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USING THE PCR METHOD TO IDENTIFY FOODBORNE PATHOGENS AND DETERMINE THEIR PREVALENCE IN UKRAINIAN FOOD PRODUCTS OF ANIMAL AND PLANT ORIGIN

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Abstract. Foodborne pathogens cause serious health problems in every country. That is why controlling the safety of the food chain includes microbiological tests. Molecular methods, mainly polymerase chain reaction (PCR), are considered highly sensitive, specific, and rapid to detect pathogens in raw materials and food. This study describes the use of specially designed and highly specific primers for PCR to detect 5 common and especially dangerous disease and food poisoning agents and to determine their occurrence in food of animal and plant origin. The studies included identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and *Cronobacter spp. (E. sakazakii)* found in raw milk, Shiga-toxin-producing *Escherichia coli* (STEC) from beef and pork carcasses, *Bacillus cereus* and *Clostridium perfringens* from various types of plant and animal raw materials and products of their processing (fruit, vegetables, berries, dried and preserved products, food concentrates, semi-preserved food). A total of 397 food samples have been investigated to detect these pathogens using classical bacteriological methods and PCR. The prevalence of foodborne pathogens in the studied products of animal and plant origin was as follows: *Staphylococcus aureus* (MRSA) and *Cronobacterspp. (E. sakazakii)* in raw cow's milk in 6.5% and 19.4% of cases, respectively; Shiga-toxin-producing *Escherichia coli* (STEC) from beef and pork carcasses in 8.1% and 5.7%, respectively; *Bacillus cereus* and *Clostridium perfringens* from different plant and animal raw materials and their processing products averaged 27.5% and 7.7%, respectively. Molecular genetic methods, which the PCR method belongs to, have such advantages as rapidity and specificity of identifying microorganisms by the features of the genetic regions that carry information about their pathogenic factors. It has been found that by the PCR method, these pathogens are detected at least 5–9 times faster than by the classical methods. These data will be useful to assess the microbiological risk and will help the governmental authorities develop strategies to reduce risks to consumers' health.

Keywords: MRSA, *Cronobacter spp.* (*E. sakazakii*), STEC, *Bacillus cereus*, *Clostridium perfringens*, raw materials of animal and plant origin and products of their processing

Introduction. Formulation of the problem

Food safety in present-day conditions is important all over the world, as it concerns public health. Microbiological hazards are a priority when assessing the risk of any food. Like other criteria in the system of food safety characteristics, the microbiological ones indicate whether food products are suitable for consumption. Besides, the qualitative and quantitative microbial composition in raw materials and ready-to-eat products indicates the processing type or mode and the level of production hygiene [1,2]. That is why, highly sensitive, specific, and rapid methods of detecting and controlling foodborne pathogens as potential health hazards are of so great practical importance [3-5].

Foodborne pathogens are traditionally monitored by classical microbiological culture-based methods. However, now PCR is becoming a more important analytical tool not only to research foodborne pathogens and food poisoning agents, but also to carry out routine analyses [4-9].

Analysis of recent research and publications

According to World Health Organisation experts, the microbiological hazards of the inappropriate quality and safety level of raw materials and food are among the most important biological threats nowadays. A recent review of the official data on this topic has shown that outbreaks of diseases caused by poor-quality or unsafe foods are registered in all countries of the world [8,10]. In order to prevent and reduce these diseases, and to control foodborne pathogens, strict measures taken and rules established in processing plants, such as hazard analysis and critical control point system (HACCP) and good manufacturing practice (GMP) [8,10].

The most common bacteria causing foodborne intoxications and toxicoinfections are *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella spp*, *Campylobacter jejuni*, *Clostridium perfringens*, *Bacillus cereus*, *Enterobacter sakazakii*, *Listeria monocytogenes*, *Yersinia enterocolitica*, etc. [9-14]. It is important to know that more than 80% of foodborne pathogens are dangerous anthropozoonotic microorganisms. They can get into raw meat and milk during slaughter and milking, or into products made from them during technological processing, or can contaminate raw vegetables, fruit, and berries when soil is fertilised with animal manure [10,12].

The **purpose** of the present study was to justify and sum up our own research results of using PCR to detect 5 common pathogens from raw materials of animal and plant origin and from products of their processing.

To achieve this purpose, the following **objectives** were set:

1. To study different food raw materials and products of their processing from different regions of Ukraine using the PCR method to identify 5 foodborne pathogens specified by international and Ukrainian food legislation.

2. To compare and evaluate the effectiveness of the classical and the PCR methods.

3. To study the levels of distribution of the above-mentioned foodborne pathogens in food products of animal and plant origin.

Research materials and methods

The work was performed in the microbiology laboratory of the Public Health Department of Sumy State University, in the Department of Biochemistry, Microbiology, and Nutrition Physiology, and in the microbiology laboratory of Odessa National Academy of Food Technologies. A total of 265 samples were collected for investigation in the years 2015–2018 from dairy farms, slaughterhouses and markets in different regions of Ukraine (the Sumy region in the northeast, the Kiev region in Central Ukraine, and the Odessa region in the south).

The raw material and food samples (raw milk, swabs taken from beef and pork carcasses, vegetables, fruit, berries, dried and preserved products, food concentrates, semi-preserved food, and spices) were investigated by classical culture-based methods. Polymerase chain reaction was performed using group-specific and species-specific primers that had been designed with consideration of the virulence genes for the five most dangerous and most common foodborne pathogens: *Staphylococcus aureus* (MRSA) and *Cronobacter spp. (E. sakazakii)* from raw milk, Shiga-toxin-producing strains of *Escherichia coli* (STEC) from beef and pork carcasses, *Bacillus cereus* and *Clostridium perfringens* from various plant and animal raw materials and products of their processing. The information about primers used in this study is snown in Table 1.

Development and optimisation of PCR to isolate methicillin-resistant Staphylococcus aureus from raw cow's milk. The procedure we used can be found in [15]. Briefly, the two primers MecA147-F and MecA147-R, which are specified in Table 1, were used for it. The DNA of *S.aureus* was isolated in the following steps: a pure culture colony was suspended in a test-tube with 0.5 cm^3 of sterile deionised water, and then heated for 10 minutes at 99°C. After centrifugation at 30.000 x g for 1 min, 2 µl of the supernatant was used as a template in a 25 µl PCR. The polymerase chain reaction was performed in termocyclers *Tertsyk* (*DNK-tekhnologiya*, Russia) and *T1* (*Biometra*, Germany). The thermal cycle parameters were as follows: 95°C for 4 min (1 cycle), then 35 cycles at 95°C for 30 s each, 50°C for 30 s, 72°C for 30 s; and the final elongation step at 72°C for 7 min.

Development and optimisation of PCR to isolate Cronobacter spp (Enterobacter sakazakii) from raw cow's milk. The milk samples were analysed by the isolation method described in [16]. In brief, first we used the culture-dependent method to isolate *Cronobacter* colonies supposed to be on VRBG agar (violet red bile glucose agar; Himedia M581). Than, we selected typical purple colonies on TSA (Trypticase Soy Agar; Himedia M290) and incubated them at 25°C for 48–72 h to achieve yellow pigmentation. Typical yellow colonies were taken from TSA, resuspended in physiological saline, and then subjected to biochemical characterisation using testing strips API 20E (*BioMerieux*, France) according to the manufacturer's instructions. After that, the PCR method with the gene 16S rRNA was used. For the purpose, three primers were selected, which are indicated in Table 1. The thermal cycle was carried out by using the initial denaturation step at 94°C for 3 min (1 cycle), followed by 35 cycles of denaturation at 94°C for 30 s each, annealing (at the given temperature) for 1 min, and elongation at 72°C for 1 min 30 s. The cycling was completed with the final elongation step at 72°C for 4 min.

Table 1 – Primers used in this study

Pathogen	Target gene	Sequence (5' → 3')	Amplicon length (bp)
Methicillin-resistant Staphylococcus aureus (MRSA)	*mecA*	MecA147-F 5'-GTG AAG ATA TAC CAA GTG ATT-3' MecA147-R 5'-ATG CGC TAT AGA TTG AAA GGA T-3'	147
Cronobacter spp. (E. sakazakii)	*16S rRNA*	2d16S-F – AGCTAATACCGCATAACGTCTACG 2d16S-R – AGGCACTCCCGCATCTCTG	863
Shiga toxin-producing strains of Escherichia coli (STEC)	*eae*	dF-eae 5'- CGCTCTTGGTATCGCTGGTAAC -3' dR2-eae 5'-TAGTCTCGCCAGTATTCGCCAC-3'	327
	Stx1	7d-stx1_F 5'-CGTGTTGCAGGGATCAGTCG-3' dstx1-R3 5'-CGCACTGAGAAGAAGAGACTGAAG-3' d-stx1-f1 5'-GCAAAGACGTATGTAGATTCGCTG-3' d-stx1-R2 5'-CAGTTACACAATCAGGCGTCG-3'	728 796
	Stx2	dF1-Stx2 5'-CCATGACAACGGACAGCAGT-3' dR3-stx2 5'-ATCTGACATTCTGGTTGACTCTCTTC-3'	466
B. cereus group	*groEL*	BCGSH - 1F 5'-GTGCGAACCCAATGGGTCTTC-3' BCGSH - 1R 5'-CCTTGTTGTACCACTTGCTC-3'	400
B. thuringiensis	*gyrB*	BTJH - 1F5'-GCTTACCAGGGAAATTGGCAG-3' BTJH - 1R5'-ATCAACGTCGGCGTCGG-3'	299
B. cereus type (*spp.*)	*nheA*	nhe A F 5'-AAGGCGAATGTACGAGAGTGG-3' nhe A R 5'-CTTCTCTCGTTTGACTATCTGCAG-3'	553
	hblD	hblD-F ACCGGTAACACTATTCATGC hblD-R GAGTCCATATGCTTAGATGC	465
	cytK	cytK-F GTAACTTTCATTGATGATCC cytK-R GAATACTAAATAATTGGTTTCC	800
	cesB	cesB-F ACCCATCTTGCGTCATT cesB-R CAGCCAAGTGAAGAATACC	154
Clostridium perfringens	*16S rRNA*	5'-AGGAGCAATCCGCTATGAGAT-3' 5'-CCTTCATCACTCACGCGGCGT-3'	200–220

Development and optimisation of PCR to isolate Shiqa-toxin-producing strains of Escherichia coli (STEC) from beef and pork carcasses. The swabs were taken randomly at the final point of the process after the carcasses were finally treated with sterile swabs in sterile saline solution. The areas of taking the samples were 100 cm² on each carcass, according to requirements ISO 17604, in the following places: distal hind limb, belly (lateral and medial) of the pork carcasses, brisket, flank and lower belly areas of the beef carcasses. The samples were examined for 2–12 hours in the following way: serial dilutions of the samples were placed on the surface of the commercial medium *Compact Dry™ EC* to isolate *E. coli* in Petri dishes (NISSUI Pharmaceutical). The Petri dishes with isolates were incubated for 24 h at 37°C. The results were interpreted by the following parameter: blue colonies were considered as *E. coli*. The specific oligonucleotide primers that were used to detect STEC are shown in Table 1.

In the test, hot-start PCR was used in the volume 0.025 cm^3. In order to minimise the formation of non-specific dimers of the primer matrix and its amplification, the method of preparation of the reaction mixture with the physical separation of PCR components was used. To prepare the "lower" reaction mixture, nucleotydtryphosfate (2 mM) was mixed with the corresponding primers in the same tube, with 0.025 cm^3 in each one (the final concentration from each primer was 10–15 pmol/sample). The thermal cycle parameters were as follows: 95°C for 3 min, followed by 35 cycles at 94°C for 30 s each, 65°C for 30 s, 72°C for 30 s; and the final elongation step at 72°C for 4 min. A non-pathogenic *E. coli* strain was the negative control [17].

Development and optimisation of PCR to isolate Bacillus cereus and Clostridium perfringens from various plant and animal raw materials and products of their processing. The swabs or weighed sample portions from different vegetable and animal raw materials and products of their processing (fruit,

vegetables, berries, dried and preserved products, food concentrates, semi-preserved food, and spices) were studied according to [9, 18–20]. Some parameters (annealing temperature) of PCR amplification were optimised taking into account that the total length of the oligonucleotide did not exceed 20–22 bases according to the formula [21]:

$$T_m = [(A + T) \times 2\ ºC] + [(G + C) \times 4ºC], \quad (1)$$

where Tm is the annealing temperature, ºC,

(A + T) is the percentage of AT bases in the primer,

(G + C) is the percentage of GC bases in the primer.

To check how sensitive PCR was in identifying toxigenic bacteria of the group *B. Cereus*, a series of ten-fold dilutions of the mixture of daily cultures of *B. cereus* strains (*B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, *B. cereus* UCM B 5671) was used.

Of the reference bacilli present in the liquid from the preservatives, the following concentrations were studied: 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8 CFU/cm^3 in the study of the contaminated preservatives and other samples. All the dilutions used were titrated to detect the number of CFU in the liquid.

To remove organic residues and concentrate microorganisms, the contaminated samples of preserved products were pre-centrifuged using the priority modes developed by us, according to the patent received. Then, a *SureFast® PREP Bacteria* F1021 kit was used to isolate DNA (*CONGEN*, Germany).

Multiplex PCR for molecular genetic diagnostics of contaminants was performed using specific primers for the nucleotide sequences of the corresponding microorganisms, which were synthesised by *Simesta VAAL* (Odessa, Ukraine). The specific oligonucleotide primers that were used to detect *Bacillus cereus* and *Clostridium perfringens* are shown in Table 1.

Electrophoresis of PCR products was performed in 1.5% agarose gel in a TBE buffer (Tris/Borate/EDTA) on a horizontal electrophoresis apparatus (*BioRad*, USA) at the constant voltage 60V for 1–2 hours.

To colour the amplification products distributed in the agarose gel, the gel plate was dipped in the TBE buffer with 0.5 μg/ml ethidium bromide (*AmpliSense*, Russia) for 15 min. When determining *Clostridium perfringens*, the electrophoresis of the amplification products was performed in 2% agarose gel at the current of 50 mA [22]. The molecular weight marker M33 was used for visual assessment of the size of the amplicons formed (SPA *Sibenzim*, Russia).

Results of the research and their discussion

Detection of methicillin-resistant *Staphylococcus aureus* in raw cow's milk. The most dangerous pathogen found in raw milk and dairy products is *Staphylococcus aureus*. This microorganism produces toxins, in particular enterotoxins, and other metabolites that enter the body together with food and cause intoxication and gastrointestinal disorders. The main sources of *S. aureus* in raw milk are a cow's udder, technological equipment, and dairy farm workers. However, one of the important problems associated with *S. aureus* is its resistance to antibiotics, especially to methicillin (an antibiotic that belongs to the penicillin class). This is due to the long and uncontrolled use of this antibiotics in livestock farming. At the international level, this staphylococcus is denoted by the abbreviation MRSA (methicillin-resistant *S. aureus*). As a result, MRSA-containing raw milk can be a potential carrier of antibiotic-resistant *S. aureus* strains to people. And all this, in turn, is a problem for the dairy industry and for public health [15,23,24].

The aim of this part of our study was to identify *S. aureus* in raw milk, focusing on methicillin-resistant isolates. For this, we use PCR with the specific primers MecA147-F/MecA147-R for the gene *mecA*.Our results show that 6.5% of raw milk samples (4 samples out of 62) had MRSA. The results of using PCR to detect MRSA are shown in Fig. 1.

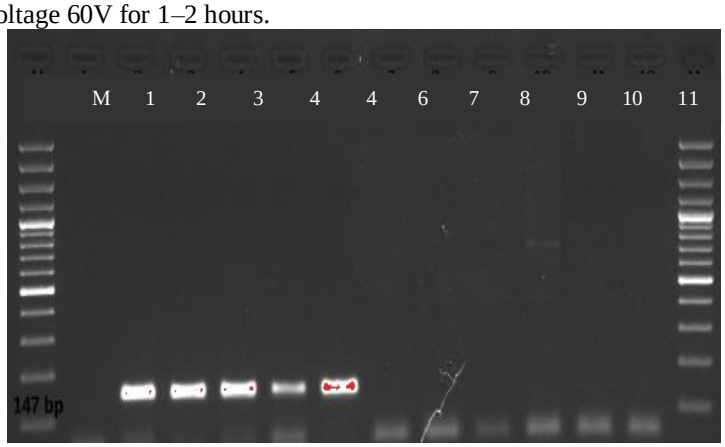

Fig. 1. Results of electrophoretic analysis of PCR products with primers for MRSA identification:
M – 100bp molecular weight marker, 1 – negative control, 2 – positive control, 3–6 – amplified product of 147bp of the gene *mecA*, 7–12 – no amplified products of the gene *mecA*

In the literature, there are many studies of the prevalence, antimicrobial resistance, virulence, and genetic diversity of *S. aureus* in raw milk. According to [25], seven (20%) of 35 isolates were identified as MRSA from raw milk samples collected from various dairy herds in the Province of Milan (northern Italy). As other researchers highlighted [26], eleven isolates of *S.aureus* out of 69 were methicillin-resistant and carried the *mecA* gene.

Although other authors in different regions of the world used PCR to detect MRSA with the *mecA* gene, this is the first Ukrainian study of how this method can be used to isolate MRSA from raw milk.

Detection of *Cronobacter spp.* (*Enterobacter sakazakii*) in raw cow's milk. The second pathogen which we studied in raw milk was *Cronobacter spp.* (*Enterobacter sakazakii*). This is an opportunistic pathogen from the family *Enterobacteriaceae* that causes fatal infection in newborns and infants up to the age of one year. It is transmitted through milk infant formula, in which this microorganism can be present. It can cause necrotising enterocolitis, bacteraemia, and meningitis in neonates, particularly those who are premature or immunocompromised, resulting in mortality 40% to 80% [27].

After searching and analysing the sequence of genes with conserved and variable regions in bacteria *Cronobacter spp.* (*Enterobacter sakazakii*), we chose several oligonucleotide primers specific to various portions of the gene 16SrRNA (Table 1). To identify *Cronobacter*, PCR analysis with the gene sequence 16S rRNA hadbeen used before [27,28]. With these primers used in our study in PCR for isolates, positive results were obtained, too (Fig. 2).

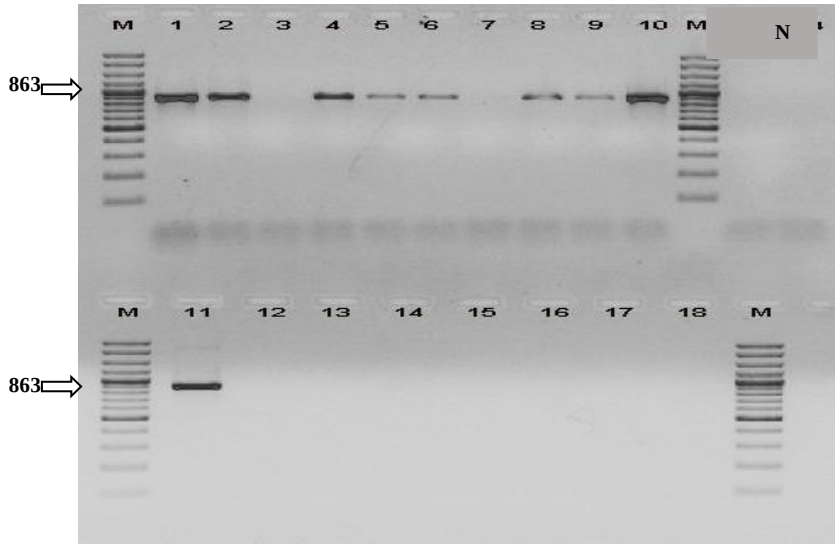

Fig. 2. Results of electrophoretic analysis of PCR products with primers 2d16S-F and 2d16S-R for *Cronobacter spp.* (*Enterobacter sakazakii*) identification:
M – 100bp molecular weight marker, 1–2 – positive controls, 3, 7 – no amplified products of 16S rRNA gene, 4, 8–11 – amplified product of 863bp of 16S rRNA gene, NC – negative contol, 12–18 – unused gel wells

Our investigations showed that 19.4% of the raw milk samples were positive for *Cronobacter spp. (Enterobacter sakazakii*) [16]. After the PCR analysis, it was determined that all these isolates had specific parts to the primers 2d16S-F and 2d16S-R. Similar results were also reported by researchers who identified these bacteria in the secretions of the affected mammary gland in heifers [28].

Detecting Shiga-toxin-producing strains of *Escherichia coli* (STEC) in beef and pork carcasses. *Escherichia coli* is a bacterium that normally lives in the intestines of people, warm-blooded animals, and birds. Its presence in raw materials and food of animal origin is considered to be faecal contamination and can be very dangerous for consumers' health. The most dangerous species among all *E. coli* is enterohaemorrhagic or Shiga-toxin-producing *E. Coli*, the causative agent of severe bloody diarrhoea and haemorrhagic uremic syndrome in humans through the production of Shiga-toxin, which is the main virulence factor responsible for the disease [17,29-32].

In our study, *E. coli* was isolated in 42.2% of cases (97 positive isolates out of a total of 230 isolates) in swabs odtined from the surface of beef and pork carcasses. Out of 97 positive isolates only seven (7.2%) were STEC. In our study, STEC was more common in the swabs from beef (8.1%), compared with the results for the pork swabs (3.0%) [17]. In some works, lower percentages of STEC were reported to be detected in beef carcasses at processing plants: 0.5%, 3.3%, 4.5% [29-31]. But 30.0% was the largest percentage of Shiga-toxin-producing strains of *E. coli* from samples of beef carcasses [31]. Even though these results differ from each other, they all agree that beef and pork can be a potential source of STEC.

As Shiga-toxin-producing *E. coli* bacteria belong to the main pathogens responsible for food poisoning outbreaks, they should be rapidly identified using specific genetic markers [3,29-31]. Our task was to develop multiplex PCR to detect *eae*, *stx1*, *stx2*, STEC virulence genes. After using the primers *eae* and *stx2* in PCR, positive results were obtained (Fig. 3).

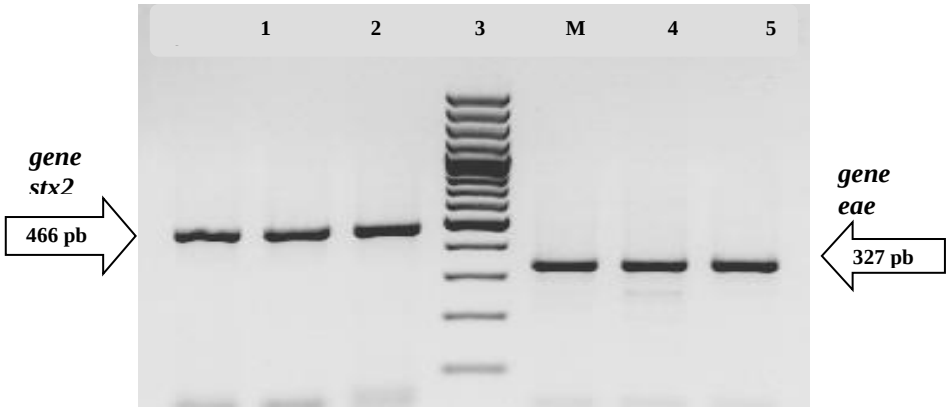

Fig. 3. Results of electrophoretic analysis of PCR products with primers for the genes *stx2* та *eae* **to identify Shiga-toxin-producing strains of *Escherichia coli (STEC)*:**
M – 100bp molecular weight marker, 1 – positive control for the gene *stx2*; 2, 3 – amplified products of the gene *stx2*; 4 – positive control for the gene *eae*; 5, 6 – amplified product of the gene *eae*

Table 2 illustrates the illness frequency and combinations of virulence genes (*stx1, stx2, eae*) of the isolated STEC strains.

Table 2 – Incidence of virulence genes of the isolated STEC from the beef and pork carcasses (n = 97)

Sample type	No. of samples studied, (%)	Combination of virulence genes (No. of positive samples/%)
Swabs from beef carcasses	5 (8.1%)	*stx1* + *stx2* (3/60) *stx1* + *eae* (1/20) *stx1* (1/20)
Swabs from pork carcasses	2 (5.7%)	*stx1* + *stx2* (2/100)
Total	7 (7.2%)	

As shown in Table 2, the *stx1* gene was the predominant gene detected in all STEC positive samples. The *eae* gene was detected in one of the examined isolates from a beef carcass. Three isolates from swabs of beef carcasses carried both the *stx1* and the *stx2* genes, one isolate showed association between the *stx1* and the *eae* genes, one isolate was only positive for the *stx1* gene. In swabs from pork carcasses (2 isolates), the *stx1* and *stx2* genes were both present.

Detection of *Bacillus cereus* and *Clostridium perfringens* from various types of plant and animal raw materials and products of their processing. Since microorganisms of the *B. cereus* group cause foodborne illnesses and are potentially enterotoxigenic for people, the ability of rapidly detecting *B. cereus* in food is critical. *B. cereus* causes diarrhoea and emetic syndromes by producing various extracellular toxins that include the three main types of enterotoxins: haemolysin BL (hbl), non-haemolytic enterotoxin (nhe), and cytotoxin K (cyt K) [2,19].

The studies were performed in 2 steps, because of a large number of the microorganisms with morphological, tinctorial, and cultural features similar to those of *B. cereus*. First, it was established whether the microorganisms belonged to the *B. cereus* group, and then, the ability to produce toxins by their certain strains was determined.

To determine the microorganisms as belonging to the *B. cereus* group, forward and reverse primers for the *groEL* gene were used. The results of studying of the food samples for the presence of microorganisms of the group *B. cereus* are shown in Fig. 6.

To determine the emetogenic and enterotoxigenic bacilli in different food raw materials and products of their processing, 4 pairs of specific oligonucleotide primers were used for *B. cereus* toxicity genes (see Table 1).

The enterotoxicity genes *nhe A*, *hbl D*, and *cyt K* were detected in 54, 44, and 30 *B. cereus* strains, respectively, where they totalled 100.0%, 81.5%, and 55.5%, respectively. The *ces B* gene, which encodes emetic toxin, was detected in 3.7% of the strains (Table 3).

The results obtained indicate that the tested concentrate products, berries, and fruit had no emetic toxin but contained enterotoxins. Some vegetables, pasteurised and tinned foods may also contain emetic toxin. Larger numbers of *B. cereus* strains tested did not change significantly the percentage of toxicity genes compared to those previously reported [19]. The maximum discrepancy is 6.4% of the *cytK* gene.

According to the literature data [33,34], the ability of *B. cereus* to produce NHE was noted in 92–100% of isolates. Of the studied and described isolates of *B.*

cereus strains, 34 to 84% can synthesise HBL, but some isolates secrete only one or two of its components, while others have *hbl* genes, but do not express proteins.The *cyt K* gene was detected as often as in 56-63% cases [33]. According to the research, the total contamination of the products with *Bacillus cereus* strains fluctuates significantly and averages 27.5%.

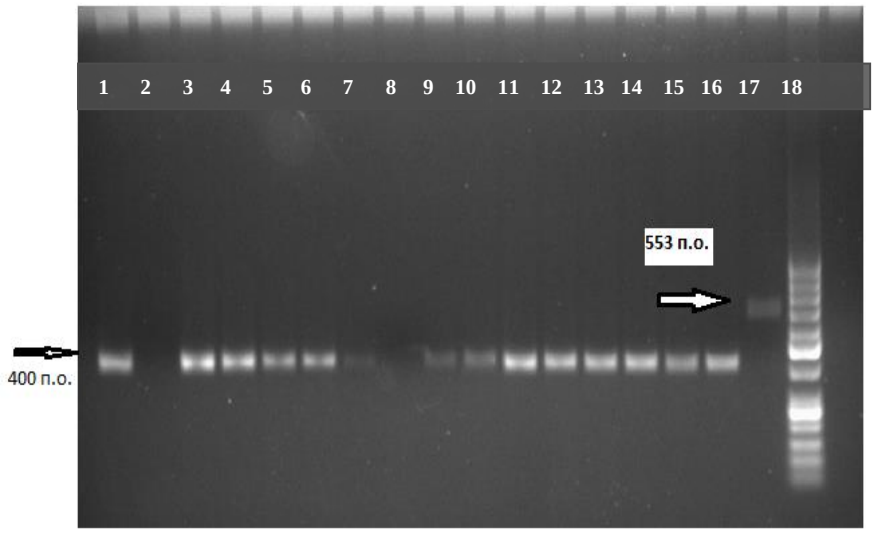

Fig. 6 – Electrophoregram of PCR products with a pair of specific oligonucleotide primers for the *groEL* gene:
1 – *B. cereus* UCM B 5671; 2 – negative control of PCR; 3–5 – *B. cereus* of reference collection strains (*B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, respectively); 6 – sample of semi-preserved spicy herring; 7 – swabs from cucumbers; 8 – sample of tinned fish fillets; 9 – sample of tinned vegetable marrow paste; 10 – sample of dried mushrooms; 11 – swabs from carrots; 12 – swabs from marrows; 13 – sample of a semi-processed meat product; 14 – food concentrates *Pea soup with bacon*; 15 – swabs from potatoes; 16 – sample of Georgian spices; 17 – *B. cereus* UCM B 5671 with a pair of primers for the *nheA* gene; 18 – molecular weight markers (pBR322/BsuRI, Fermentas)

Table 3 – Distribution of toxicity genes among *Bacillus cereus* strains isolated from different food sources in Ukraine

Toxicity gene	*Bacillus cereus* strains containing toxic genes (n = 54) isolated from:				Total, %
	vegetables	fruit and berries	pasteurised and tinned products	food concentrates	
nheA	26	10	8	10	100
hblD	20	7	8	9	81.5
cytK	14	5	8	3	55.5
cesB	1	–	1	–	3.7

Detection and rapid identification of *Clostridium perfringens* is an important component of product safety policy provided for in food regulations. Generic identification of *Clostridium* by the standard methods takes 72 hours (GOST 29185-91), but only allows roughly assessing the safety of the food under study. It should also be noted that this method is multi-stage and subjective. At the first stage (Kitt–Tarozzi medium), the morphological, physiological, and some cultural features are studied, on the basis of which generic identification is carried out. At the next stage, after reinoculation and studying a number of cultural, biochemical, and biological properties, the species is identified.

The developed method allows not only reducing the testing time, but makes it possible to identify the species of the pathogenic *Clostridium perfringens* in question [22]. The primers developed (Table 1) are only complementary to 16S rRNA of *Clostridium perfringens* and are different from all other sequences in GenBank [22].

The results of detecting *Clostridium perfringens* in samples of various types of animal and plant products are given in Table 4.

The conclusion about the presence *of Clostridium perfringens* in the sample was made upon detection of amplicons with a length of 205–215 nucleotide pairs. The *Clostridium perfringens* contamination of the tested samples averaged 7.7%.

Table 4 – Determining *Clostridium perfringens* in raw materials and various food products, (n=78)

No. of the sample	Test samples	Amplicon size, bp	Presence/absence of *Clostridium perfringens*
1	Minced meat	205	+
2	Fresh carrots	215	+
3	Tomato juice	210	+
4	Caviar	–	-
5	Ajika	–	-
6	Lecsó	–	-
7	Leaf lettuce	210	+
8	Ketchup	–	-
9	Tomato paste	–	-
10	Apple purée	–	-
11	Carrot purée (home-made)	215	+
12	Tinned green peas	–	-
13	Grape juice	–	-
14	Carrot juice	–	-
15	Apple juice	–	-

Conclusion

The article explains and presents the results of our research in the field of detecting such dangerous foodborne pathogens as methicillin-resistant *Staphylococcus aureus* (MRSA), *Cronobacter spp.* (*E. sakazakii*), Shiga-toxin-producing *Escherichia coli* (STEC), *Bacillus cereus*, and *Clostridium perfringens* in Ukrainian food of animal and plant origin.

It has been established that the presence of foodborne pathogens in the studied Ukrainian products was as follows: *Staphylococcus aureus* (MRSA) and *Cronobacter spp.* (*E. sakazakii*) was found in raw cow's milk in 6.5% and 19.4% of cases, respectively; Shiga-toxin-producing *Escherichia coli* (STEC) from beef and pork carcasses – 8.1% and 5.7%; *Bacillus cereus* and *Clostridium perfringens* from different types of plant and animal raw materials and their processing products averaged 27.5% and 7.7%, respectively.

In conclusion, the advantages of molecular-genetic methods, which PCR belongs to, are their rapidity and specificity of identifying microorganisms by the features of the genetic regions that carry information about their pathogenic factors. It has been found that by the PCR method, these pathogens are detected at least 5–9 times faster than by the classical methods. These data will be useful to assess the microbiological risk and will help the governmental authorities develop strategies to reduce risks to consumers' health.

References:

1. Elshafei AM. Role of microorganisms in food contamination, processing and safety. Journal of Food Microbiology [Internet]. 2017 August [cited 2019 August 23]; 1(1): [about 2pp.]. Available from: http://www.alliedacademies.org/journal-food-microbiology/.
2. Pylypenko L, Verkhivker Y, Pylypenko I. Microbiology, energy, packing and control of the canning production: scientific publication. LAP LAMBERT Academic Publishing: Saarbrücken; 2015.
3. Armany GA, Ibrahim HM, Amin RA, Ahmed HA. Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction. Benha Veterinary Medical Journal. 2016; 30(1):233-330. https://doi.org/10.21608/bvmj.2016.31402
4. Gill A. The Importance of Bacterial Culture to Food Microbiology in the Age of Genomics. Frontiers in Microbiology. 2017; 8 (Article 777):1-6. https://doi.org/10.3389/fmicb.2017.00777.
5. Liu Y, Cao Y, Wang T, Dong Q, Li J, Niu C. Detection of 12 Common Food-Borne Bacterial Pathogens by TaqMan Real-Time PCR Using a Single Set of Reaction Conditions. Frontiers in Microbiology. 2019; 10(Article 222):1-9. https://doi.org/10.3389/fmicb.2019.00222.
6. Wang Y, Salazar JK. Culture-independent rapid detection methods for bacterial pathogens and toxins in food matrices. Comprehensive Reviews in Food Science and Food Safety. 2016; 15(1):183-205. https://doi.org/10.1111/1541-4337.121.
7. Law JW-F., Ab Mutalib NS, Chan KG, Lee LH. Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. Frontiers in Microbiology. 2015; 5(Article 770):1-7. https://doi.org/10.3389/fmicb.2014.00770
8. Baraketi A, Salmieri S, Lacroix M. Foodborne Pathogens Detection: Persevering Worldwide Challenge. Chapter 5 from book: Biosensing Technologies for the Detection of Pathogens - A Prospective Way for Rapid Analysis. 2018. http://dx.doi.org/10.5772/intechopen.74421.
9. Pylypenko L, Pylypenko I, Yamborko A, Danylova O. Methodology for accelerated monitoring and assurance of sanitary quality and food safety. Ukrainian Food Journal. 2017; 6(2):211-226. http://dx.doi.org/10.24263/2304-974X-2017-6-2-4.
10. Yeni F, Yavaş S, Alpas H, Soyer Y. Most common foodborne pathogens and mycotoxins on fresh produce: A review of recent outbreaks. Critical Reviews in Food Science and Nutrition. 2016;56(9):1532-1544. https://doi.org/10.1080/10408398.2013.777021.
11. Boughattas S, Salehi R. Molecular approaches for detection and identification of foodborne pathogens. Journal of Food Quality and Hazards Control. 2014; 1(1):1-6.
12. Lee N, Kwon KY, Oh SK, Chang HJ, Chun HS, Choi SW. A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157: H7, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food. Foodborne Pathogens and Disease. 2014; 11(7):574-580. http://dx.doi.org/10.1089/fpd.2013.1638.
13. Priyanka B, Rajashekhar K. Patill, Dwarakanath S. A review on detection methods used for foodborne pathogens. Indian Journal of Medical Research. 2016; 144(3):327-338. http://dx.doi.org/10.4103/0971-5916.198677.

14. Alahi MEE, Mukhopadhyay SC. Detection methodologies for pathogen and toxins: A review. Sensors. 2017; 17(8):1885-1905. http://dx.doi.org/10.3390/s17081885.
15. Berhilevych OM, Kasianchuk VV, Kukhtyn MD, Lotskin IM, Garkavenko TO, Shubin PA. Characteristics of antibiotic sensitivity of *Staphylococcus aureus* isolated from dairy farms in Ukraine. Regulatory Mechanisms in Biosystems. 2017; 8(4):559-563. https://doi.org/10.15421/021786.
16. Berhilevych OM, Kasianchuk VV. Identification of *Cronobacter spp (Enterobacter sakazakii)* from raw milk and environmental samples of dairy farms. Eastern-European Journal of Enterprise Technologies. 2017; 6/11(90):4-10. https://doi.org/10.15587/1729-4061.2017.114637.
17. Berhilevych OM, Kasianchuk VV, Deriabin OM, Kukhtyn MD. Isolation of Shiga toxin-producing strains of *Escherichia coli* from beef and swine carcasses and the characterization of their genes. Regulatory Mechanisms in Biosystems Biosystems. 2018; 9(2):275-281. https://doi.org/10.15421/021840.
18. Pylypenko L, Pylypenko I, Yamborko A, Kotliar Ye. Identification of bacillary microbial contaminants and food poisoning agents from Ukrainian plant raw materials and products. Ukrainian food journal. 2017; 6(1):7-19. Available at http://nbuv.gov.ua/UJRN/UFJ_2017_6_1_3. https://doi.org/10.24263/2304-974X-2017-6-1-3
19. Pylypenko IV, Pylypenko LN, Yamborko GV, Marinova IV. Toxin production ability of *Bacillus cereus* strains from food product of Ukraine. Food Science and Technology. 2017; 11(3):93-98. https://doi.org/10.15673/fst.v11i3.612.
20. Kukhtyn MD, Horyuk YV, Horyuk VV, Yaroshenko TY, Vichko OI, Pokotylo OS. Biotype characterization of *Staphylococcus aureus* from milk and dairy products of private production in the western regions of Ukraine. Regulatory Mechanisms in Biosystems. 2017; 8(3):384-388. https://doi.org/10.15421/021759.
21. Sava VM, Pylypenko LM, Pylypenko IV, vynakhidnyky ta patentovlasnyky. Sposib vyznachennia *Clostridium perfringens* v kharchovykh produktakh. Patent Ukrainy na vynakhid № 111266. 2016 Apr 11.
22. Kyiko EY. Polymeraznaia tsepnaia reaktsyia kak metod hennoi dyahnostyky v molochnom skotovodstve. Vestnyk THU. 2001; 16(2):658-659.
23. Kukhtyn MD, Berhilevych OM, Kravcheniuk K, Shynkaruk O, Horyuk YV, Semaniuk N. Formation of biofilms on dairy equipment and the influence of disinfectants on them. Eastern-European Journal of Enterprise Technologies. 2017; 5/11(89):26-33. https://doi.org/10.15587/1729-4061.2017.110488.
24. Al-Ashmawy MA, Sallam KI, Abd-Elghany SM, Elhadidy M, Tamura T. Prevalence, molecular characterization, and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from milk and dairy products. Foodborne pathogens and disease. 2016; 13(3):156-162. http://dx.doi.org/10.1089/fpd.2015.2038.
25. Riva A, Borghi E, Cirasola D, Colmegna S, Borgo F, Amato E, et al. Methicillin-Resistant *Staphylococcus aureus* in Raw Milk: Prevalence, SCC*mec* Typing, Enterotoxin Characterization, and Antimicrobial Resistance Patterns. Journal of Food Protection. 2015; 78(6):1142-1146. https://doi.org/10.4315/0362-028X.JFP-14-531.
26. Titouche Y, Hakem A, Houali K, Meheut T, Vingadassalon N, Ruiz-Ripa L, et al. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria. Journal of Dairy Science. 2019; 102(8):6876-6884. https://doi.org/10.3168/jds.2018-16208.
27. Amer MM, Mekky HM. *Cronobacter sakazakii (Enterobacter sakazakii)*. International Journal of Research in Pharmacy and Biosciences. 2019; 6(4):4-14.
28. Vineeth B, Priyanka B, Ramesh BS, Makari HK., Vinay SMN. Isolation and partial characterization of *Cronobacter sakazakii* by 16S rRNA sequence analysis isolated from milk of dairy cows with mastitis in Chikmagalur, Karnataka, India. International Journal of Scientific and Engineering Research. 2014; 5(4):671-680.
29. Abdissa R, Haile W, Fite AT, Beyi AF, Agga GE, Edao BM, et al. Prevalence of *Escherichia coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. MC Infectious Diseases. 2017; 17(1):277-283. http://dx.doi.org/10.1186/s12879-017-2372-2.
30. Anu PJ, Latha C, Vinodkumar K, Vinod VK, Sathu T. An important approach for control of Enterohaemorrhagic *Escherichia coli* by identification of contaminating sources in beef production line. International Journal of Current Microbiology and Applied Sciences. 2018; 7(1):1921-1929. http://dx.doi.org/10.20546/ijcmas.2018.701.231.
31. Bardasi L, Taddei R, Fiocchi I, Pelliconi MF, Ramini M, Toschi E, et al. Shiga toxin-producing *Escherichia coli* in slaughtered pigs and pork products. Italian Journal of Food Safety. 2017; l(6):6579-6584. http://dx.doi.org/10.4081/ijfs.2017.6584.
32. Omoruyi IM, Uwadiae E, Mulade G, Omoruku E. Shiga Toxin Producing Strains of *Escherichia coli* (STEC) associated with beef products and its potential pathogenic effect. Microbiology Research Journal International. 2018; 23(1):1-7. http://dx.doi.org/10.9734/MRJI/2018/32959.
33. Forgani F, Kim J-B, Oh D-H. Enterotoxigenic profiling of emetic toxin- and enterotoxin-producing *Bacillus cereus*, isolated from food, environmental, and clinical samples by multiplex PCR. Journal of Food Science. 2014; 79(11):2288-2293. http://dx.doi.org/10.1111/1750-3841.12666.
34. Flores-urban KA, Natividad-Bonifacio I., Vazquez-Quinones CR, Vazquez-Salinas C, Quinones-Ramirez EI, Irma E. Detection of Toxigenic *Bacillus cereus* Strains Isolated from Vegetables in Mexico City. Journal of Food Protection. 2014; 77(12): 2144-2147. http://dx.doi.org/10.4315/0362-028X.JFP-13-479.

ІДЕНТИФІКАЦІЯ ХАРЧОВИХ ПАТОГЕНІВ ТА ВСТАНОВЛЕННЯ РІВНЯ ЇХНЬОГО ПОШИРЕННЯ В УКРАЇНСЬКІЙ ПРОДУКЦІЇ ТВАРИННОГО ТА РОСЛИННОГО ПОХОДЖЕННЯ МЕТОДОМ ПЛР

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Анотація. Збудники харчових захворювань спричиняють серйозні проблеми у сфері охорони здоров'я у кожній країні. У зв'язку з цим, мікробіологічне дослідження включено в управління безпечністю харчового ланцюга. Молекулярні методи і переважно полімеразна ланцюгова реакція (ПЛР) вважаються високочутливими, специфічними та швидкими методами виявлення збудників у сировині та продуктах харчування. У цьому дослідженні описано використання спеціально розроблених високоспецифічних ПЛР-праймерів для виявлення 5 поширених і особливо небезпечних збудників харчових отруєнь і захворювань та встановлення рівня їхнього поширення в продовольчій продукції тваринного та рослинного походження. Дослідження включали ідентифікацію стійких до метициліну *Staphylococcus aureus* (MRSA) та *Cronobacter spp.* (*E. sakazakii*) із сирого молока, шига-токсинпродукуючої кишкової палички (STEC) з туш яловичих та свинячих, *Bacillus cereus, Clostridium perfringens* з різних видів рослинної та тваринної сировини і продуктів їх переробки – фруктів, овочів, ягід, сушених та консервованих продуктів, харчоконцентратів, напівконсервів. Всього було досліджено 397 зразків продовольчої продукції для виявлення цих збудників за допомогою класичних бактеріологічних методів та ПЛР. Встановлено, що в досліджуваній вітчизняній продукції тваринного та рослинного походження поширення харчових патогенів було наступним: *Staphylococcus aureus* (MRSA) та *Cronobacterspp.* (*E. sakazakii*) в сирому молоці корів у 6,5% та 19,4% випадків відповідно; шига-токсинпродукуючих кишкових паличок (STEC) з туш яловичих та свинячих у 8,1% та 5,7%; *Bacillus cereus* та *Clostridium perfringens* в різних видах рослинної та тваринної си ровини і продуктах її переробки становлять в середньому 27,5% та 7,7% відповідно. До переваг генетично-молекулярних методів, до яких відноситься ПЛР, слід віднести їх швидкість, а також специфічність ідентифікації мікроорганізмів за особливостями генетичних ділянок генів, які несуть інформацію про їх фактори патогенності. Встановлено, що швидкість визначення наведених патогенів при використанні ПЛР порівняно з класичними методами зростає щонайменше в 5–9 разів. Ці дані будуть корисними для оцінки мікробіологічного ризику та допоможуть органам влади розробити стратегії щодо зменшення ризику для здоров'я споживачів.

Ключові слова: MRSA, *Cronobacter* spp. (*E. sakazakii*), STEC, *Bacillus cereus, Clostridium perfringens*, тваринна та рослинна сировина і продукти її переробки.

Список літератури:

1. Elshafei A. M. Role of microorganisms in food contamination, processing and safety // Journal of Food Microbiology. 2017. Vol.1 (1). P. 1-2. Available at *http://www.alliedacademies.org/journal-food-microbiology/*.
2. Pylypenko L., Verkhivker Y., Pylypenko I. Microbiology, energy, packing and control of the canning production: scientific publication. LAP LAMBERT Academic Publishing: Saarbrücken, 2015. 82 p.
3. Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction / Armany G. A., et al.// Benha Veterinary Medical Journal. 2016. Vol. 30 (1). P. 233-330.
4. Gill A. The Importance of Bacterial Culture to Food Microbiology in the Age of Genomics // Frontiers in Microbiology. 2017. Vol. 8 (Article 777). 6 p. DOI: 10.3389/fmicb.2017.00777.
5. Detection of 12 Common Food-Borne Bacterial Pathogens by TaqMan Real-Time PCR Using a Single Set of Reaction Conditions / Liu Y. et al. // Frontiers in Microbiology. 2019. Vol. 10. (Article 222). 9 p. DOI: 10.3389/fmicb.2019.00222.
6. Wang Y., Salazar J.K. Culture-independent rapid detection methods for bacterial pathogensand toxins in food matrices // Comprehensive Reviews in Food Science and Food Safety. 2016. Vol. 15(1). P. 183 – 205. DOI: https://doi.org/10.1111/1541-4337.121.
7. Rapid methods for the detection offoodborne bacterial pathogens: Principles, applications, advantages and limitations / Law J.W-F., et al. // Frontiers in Microbiology. 2015. Vol. 5. (Article 770). 7 p.
8. Baraketi A., Salmieri S., Lacroix M. Foodborne Pathogens Detection: Persevering Worldwide Challenge / Chapter 5 from book Biosensing Technologies for the Detection of Pathogens - A Prospective Way for Rapid Analysis. 2018. P. 53-72. DOI: http://dx.doi.org/10.5772/intechopen.74421.
9. Methodology for accelerated monitoring and assurance of sanitary quality and food safety / Pylypenko L., Pylypenko I. et al. // Ukrainian Food Journal. 2017. Vol. 6 (2). P. 211-226. DOI: 10.24263/2304-974X-2017-6-2-4
10. Most common foodborne pathogens and mycotoxinson fresh produce: A review of recent outbreaks / Yeni F., et al. // Critical Reviews in Food Science and Nutrition. 2016. Vol. 56(9). P.1532–1544. DOI: https://doi.org/10.1080/10408398.2013.777021.
11. Boughattas S., Salehi R. Molecular approaches for detection and identification of foodborne pathogens // Journal of Food Quality and Hazards Control. 2014. Vol.1 (1). P. 1-6.
12. A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157: H7, *Bacillus cereus*, *Vibrio Parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food / Lee N., et al. // Foodborne Pathogens and Disease. 2014. Vol. 11(7). P. 574-580. DOI: 10.1089/fpd.2013.1638.
13. A review on detection methods used for foodborne pathogens / Priyanka B., et al. // Indian Journal of Medical Reseach. 2016. Vol.144 (3). P. 327-338 DOI: 10.4103/0971-5916.198677.
14. Alahi M.E.E., Mukhopadhyay S.C. Detection methodologies for pathogen and toxins: A review // Sensors. 2017. Vol. 17(8). P.1885-1905. DOI: 10.3390/s17081885.
15. Characteristics of antibiotic sensitivity of *Staphylococcus aureus* isolated from dairy farms in Ukraine / Berhilevych O. M., et al. // Regulatory Mechanisms in Biosystems. 2017. Vol. 8 (4). P. 559-563. DOI: https://doi.org/10.15421/021786.
16. Berhilevych O. M., Kasianchuk V.V. Identification of *Cronobacter spp (Enterobacter sakazakii)* from raw milk and environmental samples of dairy farm // Eastern-European Journal of Enterprise Technologies. 2017. Vol. 6/11 (90). P.4-10. DOI: https://doi.org/10.15587/1729-4061.2017.114637.
17. Isolation of Shiga toxin-producing strains of *Escherichia coli* from beef and swine carcasses and the characterization of their genes / Berhilevych O. M., et al. // Regulatory Mechanisms in Biosystems Biosystems. 2018. Vol. 9(2). P. 275-281. DOI: https://doi.org/10.15421/021840.
18. Identification of bacillary microbial contaminantsand food poisoning agents from ukrainian plant raw materials and products / Pylypenko L., et al. // Ukrainian food journal. 2017. Vol.6 (1). P. 7-19. Available at http://nbuv.gov.ua/UJRN/UFJ_2017_6_1_3.
19. Toxin production ability of *Bacillus cereus* strains from food product of Ukraine / Pylypenko I.V., et al. // Food Science and Technology. 2017. Vol.11 (3). P.93-98. DOI: https://doi.org/10.15673/fst.v11i3.612.
20. Biotype characterization of *Staphylococcus aureus* from milk and dairy products of private production in the western regions of Ukraine / Kukhtyn M. D. et al. // Regulatory Mechanisms in Biosystems. 2017. Vol. 8(3). P. 384-388. DOI: https://doi.org/10.15421/021759.

21. Спосіб визначення *Clostridium perfringens* в харчових продуктах. Патент України на винахід № 111266 МПК C12N 15/11 (2006.01), C12Q 1/04(2006.01), C12Q 1/68 (2006.01), C12R 1/145 (2006.01), C12N 1/00 / Сава В.М., Пилипенко Л. М., Пилипенко І. В.; власники Сава В.М., Пилипенко Л. М., Пилипенко І. В. № a201409534, заявл. 29.08.2014, опубл. 11.04.2016, бюл. № 7/2016.
22. Кийко Е. И. Полимеразная цепная реакция как метод генной диагностики в молочном скотоводстве // Вестник ТГУ. 2001. Т.16. № 2. С. 658-659.
23. Formation of biofilms on dairy equipment and the influence of disinfectants on them/ Kukhtyn M. D., et al. // Eastern-European Journal of Eenterprise Technologies. 2017. Vol. 5/11(89). P. 26-33. DOI: https://doi.org/10.15587/1729-4061.2017.110488.
24. Prevalence, molecular characterization, and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from milk and dairy products / Al-Ashmawy M. A., et al. // Foodborne pathogens and disease. 2016. Vol. 13(3). P. 156-162. DOI: 10.1089/fpd.2015.2038.
25. Methicillin-Resistant *Staphylococcus aureus* in Raw Milk: Prevalence, SCC*mec* Typing, Enterotoxin Characterization, and Antimicrobial Resistance Patterns / Riva A., et al. // Journal of Food Protection. 2015. Vol. 78(6) P. 1142-1146. DOI: https://doi.org/10.4315/0362-028X.JFP-14-531.
26. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria /Titouche Y., et al // Journal of Dairy Science. 2019. Vol. 102 (8). P. 6876-6884. DOI: https://doi.org/10.3168/jds.2018-16208.
27. Amer M.M., Mekky H.M. *Cronobacter Sakazakii (Enterobacter Sakazakii)* // International Journal of Research in Pharmacy and Biosciences. 2019. Vol. 6 (4). P. 4-14.
28. Isolation and partial characterization of *Cronobacter sakazakii* by 16S rRNA sequence analysis isolated from milk of dairy cows with mastitis in Chikmagalur, Karnataka, India / Vineeth B., et al. // International Journal of Scientific and Engineering Research. 2014. Vol. 5 (4). P. 671-680.
29. Prevalence of *Escherichia coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia / Abdissa R., et al. // MC Infectious Diseases. 2017. Vol.17 (1). P. 277-283. DOI: 10.1186/s12879-017-2372-2.
30. An important approach for control of Enterohaemorrhagic *Escherichia coli* by identification of contaminating sources in beef production line / Anu P. J., et al. // International Journal of Current Microbiology and Applied Sciences. 2018. Vol. 7(1). P. 1921-1929. DOI: 10.20546/ijcmas.2018.701.231.
31. Shiga toxin-producing *Escherichia coli* in slaughtered pigs and pork products / Bardasi L., et al. // Italian Journal of Food Safety. 2017. Vol. 6. P. 6579-6584. DOI:10.4081/ijfs.2017.6584.
32. Shiga Toxin Producing Strains of *Escherichia coli* (STEC) associated with beef products and its potential pathogenic effect / Omoruyi I. M., et al. // Microbiology Research Journal International. 2018. Vol. 23(1). P. 1-7. DOI: 10.9734/MRJI/2018/32959.
33. Forgani F., Kim, J-B. Oh D-H. Enterotoxigenic profiling of emetic toxin- and enterotoxin-producing *Bacillus cereus*, Isolated from food, environmental, and clinical samples by multiplex PCR // Journal of Food Science. 2014. Vol.79 (11). P. 2288-2293. DOI: 10.1111/1750-3841.12666.
34. Detection of Toxigenic *Bacillus cereus* Strains Isolated from Vegetables in Mexico City / Flores-urban KA et al. // Journal of Food Protection. 2014. Vol. 77(12). P.2144-2147. DOI: 10.4315/0362-028X.JFP-13-479.