table 3

Calculations of the total exposure to nisin (E 234) in the first and second scenarios with all categories of food products for which the use of nisin is permitted according TR TS 029/2012 or CAC 192-1995 (adopted in 2018 year)

Food category	*F*, kg/p/y	First scenarios (TR TS 029/2012)		Second scenarios (CAC 192-1995)	
		MU, mg/kg	*C*, mg/p/d	*MU*, mg/kg	*C*, mg/p/d
Bread products (bread and pasta in terms of flour, flour, cereals and legumes)	98,7	–	–	–	–
including:					
bakery	4	0	0	6	0,066
other flour confectionery products	15,4	0	0	6	0,253
Meat and meat products in terms of meat	88,2	–	–	–	–
including:					
sausages	9,3	0	0	25	0,637
frankfurters, wieners	5,4	0	0	25	0,370
meat snack	3,4	0	0	25	0,233
meat semi-finished products and finished products	9	0	0	25	0,616
Milk and dairy products in terms of milk	272.6	–	–	–	–
including:					
flavoured fluid milk	24,5	0	0	12	0,805
flavoured fermented milk product	6,15	0	0	12	0,202
yogurt	4	0	0	12	0,132
other dairy products (cocktails, etc.)	0,7	0	0	12	0,023
cottage cheese, sweet creamed curds	7,5	10	0,205	12	0,247
cheese and bryndza	6,4	12.5	0,219	12	0,210
milk ice cream	1,5	0	0	12	0,049
Liquid egg products, kg (at the rate of 60g per 1 egg)	13,74	6.25	0,235	6	0,226
TOTAL:	–	–	0,660	–	4,117

When calculating the consumption of nisin in the second scenario, an assumption was made that, a half of fluid milk (24,5 l from 49,3 l) and fermented milk products (6,15 l from 12,3 l) could be flavored.

The calculations of the total consumption of nisin (E 234) according to the first and second scenario with all categories of food products are given in table 3.

In the first scenario, the exposure to nisin in all food products was 0,660 mg/person/day. In the second scenario, the exposure value was 4,117 mg/person/day.

At the next stage, exposure to nisin was calculated for various groups of the population (men, women, children of different age groups), depending on the scenario. The calculation considered the energy value of food diets, the average body weight of different age groups of the population, and the date on size and composition of the population of the Russian Federation as calculated by the Federal State Statistics Service in 2017 [19] (Table 4).

When calculating the exposure of different groups of the population, the following conditions were adopted: - children aged from 0 to 3 years were not considered, as in the manufacture of food products for this age group, the use of nisin is not permitted - in the calculation, the low adsorption capacity of nisin was considered - when calculating the concentration of nisin in the gastrointestinal tract, the average weight of the intestine contents was considered 220 g [41] - nisin concentration in the intestine was calculated assuming the density of the intestinal contents equals to 1 (Tables 5, 6).

Table 4

The values of the average body weight, the energy value of the diets and the number of groups of the population of the Russian Federation used in the calculations of the exposure to nisin used as food additive

Population group	Average body weight, kg	Energy intake (kcal/day)	Thousands of people
Children 0 to 3 years old	3–20	1, 267	5,910
Children 3 to 7 years old	20	1,800	5,910
Children 7 to 11 years old	30	2,100	5,910
Children 11 to 18 years old	60	2,500	13,985
Men 18–29	73	2,500	10,596
Men 30–39	73	2,500	11,548
Men 40–49	73	2,500	9,326
Men 50–59	73	2,500	9,681
Men 60++	73	2,500	10,490
Women 18–29	69	2,000	10,261
Women 30–39	69	2,000	11,769
Women 40–49	69	2,000	10,087
Women 50–59	69	2,000	11,768
Women 60++	69	2,000	19,304

DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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Table 5

Exposure to nisin (E 234) for different groups of the population of the Russian Federation (scenario 1)

Population group	Energy, kcal/day/p	Bodyweight, kg	Average intestine contents, g	Nisin consumption, mg/day	Nisin exposure, mg/day/kg	C in the intestine, mg/kg
Men, 18 to 80 years old	2,500.00	73	220	0,753	0,010	3,424
Women, 18 to 80 years old	2,000.00	69	220	0,603	0,009	2,739
Children, 3 to 7 years old	1,800.00	20	50	0,542	0,027	10,847
Children, 7 to 11 years old	2,100.00	30	70	0,633	0,021	9,039
Children, 11 to 18 years old	2,500.00	60	146.7	0,753	0,013	5,136

Table 6

Exposure to nisin (E 234) for different groups of the population of the Russian Federation (scenario 2)

Population group	Energy, kcal/day/p	Bodyweight, kg	Average intestine contents, g	Nisin consumption, mg/day	Nisin exposure, mg/day/kg	C in the intestine, mg/kg
Men, 18 to 80 years old	2,500.00	73	220	4,699	0,064	21,357
Women, 18 to 80 years old	2,000.00	69	220	3,759	0,054	17,086
Children, 3 to 7 years old	1,800.00	20	50	3,383	0,169	67,660
Children, 7 to 11 years old	2,100.00	30	70	3,947	0,132	56,383
Children, 11 to 18 years old	2,500.00	60	146.7	4,699	0,078	32,036

It has been previously reported that cytotoxicity of nisin to several eukaryotic cells was observed at a concentration of 5–640 μg/ml [23]. Thus, in scenario 1, the amount of nisin delivered with all food products was below the toxic thresholds mentioned above. In scenario 2, the levels of nisin intake were so high that a cytotoxic effect was likely to occur. Especially high levels of nisin intake were calculated for the groups of children 3 to 11 years old and, thus, these groups are exposed to a considerable risk of cytotoxicity associated with elevated exposure to nisin.

Nisin is an antibiotic that has yet to be fully evaluated for its antimicrobial effect on the intestinal microbiota. However, we have demonstrated that in scenario 1 in groups of children 3 to 11 years old and in scenario 2 (all age groups), the use of nisin can contribute to the AMR. Nisin concentration in the intestines in children 3 to 7 years old (scenario 1) was 10,847 mg/kg, and in children 7 to 11 years it was 9,039 mg/kg. According to scenario 2, nisin content in these group was between 17,086 mg/kg and 67,660 mg/kg respectively. As we mentioned in the previous section, after sub-culturing in a medium containing nisin at a concentration of 100 IU/ml, the resistance of S.agalactiae increased by the factor of 40 [21]. 1 IU is taken as the amount of nisin that suppresses the growth of one bacterial cell in 1 ml of broth. 1 IU of nisin is equal to 0.025 μg of nisin.

Assessment of the effects of nisin on the human body has shown that in both the first and second scenarios nisin can contribute to suppressing the growth of the indigenous intestinal microflora. This conclusion is in agreement with previous observations that nisin in doses of 100, 300, 900 and 2700 IU/g helps to reduce the number of bacteria of the genera Bacteroides and Enterobacteriacae in the ileum have shown that the minimum inhibitory concentration of nisin (MIC) used in the induction assays were 22,5 and 750 ng/ml for L.casei BL23 and 22,5 ng/ml for the defective mutants. Nisin can inhibit the growth of L.casei at a concentration of 25 ng/ml [42]. Nisin concentrations inhibiting the growth of conditionally pathogenic and pathogenic microorganisms cause the formation of strains multi-resistant to nisin and other antibiotics. Such a pronounced effect of nisin on the indigenous intestinal microflora can significantly influence the formation of innate immunity [42, 43].

Discussion

Risks associated with the use of nisin and their characteristics

Mechanisms of resistance to nisin. Mechanisms of resistance of microorganisms to nisin have been studied in detail [16, 29, 44, 45]. Nisin exhibits pore-forming activity and the inhibition of gram-positive microorganisms - *Listeria spp., Staphylococcus spp., Bacillus spp., Clostridium spp.* - via complexes with lipid I and II which form pores with short life span in the cell membranes, resulting in the inhibition of the cell wall biosynthesis and inducing permeability of the cytoplasmic membrane. In binding to lipid II, nisin is similar to other antibiotics [5, 27, 45, 47]. Nisin exhibits bactericidal activity in the nanomolar level *in vivo* [30].

The resistance to nisin arises from DNA and RNA mutations of microorganisms that induce changes in the membrane and cell wall composition, reducing the acidity of the extracellular medium and stimulating the binding of nisin to the cell wall, and eventually its degradation [5, 10, 46]. These facts explain the common mechanisms of gram-positive bacteria resistance to nisin, and various β-Lactam antibiotics caused by the work of certain genes localized in DNA chromosomes and plasmids [9, 48]. In non-nisin-producing L.lactis, the resistance to nisin could be conferred by a specific nisin resistance gene (NSR), which encodes a 35-kDa nisin-resistance protein (nisin-degrading protease – NSR) [30]. The antimicrobial peptide resistance mechanisms are dynamic. They can be passed from species to species via bacteriophages or horizontal gene transfer and can change specificity and function over time through evolution [16]. Evidence has been gathered proving the fact that the number of gram-positive bacteria (*L.monocytogenes*, *B.subtilis*, *S.aureus*, *Str. mutans*, E.faecalis, and *Lactobacillus spp.*) present in food and in the human body, have a common mechanisms associated with nisin resistance. It has also been shown that resistance to nisin is favored by specific genes [10, 49].

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DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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To gain immunity against its own-produced nisin, L.lactis expresses two immunity protein systems, NisI and NisFEG. The assembled nisin-Lipid II complex forms pores in the target membrane. It was shown that the NisI expressing strain displays an IC 50 of 73610 nM, and 8–10-fold increase, if compared to the non-expressing sensitive strain. NisI inhibits the nisin-mediated pore formation, even at nisin concentrations up to 1 mMol [16].

Certain bacteria produce enzymes that degrade bacteriocins, e.g., nisinase [50]. The nisin degrading protease (NSR) is non-nisin producing bacteria which can produce a novel mechanism of resistance to nisin. The NSR was shown to proteolytically cleave the C-terminal tail of nisin, thereby inactivating and reducing nisin's antimicrobial activity 100-fold [21].

Nisin can significantly decrease the antibacterial activities of many antibiotics, especially in biofilms. As the concentration of antibiotics throughout a biofilm varies, microbial cells are often exposed to levels below inhibitory concentrations and hence may develop resistance. This phenomenon coupled with irresponsible use of antibiotics stimulates the development of the whole selection of pathogens [15, 32, 47, 51]. These cell defensive mechanisms were also shown to be effective against other lantibiotics and antibiotics [13, 28, 52, 53]. It has been demonstrated that nisin and other antibiotics share common mechanisms of genome regulation in bacteria and common mechanism action of lantibiotics, peptides, and some non-peptide antibiotics.

Biological activity of nisin. In 2014-19, a physical working group of the Codex Committee on Food Additives (CCFA) introduced several amendments to General Standard on Food Additives Codex Stan 192-1995 [39] and allowed nisin to be used in several food categories (heat-treated processed meat, poultry, and game products, cream (plain), processed cheese, canned or bottled (pasteurized) or retort pouch vegetables, broths) with maximum levels between 6.25–25 ppm, based on JECFA established ADI for nisin of 0–2 mg/kg of body weight [23]. It should be emphasized that JECFA assessment was primarily based on a review of nisin's chemical properties, and when EFSA evaluated nisin and endorsed the ADI of 0.13 mg nisin/kg body weight per day, the authority did not consider nisin's antimicrobial activity [25]. On the request of the Russian Federation, nisin has been recently included by CCFA to the JECFA Priority List for a full-scale safety re-assessment and a scientific opinion on nisin's contribution to the AMR [54].

It is known that sub-inhibitory concentrations of nisin induced increased resistance of gram-positive microorganisms to nisin in food [17, 55–58). Nisin could inhibit lactobacterium growth which is the most important part of normal intestinal microbiota. For example, nisin could inhibit the growth of *L.gasseri* in the concentration of 25 ng/ml [42]. Inactivation of the signal transduction system - TCS (TC04) from the *L. casei* with which quorum-sensing histidine protein kinase genes-phosphate regulon (Pho regulon) acts in combination to increase lactic acid resistance in *L.casei*, resulted in a decreased ability to grow at pH 3.75 and increased tolerance to antibiotics that target the cell envelope of nisin [42, 59]. At the 35-day diets supplemented by nisin and salinomycin, counts of *Bacteroides spp*. and *Enterobacteriacae spp*. in ileum were significantly decreased [43].

Antimicrobial peptides play a significant role in building an innate immunity, adaptive immune response. Nisin could induce preferential apoptosis, cell cycle arrest, and reduces cell proliferation in some cancer's cells. It also shown mitogenic activity [12, 60, 61].

The Pho regulon is responsible for inorganic phosphate uptake by phosphate ATP-binding protein (Pst proteins) and is controlled by TCS in all bacteria containing a Pho regulon. Studies of *E.coli* have revealed that this regulon is not only important for the regulation of phosphate uptake but also affects genes involved in virulence and pathogenesis, secondary metabolite production, nutritional regulation, and stress responses, including the acid tolerance response. The Pho regulon in *E.coli* is tied to the expression of acid shock proteins, sigma factors, chaperones, and acid resistance systems, including glutamate-dependent acid resistance (GDAR) [57]. It has been reported that ribosomal biosynthesis of peptides such as nisin is positively controlled by phosphate too [50, 62].

At the same time, enhanced nisin resistance in some mutants was shown to be associated with increased expression of three genes: pbp2229, hpk1021, and lmo2487, encoding a penicillin-binding protein, a histidine kinase, and a protein of unknown function. The expression of virulence genes in one nisin-resistant mutant was analyzed, and each mutant consistently showed either an increase or a decrease in the expression of virulence genes (prfA-regulated as well as prfA-independent genes). The direct role of the three genes in nisin resistance was determined. It fact indicated that a mutant-specific change in virulence may occur concomitantly with bacteriocin resistance development [63].

The effect of nisin on the intestinal (gut) microflora. AMR issue. The normal intestinal microflora is an evolutionarily developed ecological system of various symbiotic microorganisms which inhibits open cavities of the human body and maintain the biochemical, metabolic, and immunological balance necessary for the preservation of human health. However, at present, due to extensive use of antibiotics, this balance is disturbed in most of the population, leading to changes in the composition and number of intestinal microflora, i.e. to the development of unbalance of intestinal microflora. Not only the composition, number, pathogenic potential, the virulence of intestinal microflora change, but also there is a translocation of microorganisms, their movement to unusual habitats, for example, from the colon to the small intestine and, most importantly, from the intestine to the lymph and blood flow. Optional opportunistic microorganisms of intestinal microflora can cause the development of acute intestinal infection and the rapid growth of bacteria may become a source of endogenous infection with different localization of the inflammatory process. As a result, this process can inhibit the non-specific immunity status of the population [50, 64].

Nisin modulated the gastrointestinal microbiota and may induce pathogenic properties of microorganisms present in the composition of the intestinal microflora. The disruption of the qualitative and quantitative characteristics of the intestinal microflora, in turn, leads to a shift in the acid balance of intestinal lymphoid tissue in the intestinal lumen, the proliferation of inflammatory processes, and the development of putrefactive processes. These effects can lead to significant changes in the proliferation of immunocompetent cells, production of pro- and anti-inflammatory cytokines andmultiple chemokines absorption of nutrients and biologically active metabolites of microflora, which together can lead to the development of various alimentary-dependent pathologies [43, 46, 50, 60, 61, 65].

The facts indicate nisin to be an antibiotic and its use can contribute to the development of AMR.

The AMR presents a global issue which (World Health Organization 2014): threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. AMR is an increasingly serious threat to global public health that requires action across all government sectors and society [7].

Issues related to the use of nisin of biotechnological origin. The gene-encoded nature of nisin makes it more amenable to bioengineering strategies to further enhance their antimicrobial and physicochemical properties [56]. Nisin obtained by biotechnological methods suppresses the growth of gram-positive and gram-negative bacteria [56, 66, 67]. Thus, nisin A is largely different in its properties from other forms of nisin. It becomes a matter of the outmost importance to conduct the risk assessment and establish parameters of the safe use and maximum levels for each type of nisin obtained by biotechnological methods and used in the food industry.

The use of nisin as a therapeutic drug. Several recent studies highlighted multiple ways in which nisin could potentially be used as a therapeutic agent. As a result of the antibacterial activity, nisin-based bacteriocins are regarded as candidates in the therapy of infectious diseases caused by microorganisms with multiresistance to antibiotics. It has been shown that nisin possessed immunomodulatory properties, giving rise to the suggestion of a pos-

DOI: https://dx.doi.org/10.33029/0016-9900-2020-99-7-704-711
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sible role for nisin as a novel immunomodulatory therapeutic drug. Several studies reported high activity of novel bioengineered derivatives of nisin against *Mycobacterium spp*. [5, 67–69]. Nisin has the potential to serve as a novel therapeutic with antitumor effect [12, 33, 34, 46]. However, to establish the safety of bacteriocins, in the case of their use as medicines, many tests will be conducted [50]. Nisin is also used broadly in the veterinary industry [55, 56]. Nisin supplementation improved broiler growth performance in a dose-dependent manner [43]. In general, one needs to consider a widely accepted principle that clinically used medicines should not be used in the food industry.

Conclusion

This study demonstrated that nisin, a widely used food preservative, possesses antibiotic properties, and contributes to the AMR. Our conclusions were based on the following considerations:

1. Nisin and other antibiotics have been shown to have common mechanisms of the regulation DNA and RNA, protein, and polysaccharides of the microorganisms;
2. The studies conducted in recent years indicate that the use of nisin, even in sub-inhibitory concentrations, leads to an increase in the AMR of microorganisms which are contaminants of foods as well as the causative agents of acute intestinal diseases and food poisoning.

It follows that the use of nisin can contribute to the AMR of microorganisms in intestinal microflora and increase the virulence and pathogenic potential of microorganisms which cause foodborne diseases and the harm the human immune system and intestinal microbiota of the humans.

At the same time, new lantibiotics become available as the development of bioinformatics software and search engines have allowed the identification of nucleotide sequences of bacterial genomes and their biosynthetic clusters. In this regard, nisin is at the forefront of research related to the search for effective antimicrobial agents for their use not only in the food industry but also in the treatment of inflammatory diseases, cancer, as well as in agriculture as an anti-inflammatory agent and a growth promoter for farm animals.

Most importantly, recent risk assessments of nisin used as a food additive by JECFA and EFSA were carried out only for nisin A, which was produced by non-GMO strains, and did not account for its antibiotic properties, including those in the intestinal microbiota. Thus, nisin A is largely different in its biological properties from other forms of nisin.

The risk assessment described here takes into consideration the antibiotic effect of nisin on the human body, demonstrating that potential adverse effect of food additives E234 on the intestinal microbiota which can occur when nisin is consumed with food, both at the minimum and at the maximum level of consumption in all age groups. Estimated amounts of nisin consumed exceed by a factor from 40 to 27064 times (depending on the dose) the levels established in scientific studies, which have an inhibitory effect on the representatives of the indigenous microflora (bacteroides, enterobacteria, and lactobacilli) its used have a great potential direct risk for consumers of all population groups. It should be noted that even taking into account hypothetically possible losses of nisin in the process of food preparation and digestion – 50%, the consumption of nisin in both scenarios (with minimum and maximum levels of consumption) will exceed the level affecting the intestinal microbiota from 20 to 13,535 times.

The data collected on the negative role of nisin (E 234) in the promotion of AMR of pathogenic and opportunistic microorganisms, as well as in the formation of the immune response of the body, highlight the need to re-evaluate this preservative from AMR position.

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