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The importance and necessity of controlling food microbiological safety in the Republic of Armenia

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KEY CONTRIBUTION

The presence of *E. coli* in the ready-to-eat dishes suggests the possibility of secondary contamination. *Pseudomonas aeruginosa* was predominantly present in water samples, including both tap and bottled water. The examination of food products and beverages showed that each tested sample could fail to meet regulatory standards based on multiple criteria.

ABSTRACT

Foodborne poisoning occurs when individuals consume food contaminated with various bacteria, including *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium difficile*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and *Enterococcus* spp. These bacteria pose significant public health risks worldwide. This study aimed to identify the causes of food poisoning incidents by examining food products and beverages given to the Department of Food and Beverage Expertise of the National Bureau of Expertise of the Republic of Armenia in accordance with regulatory documents approved by the Armenian government.A total of 138 samples were found to be non-compliant with microbiological standards, corresponding to 242 non-compliant indicators, as several samples failed multiple criteria simultaneously. Non-compliance related to mesophilic aerobic and facultative anaerobic bacteria (QMAFAnM), yeast counts, fungal counts, and combined yeast and fungal counts was observed in 25.6% (95% CI: 20.1–31.1), 2.5% (95% CI: 0.5–4.4), 5.8% (95% CI: 1.3–10.3), and 2.1% (95% CI: 0.3–3.9) of samples, respectively. Coliforms were detected in 42.1% (95% CI: 35.8-48.4) of the samples, in particular *Escherichia coli* was revealed in 9.5% (95% CI: 5.8- 13.2) of the samples. *Staphylococcus aureus* was found in 5.8% (95% CI: 2.8-8.74). *Proteus* spp. was present in 3.3% (95% CI: 1.1-5.5) of cases. Sulfite-reducing clostridia (presumably *Clostridium perfringens*) appeared in 1.2% of the samples, *Pseudomonas aeruginosa* was identified in 1.7% (95% CI: 0.1-3.3) of cases, and *Bacillus cereus* was detected in 0.4% of the total samples. The research indicated that food products contaminated with pathogenic or opportunistic microorganisms can lead to foodborne illness. Ensuring food safety is a collective responsibility that requires the combined efforts of all stakeholders involved.

Introduction

Improperly managed food can become a breeding ground for various pathogenic bacteria, which pose significant challenges to public health by causing a high number of illnesses. Bacterial food poisoning is most commonly associated with *Salmonella* spp., *Campylobacter* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*, among others (Hernández-Cortez et al., 2017). These bacteria possess characteristics that enable them to evade the host immune system, leading to various health disorders.

Cooking food before consumption reduces the risk of foodborne illness by killing harmful bacteria. However, some bacterial genera, such as *Staphylococcus*, *Bacillus*, and *Clostridium*, produce heat-stable toxins that can remain harmful even after cooking (Hernández-Cortez et al., 2017, Merry, 1997). Outbreaks of foodborne illness have been linked to a variety of food sources, including meat, dairy products, vegetables, fish, cereals, and legumes.

Poultry, in particular, is often contaminated with *Salmonella* spp., *Staphylococcus aureus*, and occasionally *Bacillus cereus*, as well as psychrotrophic pathogens such as *Listeria monocytogenes*. In the European Union, eggs and egg products are major reservoirs of human salmonellosis (Mor-Mur et al., 2010). The levels of certain pathogenic species, such as *Listeria monocytogenes* and *Staphylococcus aureus*, found in raw milk are generally lower than disease-causing concentrations.

Between the 1950s and 1970s, there were several incidents involving pathogenic microbes in processed dairy products, including *Salmonella* species in dried milk products and *Staphylococcus aureus* in cheese (Collins et al., 1968; Hendricks et al., 1959). Ready-to-eat foods may be contaminated by various bacteria, including species of *Bacillus*, coagulase-negative staphylococci, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas* spp., and *Staphylococcus aureus* (Annan-Prah, 2011; Festus and Damilola, 2018; Gdoura-Ben Amor et al., 2018). Therefore, it is crucial to analyse data related to foodborne illness incidents that have occurred in the Republic of Armenia.

Materials and methods

Sample collection

The material for microbiological analysis consisted of food products submitted for examination to the National Bureau of Expertise of the Republic of Armenia.he material for microbiological studies was composed of food products forwarded for the examination to the National Bureau of Expertise of the Republic of Armenia. These included vegetable and meat salads, cheese and meat appetisers, pork, lamb, fish dishes, and other food products. A total of 23 forensic investigations were conducted as part of the cases related to food poisoning incidents that occurred in various institutions throughout the Republic of Armenia, such as hotels, restaurants, and kindergartens.

State Standard Specifications (GOST in Russian) applied for the study

The bacteriological study of the material involved the isolation and identification of microbial cultures. These procedures were performed using specific methodologies to determine each group of microorganisms, in accordance with GOST standards. For isolation and determination of coliforms, QMAFAnM, yeasts and fungi, sulfite-reducing clostridia, *Staphylococcus aureus*, *Proteus* spp., and *Pseudomonas aeruginosa* were applied GOST 31747-2012, GOST 10444.15-94, GOST 10444.12-2013, GOST 29185-2014, GOST 31746-2012, GOST 7702.2.7-2013, and GOST R 54755-2011, respectively . The

collected data were compared with the permissible units outlined in TR CU 021/2011 ("Technical Regulation of the Customs Union on Food Safety") (Table 1).

Table 1. The measurement units and permissible standards of microbial indicators as outlined in TR CU 021/2011 ("Technical Regulation of the Customs Union on Food Safety")

The indicator	The full name	The measurement unit (per GOST, TR CU)	The notes / standard form of expression
QMAFAnM	Total viable count (Mesophilic aerobic and facultative anaerobic microorganisms)	CFU/g or CFU/cm³	CFU — colony forming units; record as *QMAFAnM, CFU/g*
Coliforms	Coliform bacteria (*Enterobacteriaceae* group)	CFU/g, CFU/cm³ or in 0.01 g (cm³) of product	Depending on method and standard; often *absence in 0.01 g*
***Proteus* spp.**	Bacteria of the genus *Proteus*	Presence/absence in 0.1 g (cm³) or CFU/g	Usually expressed as *absence in 0.1 g*
Yeast-like fungi	Yeast-like fungi	CFU/g or CFU/cm³	*Yeasts, CFU/g*
Mold fungi	Mold fungi	CFU/g or CFU/cm³	*Molds, CFU/g*
Staphylococcus aureus	*Staphylococcus aureus* (pathogenic staphylococci)	Presence/absence in 1.0 g (cm³) or CFU/g	Typically *absence in 1.0 g (cm³)*
Sulfite-reducing clostridia	Sulfite-reducing clostridia	Presence/absence in 0.01–0.1 g (cm³)	For canned or anaerobic products — *absence in 0.01 g*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	Presence/absence in 0.1 g (cm³)	*Absence in 0.1 g*
Bacillus cereus	*Bacillus cereus*	CFU/g or CFU/cm³	Often regulated as *not more than 10² CFU/g*

Data processing

Data evaluation was performed using the appropriate function in Microsoft Excel 2016. Each procedure throughout the analysis has been carried out in duplicate. The statistical analysis of the results included the standard error (m) and the confidence interval (CI) with a 95% probability.

Results and discussion

The National Bureau of Expertise of the National Academy of Sciences of the Republic of Armenia received over 23 cases for examination related to food poisoning. The examination of food products primarily revealed the presence of coliform bacteria (particularly *Escherichia coli*), *Staphylococcus aureus*, and other bacterial species at levels exceeding the permissible limits set by regulatory documents (TR CU 021/2011).

A total of 16 types of food products and drinking water samples were tested to evaluate their compliance with current regulatory requirements. These included nine thermally processed food products and seven non-thermally processed products. It is important to note that the same product could be tested for multiple parameters at the same time.

The total number of tested samples is 205, with 138 (67.3%) not meeting the permissible units specified in the regulatory documents of the Republic of Armenia (Table 2) corresponding to 242 non-compliant indicators, as some samples failed multiple criteria simultaneously (Table 3).

It is important to note that soups (8 samples, 100%), which are typically cooked at high temperatures, can still become contaminated with harmful microbes, especially coliforms (refer to Table 3, Supplementary 1), due to secondary contamination (Valero et al., 2016). Coliforms are known to be sensitive to elevated temperatures and generally do not survive temperatures exceeding 60 °C (Denis et

al., 2006). Regarding meat products, including thermally treated ones (40 samples, 93%), the failure to comply with permissible limits in both thermally non-processed and thermally processed samples reflects inadequate hygienic and thermal conditions during preparation or storage (Orta-Ramirez and Smith, 2002). The rates of non-compliance with QMAFAnM, coliform bacteria, *E. coli*, *S. aureus*, *Proteus* spp., sulfite-reducing clostridia (*Cl. perfringens*), *P. aeruginosa*, yeast count, fungal count, total yeast and fungal count, and B. cereus were 25.6% (95% CI: 20.1–31.1), 42.1% (95% CI: 35.8–48.4), 9.5% (95% CI: 5.8–13.2), 5.8% (95% CI: 2.8–8.7), 3.3% (95% CI: 1.1–5.5), 1.2%, 1.7% (95% CI: 0.1–3.3), 2.5% (95% CI: 0.5–4.4), 5.8% (95% CI: 1.3–10.3), 2.1% (95% CI: 0.3–3.9), and 0.4%, respectively.A typical representative of coliform bacteria is *E. coli*, which is associated with sanitary conditions. The presence of *E. coli* in food samples indicates recent fecal contamination. In our analysis, *E. coli* was isolated from the following food items: three samples of soups, five samples of thermally processed meat products, two samples of thermally non-processed meat products, six samples of sausages, five samples of salads containing vegetables and meat, and one sample each of thermally processed and thermally non-processed egg products. Since the samples submitted to our laboratory are linked to forensic cases, we have access to comprehensive information about each case, including details about other laboratory experiments conducted. It is important to note that personnel involved in handling these food items, as well as associated surfaces, were also examined.

Table 2. The overview of the microbial contaminates in analysed food and beverage samples

	The name of a food product	The absolute number of samples studied	The absolute number of tested food product samples that meet the standards	The absolute number of tested samples of food products that do not meet the standards		95% CI	The total number of studied indicators that do not meet regulatory standards
				Abs.	%± m		
1	Soups	8	-	8	-	15	15
2	Meat products	43 (22 heat-treated, 21 not heat-treated)	3	40	85.4-100.6	75 (41 heat-treated, 4 not heat-treated)	75 (41 heat-treated, 34 not heat-treated)
3	Fermented milk products	17	4	13	56.3-96.7	25	25
4	Sausages	9	1	8	68.3-109.5	20	20
5	Pilafs	12	3	9	50.5-99.5	15	15
6	Salads incorporated with vegetables and meat	8	-	8		24	24
7	Confectionery and bakery products	17	9	8	23.4-70.8	11	11
8	Canned food	25	11	14	36.6-75.4	15	15
9	Eggs, egg products	5	-	5		7	7
10	Industrial juices and drinks	14	10	4	4.9-52.3	4	4
11	Spices	3	1	2	13.4-120	3	3
12	Tea, coffee, cocoa	5	2	3	14.1-102.9	3	3
13	Flour	6	4	2	-	3	3
14	Sugar	6	4	2	-	2	2
15	Raw vegetables	13	13	-		-	-
17	Drinking water	14	2	12	64.3-104.1	20	20
	Total number	**205**	**67**	**138**	**60.8-73.8**	**242**	**242**

It is important to note that the staff handling these food items, as well as the surfaces associated with them, have also been examined. Coliform bacteria were detected on the hands of the personnel.It was evident that coliform bacteria were present on their hands. As anticipated, coliform bacteria were also detected on food-contact surfaces and related items.

Table 3. Indicators of microbial contamination in the analyzed food and beverage samples not complying with regulatory standards

The name of a microbe or group of microbes	The absolute number of indicators of the studied food samples that do not meet the permissible standards	%± m	95% CI
QMAFAnM	62	25.6±2.8	20.1-31.1
Coliform bacteria	102	42.1±3.2	35.8-48.4
E .coli	23	9.5±1.9	5.8-13.2
Staphylococcus aureus	14	5.8±1.5	2.8-8.74
Proteus spp.	8	3.3±1.1	1.1-5.5
Sulfite-reducing clostridia	3	1.2±0.7	-
Pseudomonas aeruginosa	4	1.7±0.8	0.1-3.3
Yeasts count	6	2.5±1.0	0.5-4.4
Fungi count	14	5.8±2.3	1.3-10.3
Total fungi and yeasts count	5	2.1±0.9	0.3-3.9
Bacillus cereus	1	0.4±0.4	-
The total number	**242**	**100**	

The isolation of *E. coli* from ready-to-eat food samples, such as soups, barbecue, pilafs, and salads, highlights its significant importance (Table 4). The presence of coliforms in these items indicates potential secondary contamination (Oh et al., 2018, Herawati et al., 2023). Furthermore, the prevalence of *E. coli* in industrially produced foods, including sausages, basturma, and sujukh, suggests that inadequate hygienic conditions during production may be responsible (Shaltout et al., 2022). It is worth noting that while non-pathogenic strains of *E. coli* are generally harmless, they can still lead to gastrointestinal issues in individuals with a weakened immune response (Kashima et al., 2021). The presence of *Bacillus cereus* bacteria was detected in a canned poultry product sample. Therefore, among various harmful bacteria, *Bacillus cereus* can survive in sterilized canned food (Bergey, 1994).

A higher prevalence of *Staphylococcus aureus* was found in salads with addition of mayonnaises. This was followed by ready-to-eat meals, confectioneries, raw chicken meat, and sausages. One of the most severe cases of food poisoning occurred at a wedding, where more than 50 guests became ill, including the bride and groom, who were hospitalized on their wedding day at the Nork Infectious Diseases Hospital. Medical assistance was provided based on the severity of their symptoms. Individuals began to feel unwell 1.5 to 2 hours after consuming the food, which is characteristic of *Staphylococcus aureus* poisoning. These cases included one sample each from bakery and meat products, as well as five salad samples (Figure 1).

These samples did not meet regulatory standards. The bacteria were primarily detected in salads prepared with homemade mayonnaise, including Olivier salad, Caesar salad, chicken salad, and salad with boiled beef tongue, among others.

During the epidemiological investigation, it was determined that the individual preparing the mayonnaise was a carrier of *Staphylococcus aureus*. Additionally, non-compliance was identified in terms of QMAFAnM (21 out of 25 samples) and coliform bacteria (23 out of 25 samples). The most affected individuals were children and women who had not consumed alcohol. Individuals who consumed alcohol together with the contaminated food exhibited less severe signs of intoxication.

Individuals who consumed alcohol together with the contaminated food exhibited less severe signs of intoxication.

As discussed in one research, commercial mayonnaise has been reported to prevent growth of *Staphylococcus aureus* (Doyle et al., 1982). In many countries, mayonnaise is mainly made at home and has higher pH values compared to commercial varieties. Additionally, mayonnaise and similar foods may be stored at inappropriate temperatures (Gomez-Lucia et al., 1987).

Proteus species were detected in six raw meat samples and two thermally processed meat products (Figure 1). Wang et al. (2010) reported a case of food poisoning at a restaurant in Beijing, China, caused by *Proteus mirabilis*. Genotyping and the Dienes method identified the same bacterial clone in the food consumed, as well as in stool samples from patrons, the cook, and the waiter. It is likely that the lack of hygiene among the staff facilitated the transmission of the bacteria to the meal.

Sulfite-reducing clostridia were detected in 3 of 138 samples, including one thermally processed meat product and two canned food samples. These bacteria were primarily found in samples of ready-to-eat meat. This finding is consistent with data from other studies indicating that storing cooked meat at room temperature can promote the germination and growth of *Clostridia* spores, potentially leading to foodborne illness (Bataeva et al., 2020).

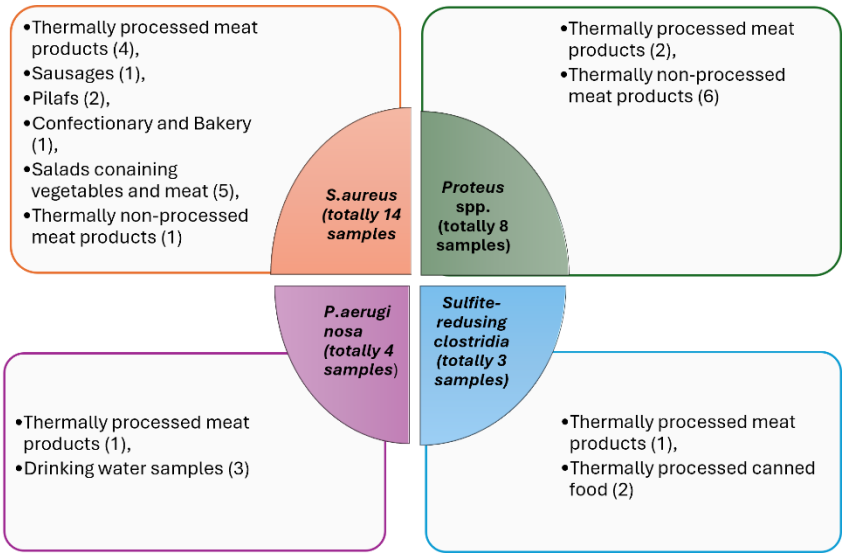

Figure 1. The prevalence of *S.aureus*, *Proteus* spp., *P.aeruginosa*, and Sulfite-reducing clostridia bacteria in various food products examined in our research

Pseudomonas aeruginosa was predominantly detected in three water samples, including both tap and bottled water. It was also detected in one sample of thermally processed meat product. The highest prevalence of *P. aeruginosa* in drinking water samples were recorded in Croatia, Serbia and Montenegro (Vukić Lušić et al., 2021).

Conclusion

The examination of food products and beverages showed that each tested sample could fail to meet regulatory standards based on multiple criteria. Overall, the findings of this study indicate that the samples submitted for examination were associated with food poisoning incidents, which explains the high prevalence of microorganisms detected in the analysed food products and beverages.

Author Contributions: AT and SN conceived and designed the study. SN and AT performed the experiments. NA and SM provided reagents and analytical tools. SN and AT wrote the manuscript. NA and SM edited the manuscript and gave recommendations for the experiment. All authors read and approved the final manuscript.

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***Conflicts of Interest*:** The authors declare no conflict of interest.

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