DYNAMICS OF FOODBORNE DISEASES IN LATVIA AND MICROBIOLOGICAL INVESTIGATIONS OF READY-TO-EAT FOODS FROM CATERING ESTABLISHMENTS

PER MAISTĄ PLINTANČIŲ LIGŲ DINAMIKA LATVIJOJE IR GATAVŲ MAISTO PRODUKTŲ MAITINIMO ĮMONĖSE MIKROBIOLOGINIAI TYRIMAI

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SANTRAUKA

Reikšminiai žodžiai: per maistą plintančios ligos (MPL), gatavi maisto produktai (GMP), maisto sauga, mikrobiologiniai tyrimai, maisto higienos reikalavimai.

Tyrimo tikslas. Išsiaiškinti gatavų maisto produktų mikrobiologinę kokybę maitinimo įmonėse.

Metodai. Tradicinėse ir netradicinėse (rytietiškose) viešojo maitinimo įmonėse Latvijos sostinėje Rygoje atrinkti 276 maisto mėginiai ir atlikti aerobinių kolonijų skaičiaus (AKS) ir koliforminių bakterijų skaičiaus (KBS) tyrimai. Įvertinta higieninės situacijos viešojo maitinimo įmonėse analizė. Mikroorganizmų augimo GMP mėginiuose priežastys analizuotos pagal maisto kategoriją, maisto temperatūrą, perdirbimo būdą ir sudėtį.

Rezultatai. GMP mėginiuose nustatyti įvairūs indikatoriniai mikroorganizmai, tokie kaip Staphylococcus, Enterobacter, Escherichia, Klebsiella, Citrobacter, mielės, pelėsiai ir kiti. Daugiausia mikroorganizmų aptikta mėginiuose, kurie nebuvo termiškai apdoroti (III kategorijos maisto produktai) – 61,1 proc. mėginių AKS siekė nuo 10⁴ iki 10⁷ ksv g⁻¹. Šioje kategorijoje nustatyta KBS siekė nuo 10² iki ≥ 10⁴ ksv g⁻¹. Šie duomenys parodo III kategorijos maisto produktų kaip galimo maistinės kilmės patogenų ar potencialiai pavojingų gedimo mikroorganizmų šaltinio epidemiologinę reikšmę.

INTRODUCTION

The quality and safety norms for ready-to-eat foods (RTE) prepared in catering establishments are not regulated at present in Latvia and consequently there are not previously required quality and safety criteria which would be compared with appropriate and non-appropriate microbiological quality of RTE.

Food safety legislation of the European Community requires quality and safety food circulation politics, based on the latest scientific achievements and risk analysis, which include risk assessment, risk communication and risk management [8, 10, 11, 15–18, 21, 24].

In the food safety competent Authority – Food and Veterinary Service Republic of the Latvia (hereinafter – FVS) the state monitoring programme on microbiological investigations of food samples is only additional mechanism for realization of food safety surveillance measures. Actually this is one of the risk assessment’s components – risk analysis.

The latest European Union hygiene regulations state mandatory microbiological safety criteria for the end products and separate voluntary quality criteria for products processed during the stages of the technological process. Microbiological criteria can be used to design products and processes and to indicate the required microbiological status of raw materials, ingredients or end-products at any stage of the food chain, as appropriate [4–7, 18, 24]. Unfortunately these norms are appropriate mostly for industrially processed products not for RTE that are prepared in catering establishments.

Indicator groups of bacteria are widely used as measure of the hygienic characteristics of food and beverage. They have the advantage of being enumerated inexpensively and easily for quantifying the performance of a production process, when particular pathogens or spoilage organisms might be difficult to detect. In general, the most reliable indicators of product quality tend to be product specific. Total *Enterobacteriaceae* are used as a measure of faecal contamination during meat production in Europe, although coliforms are also encountered in research. In effect, microbial quality indicators mainly are spoilage organisms whose increasing numbers result in loss of product quality [1, 2, 121, 13, and 18]. The role of catering establishments and offered services and the number of the products produced there has substantially increased in the past few years. The food processing technologies are improving. The closer attention of food operators and customers is paid to the nutrition value of RTE, their composition, and visual decoration as well as on the quality of raw materials, safety of used processing methods and other specific aspects. Greater numbers of people go out and eat meals prepared in restaurants, canteens, fast food outlets, and by street food vendors. About 78 % of consumers eat out at a restaurant, bar, pub or café at least once a month, which 38 % eating out at least once at week or more often [27, 28]. In many countries, the boom in food service establishments is not matched by effective food safety education and control. Unhygienic preparation of food provides ample opportunities for contamination, growth, or survival of foodborne pathogens. Foodborne diseases pose a considerable threat to human health and the economy of individuals, families and nations. Their control requires a concerted effort on the part of the three principal partners, namely governments, the food industry and consumers [14, 20, 25, 26].

However RTE quite often are potentially hazardous for human health. Despite the positive dynamics of the past few years, the outbreaks of foodborne diseases, that were caused by foods prepared in this establishments are registe-
red relatively often in Latvia. The information provided by Public Health Agency of Latvia has concluded that greatest number of outbreaks of foodborne diseases or acute enteric infections (AEI) during the last 5 years has been registered particularly in the catering establishments including the canteens of the schools and kindergartens, health care institutions, National Forces, etc. [20, 23].

Annually in the country are registered about 9 till 10 thousands cases of AEI, where main part are virus initiated from 25 % in 2005 to 38.6 % in 2008; salmonella initiated from 9 % in 2005 to 13.9 % in 2008 and undetermined diarrhea’s from 60 % in 2005 to 38.5 % in 2008 (SVA, 2005–2008). Comparison with neighboured Europe country showed that in Lithuania, Latvia and Estonia in 2006 year the highest incidence rates (incidence rate at 100 thousands inhabitants) on rotavirus infection were registered – respectively 143.7, 115.58 and 100.6. Last year the highest level of AEI were registered [6, 22, 23] – Figure 1.

Thus 47 % of outbreaks (with 5 and more illness cases) registered in 2004–2007 were related to the catering establishments and children care institutions, and respectively 63 % were registered in 2008. The 39 % cases of outbreaks in 2005 year have viral aetiology agent and 45 % in 2007. In 2006, the prevalent agent was salmonella in 69 % cases of outbreaks, viral agents and non-identified agents were identified only in 8 %, other bacterial agents in 15 % [22, 23].

Active and focused preventive activities at food establishments performed by Food and Veterinary service (FVS) since 2002 year have influenced the number of total food related outbreaks and it has diminished substantially till 2007. However in 2008 year instant cyclic elevation of FBD was registered (see Figure 2).

MATERIALS AND METHODS

During the years 2005–2007, 276 samples of RTE from public catering establishments were investigated, including identification of total amount of microorganisms – aerobic colony count (ACC), indicator microorganisms – total coliforms (TC), if necessary – genus determination of separate isolated cultures, determination of total amount of isolated pathogens, identification and validation of other necessary RTE microbial quality characterization parameters.

Fourteen catering establishments from Riga city (capital of Latvia) with relatively equal hygienic situation were selected for sampling. Conventionally all samples were divided in three categories of food in dependence on the components of the food products, their processing methods and microbiological nature of the food products.

The method LVS ISO 4832:2003 „General directions for counting of Coliforms bacteria. Colony counting method”. for determination of total coliforms was used. The method LVS EN ISO 4833:2003 “The food and feed microbiology. Horizontal method of microorganisms counting. Colony counting method under 30 °C”, for determination of aerobic colony count was used. For detection of biochemical property of isolated cultures of microorganisms API biochemical test systems (API ® strips) were used.

Sampling was carried out according to the quality management procedures of FVS, samples were taken by trained persons – food inspectors. Microbiological investigations of the samples were carried out in the accredited laboratory – National Diagnostic Centre of FVS.

Guidelines of the Irish Food Safety Agency, Australia New Zealand Food Authority and internal guidelines of the Latvia Food and Veterinary Service on evaluation of the...
Results on microbiological investigation samples (microbiological criteria) of some RTE were used. The publication contains the main part from the biggest investigation data [12, 13].

Data analysis, including statistical analysis of the data was undertaken using Microsoft Excel, SPSS (statistical package for the social science) software package 15.0 version were performed to assess the relationships between variables of interest.

RESULTS AND DISCUSSION

As a result of microbiological investigations in 83 or 30.0% from the total investigated samples (n=276) non-conformities with stated or control criteria concerning identification on the aerobic colony count (ACC) and total coliforms (TC) in cfu g⁻¹ (colony forming units per gram) there were identified.

Non-conformities with stated criteria were recognised more often in samples tested for TC in 68 samples or 24.6% from total investigated (Table 1).

Comparison with stated criteria for ACC in the first category food group showed that 100 or 92.6% samples had satisfactory results and 8 (7.4%) had acceptable results. Whereas investigations on TC showed that 101 or 93.5% samples had satisfactory results and only seven (6.5%) samples had acceptable results. Non-conformities with stated criteria were recognised more often in traditional meals (10.2%) than in non-traditional meals (3.7%).

In second category food, the biggest numbers of cfu g⁻¹ on ACC investigated samples were recognised in 8 (7.4%) – more than 10⁴ cfu g⁻¹ and on TC investigated in 7 (6.5%) – more than 10² cfu g⁻¹ were recognised (Table 1). The highest lg10 average values of ACC in traditional meals such as cabbage rolls with meat and rice, roasted pork and boiled buckwheat were identified from 2.94 till 3.2 (at total lg10 average value for traditional meals – 2.14).

Table 1. RTE category and the results of samples conformity with stated criteria

<table>
<thead>
<tr>
<th>Category name of RTE and short description of control criteria</th>
<th>RTE subgroup name</th>
<th>Total investigated samples</th>
<th>Samples (%), which doesn’t comply with evaluation “satisfactory testing result”</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. category</td>
<td>Traditional meals</td>
<td>50</td>
<td>ACC: 5 (10%)</td>
</tr>
<tr>
<td>Satisfactory testing results: ACC: &lt;10⁴ cfu g⁻¹; TC: &lt;10² cfu g⁻¹; Staphylococcus aureus: &lt;10⁵ cfu g⁻¹.</td>
<td>Total 1st category meals</td>
<td>108</td>
<td>TC: 6 (7.4%)</td>
</tr>
<tr>
<td>Non-traditional meals</td>
<td>58</td>
<td>3 (5.2%)</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>2. category</td>
<td>Traditional meals</td>
<td>40</td>
<td>ACC: 6 (15%)</td>
</tr>
<tr>
<td>Satisfactory testing results: ACC: &lt;10⁶ cfu g⁻¹; TC: &lt;10² cfu g⁻¹; Staphylococcus aureus: &lt;10³ cfu g⁻¹.</td>
<td>Total 2nd category meals</td>
<td>83</td>
<td>TC: 19 (47.5%)</td>
</tr>
<tr>
<td>Non-traditional meals</td>
<td>43</td>
<td>0</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3. category</td>
<td>Traditional meals</td>
<td>40</td>
<td>ACC: 1 (26%)</td>
</tr>
<tr>
<td>Satisfactory testing results: ACC: criteria not applicable; TC: &lt;10² cfu g⁻¹; Staphylococcus aureus: 10² cfu g⁻¹.</td>
<td>Total 3rd category meals</td>
<td>85</td>
<td>TC: 15 (48.2%)</td>
</tr>
<tr>
<td>Non-traditional meals</td>
<td>45</td>
<td>0</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Total traditional meals</td>
<td>130</td>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>Total non-traditional meals</td>
<td>146</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Total all categories meals</td>
<td>276</td>
<td>15 (5.4%)</td>
<td>68 (24.6%)</td>
</tr>
</tbody>
</table>

Note*: include 1 sample where was identified Staphylococcus aureus
The highest log10 average values of ACC in non-traditional meals such as roasted vegetables, chicken meat with vegetables, "Taugogipakom" and boiled bean with sesame and soya sauce from 2.2 till 3.9 (at total log10 average value in this group 1.99).

The highest log10 average values of TC in traditional meals such as fresh vegetable salad with vegetable oil, fresh cabbage salad with vegetable oil and fresh vegetable salad "Rozmarija" were identified from 3.59 till 3.19 (at total log10 average value in this group – 2.20).

The highest log10 average values of TC in non-traditional meals such as chicken meat with vegetables, "Taugogipakom" was 1.3 (at total log10 average value in this group – 1.03) see Figure 4.

Comparison with stated criteria for ACC in the second category food group showed that 75 (90.4 %) samples had satisfactory results and only eight (9.7 %) samples had acceptable results. Whereas investigations on TC showed that 63 (75.9 %) samples had satisfactory results, 15 (18.07 %) samples had acceptable results, and 5 (6.02 %) samples had unsatisfactory results. Non-conformities with stated criteria were recognised more often in traditional meals (30.1 %) than in non-traditional meals (1.2 %).

In third category food, the biggest amount of cfu g⁻¹ on

Figure 3. ACC and TC log10 average values among 1st category RTE samples

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"Rozmarija" were identified from 5.38 till 5.0 (at total log10 average value in this group – 4.08).

The highest log10 average values of ACC in non-traditional meals such as roasted vegetables, chicken meat with vegetables, "Taugogipakom" and boiled bean with sesame and soya sauce from 2.2 till 3.9 (at total log10 average value in this group 1.99).

The highest log10 average values of TC in traditional meals such as fresh vegetable salad with vegetable oil, fresh cabbage salad with vegetable oil and fresh vegetable salad "Rozmarija" were identified from 3.59 till 3.19 (at total log10 average value in this group – 2.20).

The highest log10 average values of TC in non-traditional meals such as chicken meat with vegetables, "Taugogipakom" was 1.3 (at total log10 average value in this group – 1.03) see Figure 4.

Comparison with stated criteria for ACC in the second category food group showed that 75 (90.4 %) samples had satisfactory results and only eight (9.7 %) samples had acceptable results. Whereas investigations on TC showed that 63 (75.9 %) samples had satisfactory results, 15 (18.07 %) samples had acceptable results, and 5 (6.02 %) samples had unsatisfactory results. Non-conformities with stated criteria were recognised more often in traditional meals (30.1 %) than in non-traditional meals (1.2 %).

In third category food, the biggest amount of cfu g⁻¹ on
ACC investigated samples in 1 (1.2 %) sample were recognised – 9.3x10^6 cfu g^{-1} (meat salad) and on TC investigated in 41 (48.2 %) were recognised (Table 1).

The highest lg10 average values of ACC (more than 10^6 cfu g^{-1}) in traditional meals such as freshly made carrot juice, meat salad with mayonnaise and boiled vegetable salad with mayonnaise were identified from 5.6 till 6.47 (at total lg10 average value for traditional meals – 4.66). As well as highest lg10 average values of ACC were identified in non-traditional meals such as ,,Greek'' salad with Feta cheese, Italian pasta salad with chicken ham and Roman salad from 5.5 till 5.8 (at total lg10 average value for non-traditional meals – 4.24).

The highest lg10 average values of TC in traditional meals such as carrot juice, meat salad with mayonnaise and boiled vegetable salad with mayonnaise were identified from 3.5 till 4.1 (at total lg10 average value in this group – 2.8). As well as highest lg10 average values of TC were identified in non-traditional meals such as Italian pasta salad with chicken ham, Roman salad and meat salad from 2.4 till 3.3 (at total lg10 average value for non-traditional meals – 1.7) see Figure 5.

Comparison with stated criteria for ACC in the third category food group showed that 33 (38.8 %) samples had results with cfu g^{-1} from 10 till 10^4 cfu g^{-1}, 33 (38.8 %) samples had high level results with 10^4 till 10^6 cfu g^{-1} and

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**Figure 4.** ACC and TC lg10 average values among 2\(^{nd}\) category RTE samples
19 (22.3 %) samples had highest numbers of detected cfu g⁻¹ from >10⁶ till 10⁷ cfu g⁻¹.

Whereas investigations on TC showed that 43 (50.6 %) samples had satisfactory results (<10² cfu g⁻¹), 30 (35.3 %) samples had acceptable results (10² till 10⁴ cfu g⁻¹) and 12 (14.1 %) had unsatisfactory results (more than 10⁴ cfu g⁻¹). Non-conformities with stated criteria were recognised more often in traditional meals (25 %) than in non-traditional meals (4.4 %).

The highest lg10 average values for ACC were recognised in third category RTE – 4.44 and more significant values were detected in traditional meal subgroup – 4.66.

Also high lg10 average values for TC were recognised in third category RTE – 2.23 and more significant values detected also in traditional meals subgroup – 2.83.

The lowest lg10 average values were detected in first category RTE – total in category for ACC – 2.09 and TC – 1.12. For second category RTE – total for ACC – 3.0 and TC – 1.6. In both previously listed categories also highest values in traditional meals subgroup were detected (Table 2).
During study 111 different genera of separate isolated cultures were recognized those was identified in 89 (32.2\%) of samples from total investigated (n=276). Among isolated cultures there were 23.4% Bacillus, 22.5\% Entero- bacter and 14.4 \% Staphylococcus, 11.7 \% Klebsiella, 7.21 \% Pantoea, 3.6 \% Citrobacter and 2.7 \% Escherichia genus microorganisms (Figure 6).

Generally identified Bacillus genera includes three species and two of them Bacillus subtilis and Bacillus licheniformis have occasionally shown to be responsible for incidents of food-poisoning resembling one or other of the syndromes typical of Bacillus cereus, which are well known foodborne pathogen [2, 6, 14].

The average values of different identified microorganisms are showed below (Table 3).

The highest average values were detected from 3. category RTE samples that were investigated for Bacillus, Staphylococcus, Entero bacter, Klebsiella and Escherichia species. Unfortunately in the 1. and 2. category RTE samples the high levels of Entero bacter spp. Klebsiella spp. also were recognised.

Comparison of \(p\)-values in ANOVA analysis showed that significant values were recognised in 3\textsuperscript{rd} category of RTE samples; also there more significant were investigation results on TC. The high values (up to \(10^3\) cfu g\(^{-1}\)) of total coliforms should be used as active indicator of non-hygienic practices, they are the best component of a safety program such as the HACCP system especially in catering establishment where prepared different chilled RTE. Notwithstanding that total coliforms should not used for meals who undergoes heat treatment [2, 3, 18] – Table 4.

During the study the different non-compliances with hygienic requirements were identified in food chain. In the
case of traditional meal non-conformities concerning food internal temperatures during different processing stages were detected. In few cases food temperatures were much higher than stated critical limits according the establishment’s self-control system procedures (based on HACCP principles); however the storage and delivery time of these products was not longer than stated critical time limits. Generally at 9 from 14 establishments or 64.3 % establishments there were identified problems concerning temperature requirements (predominantly chilled meals, including salads, and fresh made juice). As a result of this evaluation it was detected that the worst non-complied microbiological samples were taken from establishments with the lowest hygiene level [3, 9, and 20].

CONCLUSIONS
The highest count of microorganisms was detected in RTE samples whose components did not undergo heat treatment (3rd category meals) – ACC varied from $10^0$ up to $10^3$ cfu g$^{-1}$ in 61.1 % samples; TC in this category meals was detected in level from $10^0$ up to $10^4$ cfu g$^{-1}$. However 12 of 85 (14.1 %) samples investigated on TC were not in compliance with stated criteria. That demonstrates the epidemiological significance of these category meals, as probable source of foodborne pathogens or potentially hazardous spoilage microorganisms.

RTE samples which were not in compliance with stated criteria were recognised often in 64.3 % catering establishments with unhygienic practices. There was observed food cross-contamination risk and different non-conformities on cooking, chilling, defrosting and storage requirements.

89 different genera of separate isolated cultures of microorganisms were identified in 32.2 % from total investigated samples (n=276). The main isolated cultures were Bacillus, Enterobacter, Staphylococcus, Citrobacter and Klebsiella genera. Some of these microorganisms are well known food pathogens and may cause infectious enteric diseases or food intoxications.

The total amount of identified microorganisms depends on the category of the products, processing methods, hygienic situation in the catering establishment and on the other factors influencing growth of microorganisms. In the 1st category meals (n=31) more often were identified Bacillus spp. (51.6 %), Staphylococcus spp. (25.8 %) and Enterobacter spp. (9.7 %) microorganisms. In the 2nd category meals (n=33) more often were identified – Enterobacter spp. (36.6 %), Klebsiella spp. (15.15 %), Staphylococcus spp. (15.15 %) and Citrobacter spp. (9.09 %). In the 3rd category meals (n=25)

<table>
<thead>
<tr>
<th>Names of microorganisms species</th>
<th>1st category</th>
<th>2nd category</th>
<th>3rd category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus spp.</td>
<td>$5.1 \times 10^2$</td>
<td>$1.2 \times 10^4$</td>
<td>$9.3 \times 10^5$</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>$1.2 \times 10^2$</td>
<td>$2.2 \times 10^4$</td>
<td>$4.2 \times 10^5$</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>$2.7 \times 10^5$</td>
<td>$7.5 \times 10^4$</td>
<td>$3.2 \times 10^5$</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>$6.1 \times 10^5$</td>
<td>$9.5 \times 10^5$</td>
<td>$9.5 \times 10^5$</td>
</tr>
<tr>
<td>Esherichia spp.</td>
<td>$3.8 \times 10^4$</td>
<td>-</td>
<td>$3.2 \times 10^5$</td>
</tr>
</tbody>
</table>

Table 3. Average values (cfu g$^{-1}$) on identification results of microorganism’s species

<table>
<thead>
<tr>
<th>Food category/ name of investigated parameters</th>
<th>df*</th>
<th>F**</th>
<th>Sig.***</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>0.541</td>
<td>0.464</td>
</tr>
<tr>
<td>lg(ACC) between groups</td>
<td>1</td>
<td>0.177</td>
<td>0.675</td>
</tr>
<tr>
<td>TC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>0.280</td>
<td>0.598</td>
</tr>
<tr>
<td>lg(TC) between groups</td>
<td>1</td>
<td>3.981</td>
<td>0.049</td>
</tr>
<tr>
<td>ACC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>5.881</td>
<td>0.018</td>
</tr>
<tr>
<td>lg(ACC) between groups</td>
<td>1</td>
<td>42.153</td>
<td>0.000</td>
</tr>
<tr>
<td>TC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>4.329</td>
<td>0.041</td>
</tr>
<tr>
<td>lg(TC) between groups</td>
<td>1</td>
<td>28.943</td>
<td>0.000</td>
</tr>
<tr>
<td>ACC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>3.206</td>
<td>0.077</td>
</tr>
<tr>
<td>lg(ACC) between groups</td>
<td>1</td>
<td>1.402</td>
<td>0.240</td>
</tr>
<tr>
<td>TC (cfu g$^{-1}$) between groups</td>
<td>1</td>
<td>5.175</td>
<td>0.025</td>
</tr>
<tr>
<td>lg(TC) between groups</td>
<td>1</td>
<td>15.975</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 4. Comparison of p-values between samples from traditional and non-traditional meals (ANOVA analysis)

Notes: * freedom grade count; ** Fisher’s value; *** p-value
more often were identified Enterobacter spp. (36 %), Klebsiella spp. (28 %), Pantoaea spp. (24 %) and Bacillus spp. (16 %).

The high levels of potentially hazardous microorganisms in investigated RTE samples showed really possibility for consumers to become ill via meals from unhygienic catering establishments.

The recommendations for FVS and background analysis for hereafter study stages during this study were prepared.

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