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Full Length Research Paper

Determination of typical house flora during production process of the Petrovac Sausage (*Petrovská klobása*)

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In the meat sector, different crises, but also the recurring food-poisoning cases, have undermined public confidence in intensive or industrial meat producing systems. Consumers are, therefore, turning to traditional products. Traditional fermented dry sausages account for a significant part in such a domain. Traditional dry sausages rely on natural contamination by environmental flora. This microbiota is usually referred to as "house flora". This paper reviews the diversity of microbiota in small-scale processing units, during production process of the traditional fermented dry sausages *Petrovac sausage*. A total of 62 samples from two households in Vojvodina, Serbia were tested. Testing comprised microbiological, immunoenzimatic (Vidas, *L. monocytogenes* Xpress (LMX), bioMérieux) and molecular tests (PCR). The presence of aerobic bacteria, *Escherichia coli*, *Enteroccocus* spp., *Staphylococcus aureus*, Aerobic spore-forming bacteria, *Enterobacteriaceae* and *Listeria* spp. was detected. Regardless of the microorganisms, knives had the lowest contamination level (<2 logcfu/cm^2), while the saw after cutting had the highest ones (>8 logcfu/cm^2). *Listeria monocytogenes* and *Staphylococcus aureus* were detected in 2.77%, while *E. coli* was enumerated in 6.7%. Presence of *Listeria monocytogenes* was detected in swabs from the drain (2.28±0.02 log$_{10}$CFU/cm^2), the mincing (2.02±0.46 log$_{10}$CFU/cm^2) and stuffing (2.30±0 log$_{10}$CFU/cm^2) machines. The knowledge is crucial for the improvement of hygiene control system in traditional meat processing industries.

Key words: *Petrovac sausage*, house flora, *Listeria monocytogenes*, critical points.

INTRODUCTION

The *Petrovac sausage* (Petrovská klobása), is a traditional and autochthonous fermented pork meat product, which is a part of gastronomic heritage of Slovaks in Vojvodina, and which is produced in a traditional way in rural households in the Municipality of Bački Petrovac. In rural households, this sausage is made by the end of November and during December. The *Petrovac sausage* is made by mixing partly cooled (cca 4 h p.m) or cold (cca 24 h p.m) medium chopped lean pork and fat (up to 10 mm) with addition of powdered red hot spicy paprika, salt, crushed garlic, caraway and sugar. A well-mixed filling, which is prepared within 15-30 minutes by using a unique technique of manual mixing with kneading and overturning, is stuffed into natural casings consisting of the rear part of pig intestines (rectum), forming units 35-45 cm long and 4.5-5.0 cm in diameter. After stuffing, the sausages are left to drain for a while and then they are smoked by a cold process for about 10-15 days with pauses, using specific kinds of wood (cherry wood in

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particular). When a smoking process is finished, the sausage is kept in a dry and well ventilated place to dry and ripen, until it achieves an optimum quality, which takes about four months (Tasić, 2012). The *Petrovac sausage* (Petrovská klobása) is a product with a protected designation of its geographical origin, under number 44, based on the order issued by the Republic Bureau for Intellectual Property, number 9652/06 G-03/06, on 21/05/2007. Due to the above said, and in order to achieve a recognizable product of standardized supreme quality which will be continually produced in the controlled conditions to be sold on the domestic and world markets, the aim of this study was to determine the parameters of typical house flora during the production process of the *Petrovac sausage*, which is crucial because of the safety (pathogenic flora), acceptability (spoilage flora) and sensorial quality (technological flora) (Leroy et al., 2010).

MATERIALS AND METHODS

Samples

Test samples were collected from two village households (A and B) at Bački Petrovac, where the preparation of *Petrovac sausage* samples was performed in a traditional manner. Testing included examination of 62 samples, such as: Smears of workers' hands (n=11), smears of working surfaces (n=1), smears of equipment before beginning the operation (n=9), smears of equipment after the operation (n=9), other smears from the working area - wall, drain (n=7), smears of pig halves (n=3), samples of nutrients (n=2), samples of water for rinsing the equipment (n=2), samples of spices (n=6), samples of intestines (n=2), samples of meat chunks (n=4), samples of the filling (n=2) and samples of sausages after the drying process (n=4). All samples were kept refrigerated and analyzed within 2 h.

Microbiological tests

Each sample was tested on the presence of the following bacteria: (1) total viable count (ISO 4833), Plate Count Agar -PCA, Oxoid incubated at 30°C for 72h; (2) total count of bacteria of the *Micrococaceae* family, Manitol salt phenol - red agar, Oxoid, incubated at 30°C for 72 h; (3) total count of Enterobacteriaceae (ISO 21528-2), Violet Red Bile agar with glucose - VRBG, Oxoid, incubated at 30°C for 72 h; (4) total count of β - glucuronidase positive *E. coli* (ISO 16649-2), Tripton Bile x Glucuronide agar (TBX), Oxoid, incubated at 44°C for 24 h; (5) Enterococcus on Bile esculin azide agar, Biokar diagnostics, incubated at 37°C for 48 h; (6) total count of coagulase positive staphylococci (ISO 6888 - 1), Baird Parker, Oxoid, incubated at 37°C for 24 h; (7) *Pseudomonas* spp., on Pseudomonas Selective Agar - Cetrimide Agar, Merck, incubated at 35°C for 48 h; (8) A total count of aerobic spore-forming bacteria was performed in accordance with the Rule Book on Methods of Doing Microbiological Analyses and Superanalyses of Foods (Off. Gazette of SFRJ, No. 25/80); (9) total count of sulphate-reducing bacteria which grow in anaerobic conditions (ISO 15213), Iron Sulfite Agar, Oxoid, incubated at 37°C for 48 h; (10) total count of *Clostridium perfringens (* ISO 7937), Sulfite cycloserine Agar, Oxoid, incubated at 37°C for 20 h; (11) total count of *Salmonella* spp. (ISO 6579), on modified Rappaport Vasilidis Soft Agar incubated at 42°C for 24 h, Rambach, Merck, incubated at 37°C for 24 h; (12) determination of the presence of lactic acid bacteria in samples of chunk meat and filling (ISO 15241), Man - Ragosa Sharpe (MRS), incubated at 30°C for 48-72 h Merck, Darmstad, Germany (13) presence and total count of *Listeria monocytogenes* **(**ISO 11290-1, ISO 11290-2), ALOA, Merck. Tests were done in three repeats.

Immunoenzimatic tests

For detection of *Listeria monocytogenes*, a Vidas was used, *L. monocytogenes* Xpress (LMX), bioMérieux. For food samples, 25 g of sample (analytical unit) was aseptically added to 225 mL of LX broth in a stomacher bag. For environmental samples, for each swab, 10 mL of LX broth was aseptically added for each swab. Incubation period was 30 ± 1°C for 22 - 24 h for food samples or 24 - 26 h for environmental samples.

After a specific period of incubation, about 1- 2 ml broth was removed into a sterile test-tube, which was then 5 ± 1 min heated at 95 to 100°C. The tube was cooled down and 250 µl of the enriched sample was taken to test. All positive results obtained were confirmed by the reference ISO 11290 method or by using the ALOA chromogenic agar.

Molecular tests

A PCR analysis was performed to confirm *Listeria* spp. colonies. DNA was extracted using the DNeasy Tissue kit (Qiagen GmbH, Germany) according to the manufacturer's protocol for Gram-positive bacteria). PCR was performed in a final volume of 50 µl containing 1xPCR buffer (10xPCR buffer: 500 mM KCl, 100 mM Tris-HCl, 0.8% Nonidet P40), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 2.5 µM of each primer, 1 U of *Taq* polymerase (Fermentas UAB, Lithuania) and 0.1-1 µg of DNA template. The samples were amplified in a DNA thermal cycler (Flexigene, Techne, UK) with primers complementary to the *iap* gene for 5 min at 95°C; 35 cycles of 1 min at 95°C, 2 min at 36°C and 3 min at 72°C; and, finally, 7 min at 72°C (Cocolin et al., 2002). Sequence of primers is shown in Table 1. All PCR products were analyzed by agarose gel electrophoresis on 2% (wt/vol) agarose gels in a 1xTBE buffer (10xTBE: 89 mM Tris, 89 mM boric acid, 2 mM EDTA) (Fermentas). All PCR products were run next to the DNA molecular standards "MassRulerTM DNA Ladder" (Fermentas) and „GeneRulerTM DNA Ladder Mix" (Fermentas).

Statistical analysis was performed on the data using Statistica 7.1.

RESULTS

Results of testing are shown in Tables 1, 2, 3, 4, 5, 6, 7 and Figure 1. Our results review the diversity of microbiota, both in the environment and on the equipment in village households A and B (Tables 2, 3, 4 and 5). The environment of processing units was colonized at variable levels by resident spoilage and technological microbiota, with sporadic contamination by pathogenic mycobiota. In the households A and B (Tables 2, 3, 4 and 5) the presence of aerobic bacteria, *E. coli*, enterococci, *Staphylococcus aureus*, aerobic spore-forming bacteria, *Enterobacteriaceae* and *Listeria* spp. was detected (Figure 1). In household A (Table 2), the aerobic bacteria counts ranged from 1.26±0.17 $\log_{10}$CFU/cm^2 (knife) to 8.04±0.91 $\log_{10}$CFU/cm^2 (saw after cutting). *E. coli* was present in two samples (saw after cutting and table), while enterococci were found in all experimental samples between 2±0 $\log_{10}$CFU/cm^2

Table 1. Sequence of primers used in the working process.

Designation of primer	Sequence of primer	Region surrounded	Expected length of PCR product
List-univ. 1‡	5′-GCCAGCGGCCCGGCGCGGGC CCGGCGGGGGCCGCGGCATGTC ATGGAATAA-3′	*iap*	600 - 610 bp[1] 472 bp[2]
List-univ. 2	5′-GCTTTTCCAAGGTGTTTTT-3′		457 bp[3]

‡ Length of fragment depends on the type of bacteria of *Listeria* strain. [1]Expected lengths of PCR product for *L. ivanovii*, *L. seeligeri* and *L. Welshimeri.* [2]Expected length of PCR product for *L. Monocytogenes.* [3]Expected lengths of PCR products for *L. Innocua.*

Table 2. Microbiological contamination of swabs taken from the working surfaces, machines, tools and workers' hands in the household A during the meat production process (MS±Sd, $log_{10}CFU/cm^2$).

Type of bacteria	Workers' hands	Workers' hands after slaughtering	Saw	Saw after cutting	Knife	Knife after cutting	Table	Wall
Aerobic bacteria	4.13±0.16	6.09±0.52	7±0	8.04±0.91	1.26±0.17	1.33±0.17	6.83±0.02	7±0
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	3.11±0.16	ND	ND	1.98±003	ND
Enterococcus	2±0	5.67±0.06	3.04±0.07	3.35±0.31	2.13±0.08	4.74±0.04	5.04±0.04	3.94±0.03
Staphylococcusa ureus	2.24±0.21	ND	ND	ND	ND	ND	ND	ND
Pseudomonas	ND	ND	ND	ND	ND	ND	ND	ND
Aerobic spore-forming bacteria-AES	ND	ND	ND	2.19±0.07	ND	1.89±0.20	2±0	ND
Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriace	ND	ND	ND	5.04±0.4	ND	3.8±0.29	2.67±0.31	ND
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND

ND, Presence not detected.

(workers hands) and 5.67±0.06 $log_{10}CFU/cm^2$. *Staphylococcus aureus* was found in only one sample (workers hands). Aerobic spore forming bacteria were detected in three samples, with contamination around 2 $log_{10}CFU/cm^2$. *Enterobacteriaceae* had total counts between 2.67±0.31 $log_{10}CFU/cm^2$ (table) and saw after cutting (5.04±0.4 $log_{10}CFU/cm^2$). Other groups of bacteria were not detected. Household B (Table 3) defined similar situation with regard to the presence of microorganisms (aerobic bacteria, *E. coli*, enterococci, aerobic spore-forming bacteria, *Enterobacteriaceae)*. The working surfaces, machines, tools and worker's hands had total aerobic counts between 2.57±0.24 $log_{10}CFU/cm^2$ (chopper) and 7.19±0.15 $log_{10}CFU/cm^2$. For *E. coli*, contamination level was 1.87±00 log $log_{10}CFU/cm^2$, 3.41±0.23 $log_{10}CFU/cm^2$, 3±0 $log_{10}CFU/cm^2$, respectively. *Enterobacteriaceae* were found in six samples, with maximum of 5.73±0.2 $log_{10}CFU/cm^2$ (knife after cutting). The presence of *Listeria monocytogenes* was detected in swabs from the drain 2.28±0.2 $log_{10}CFU/cm^2$ (Table 3). In households A and B, during preparation of the filling (Tables 4 and 5), the presence of *Listeria monocytogenes* was detected in swabs from the stuffing (2.3 ±0 $log_{10}CFU/cm^2$) and mincing machine (2.02±0.46 $log_{10}CFU/cm^2$). As regards the raw materials, filling and sausage after drying process (Table 6), the presence of aerobic bacteria, micrococci, enterococci, *Enterobacteriaceae, E. coli*, coagulase positive staphylococci, *Listeria monocytogenes* and Lactic Acid Bacteria was detected while other groups of bacteria were not detected. *L. monocytogenes* was detected *in* sausage filling A (2.075 ±0.07 $log_{10}CFU/cm^{2)}$ and sausage filling B (2.085 ±0.08 $log_{10}CFU/cm^2$).

DISCUSSION

Many authors support the belief that the microorganisms present in traditional sausages are derived from the raw materials or from the manufacturing (Talon et al., 2007).

Table 3. Microbiological contamination of swabs taken from the working surfaces, machines, tools and workers' hands in the household B during the meat production process (MS±Sd, $log_{10}CFU/cm^2$).

Type of bacteria	Workers' hands	Workers' hands	Workers' hands	Saw	Saw after cutting	Knife	Knife after cutting	Chopper	Chopper after cutting	Apron	Drain
Aerobic bacteria	3.6±0.53	4.05±1.69	3.72±0.09	5.83±0.56	6.29±0.03	5.58±0.17	6.1±0.35	2.57±0.24	6.44±0.17	6.46±0.19	7.19±0.15
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	3.41±0.36	ND	1.87±0	ND	3.41±0.23	ND	3±0
Enterococcus	ND	ND	ND	4.28±0.25	4.45±0.08	ND	3.66±0.16	ND	4.39±0.05	ND	3.33±0.28
S. aureus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aerobic spore-forming bacteria-AES	ND	ND	ND	2.19±0.27	ND	<1	1.2±0	<1	ND	ND	2.2±0
Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriacea	<1	ND	ND	3.52±0.17	5.07±0.5	ND	5.73±0.2	ND	4.64±0.06	ND	2.37±0
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L.monocytogenes.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.28±0.02

ND- presence not detected

Table 4. Microbiological contamination of swabs taken from the working surfaces, machines, tools and workers' hands in the household A during preparation of the filling (MS±Sd, $log_{10}CFU/cm^2$).

Type of bacteria	Mincing machine (beginning)	Mincing machine (operation)	Stuffing machine (beginning)	Stuffing machine (operation)	Casing	Casing with the filling	Workers' hands during grinding	Workers' hands with spices	Drain
Aerobic bacteria	4.83±0.26	6.64±0.05	3.23±0.06	6.77±0.07	6.75±0.13	6.75±0.13	6.69±0.05	6.94±0.1	6.84±0.06
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	1.97±0.03	ND	ND	ND	2±0	ND
Enterococcus	ND	5.27±0.25	ND	4.52±0.06	4.03±0.05	4.89±0.06	4.72±0.05	5±0	3.28±0.12
Staphylococcus aureus	ND	2±0	ND	ND	ND	ND	3.31±0.21	3±0	ND
Pseudomonas	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aerobic spore-forming bacteria	ND	ND	ND	<1	ND	<1	ND	ND	<1

Table 4. Contd.

Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	ND	ND	ND	1.32±0. 4	ND	ND	ND	ND	1.33±0.5
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	2. 3±0	ND	ND	ND	ND	ND

ND- presence not detected

Table 5. Microbiological contamination of swabs taken from the working surfaces, machines, tools and workers' hands in the household B during preparation of the filling (MS±Sd, $log_{10}CFU/cm^2$).

Type of bacteria	Mincing machine (beginning)	Mincing machine (operation)	Stuffing machine (beginning)	Stuffing machine (operation)	Workers' handsafter cutting meat	Workers' hands after cutting meat	Workers' hands after cutting meat	Workers' hands after mixing the filling	Drain
Aerobic bacteria	3.18±0.14	6.56±0.08	2.21±0.02	4.21±0.26	6.21±0.62	6.1±0.19	5.2±0.17	6.75±0.12	7.19±0.15
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	ND	ND	3±0
Enterococcus	ND	3.33±0.24	ND	3.57±0.27	ND	4.3±0.11	4.14±0.36	4.3±0.3	3.33±0.28
Staphylococcus aureus	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aerobic spore-forming bacteria	ND	ND	ND	3.16±0.13	ND	ND	ND	<1	2.15±0
Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	ND	2.33±0.58	ND	ND	2.33±0	2.33±0	ND	2.11±0.58	2.37±0
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	2.02±0.46	ND	ND	ND	ND	ND	ND	ND

ND- presence not detected.

Table 6. Microbological contamination of samples of nutrients, water for equipment rinsing, swabs from pig bodies, chunk meat, spices, intestines, filling and sausages after a drying process *(*MS±Sd, $log_{10}CFU/cm^2$*).*

Sample	Aerobic bacteria	*Micrococcaceae*	*Enterococcus*	*Enterobacteriaceae*	*E.coli*	AES	SA	PS	SRC	CPS	LM	LAB
Nutrient A	5.42±0.52	3.45±0.78	2.77±0.68	3.59±0.59	ND	ND	ND	ND	ND	ND	ND	-
Nutrient B	5.78±0.02	3.69±0.32	3.41±0.1	4.55±0.1	ND	ND	ND	ND	ND	ND	ND	-
Water A	5.12±0.41	ND	ND	4.34±0.12	ND	-	-	-	-	-	-	-
Water B	7.26±0.58	ND	ND	6.87±0.24	2.18±0.16	-	-	-	-	-	-	-

Table 6. Contd.

Body A	5.46±0.3	3.66±0.35	4.43±0.68	2.08±0.33	ND	ND	ND	ND	ND	ND	ND	-
Body B1	5.37±0.24	3.59±0.35	3.9±0.64	2.33±0.9	<2	ND	ND	ND	ND	ND	ND	-
Body B2	4.22±0.2	3.12±0.67	4.06±0.58	3.35±0.08	<2	ND	ND	ND	ND	ND	ND	-
KM A1	3.91±0.79	3.66±0.04	2.16±0.28	ND	ND	ND	ND	ND	ND	ND	ND	2.014±0.04
KM A2	3.51±0.17	3.31±0.31	2.11±0.19	ND	ND	ND	ND	ND	ND	ND	ND	2.02±0.05
KM B1	4.37±0.48	3.48±0.28	2.19±0.17	2.5±0.63	ND	ND	ND	ND	ND	ND	ND	2±0
KM B2	4.58±0.1	3.79±0.04	3.28±0.06	2.19±0.17	ND	ND	ND	ND	ND	ND	ND	2±0
Garlic	3.3±0.83	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Garlic	3.74±0.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Caraway	4.95±0.92	2.71±2.36	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Pika	6.15±0.47	1.33±1.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Sugar	1±0.28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Salt	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
Intestine pr.	5.44±0.05	ND	2.77±0.42	3.86±0.77	ND	ND	ND	ND	ND	3.3±0.26	ND	-
Intestine ve.	2.01±0.02	ND	1.92±0.33	ND	ND	ND	ND	ND	ND	ND	ND	-
Filling A	7.03±0.05	4.23±0.48	3.24±0.24	3.89±0.06	ND	ND	ND	ND	ND	ND	2.075±0.07	-
Filling B	7.08±0.07	4.51±0.45	3.2±0.17	4.18±0.13	ND	ND	ND	ND	ND	ND	2. 085±0.08	-
Sausage A1	4.19±0.22	2.75±0.35	<2	ND	ND	ND	ND	ND	ND	ND	ND	5.3±0.15
Sausage A2	4.28±0.23	3.4±0.22	<2	ND	ND	ND	ND	ND	ND	ND	ND	6.4±0.22
Sausage B1	4.5±0.05	2.64±0.1	<2	ND	ND	ND	ND	ND	ND	ND	ND	5.7±0.15
Sausage B2	4.31±0.02	2.62±019	<2	ND	ND	ND	ND	ND	ND	ND	ND	6.2±0.30

A1, pork chunk meat A (sample 1); MA2, pork chunk meat A (sample 2); MB1, pork chunk meat B1; MB2, pork chunk meat B2; NA, pork meat filling A; NA, pork meat filling B1 and B2; ND, presence not detected;, not a subject of testing; AES, Aerobic spore,forming bacteria; SA, *Salmonella* spp.; PS, *Pseudomonas* spp; SRC, Sulphite, reducing clostridia; CPS, Coagulase positive, staphylococci; LM, *Listeria monocytogenes*; LAB, Lactic Acid Bacteria.

Table 7. Presence of *L. monocytogenes* in contaminated samples by Vidas.

Sample	RFV value
Drain	12237
Stuffing machine (operation)	12296
Mincing machine (operation)	11740
Filling A	10721
Filling B	11276

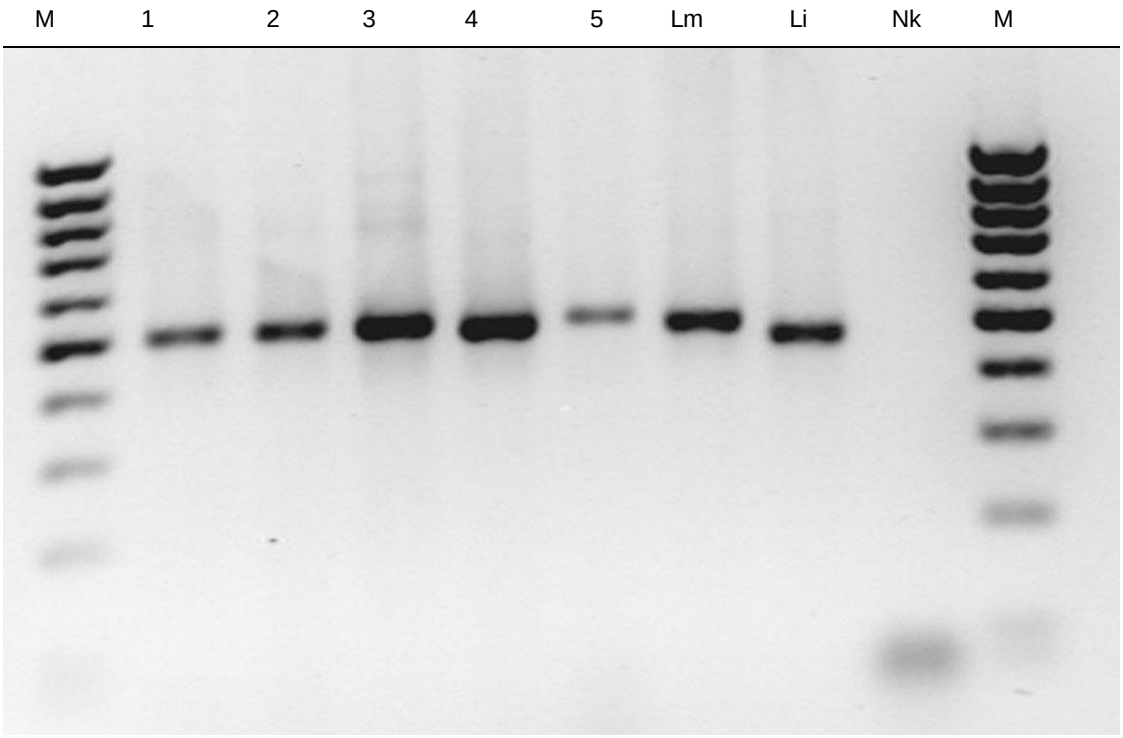

Figure 1. Agarose gel electrophoresis of the PCR products obtained by using List-univ. 1/List-univ; 2 primers from samples obtained during food processing establishment: Lane M, MassRuler^{TM} DNA Ladder; lane 1, sample from drain; lane 2, sample from stuffing machine (operation); lane 3, sample from grinding machine (operation); lane 4, sample from filling A; lane 5, sample from Filling B, *Lm* - *L. monocytogenes* 4b ATCC 19115, *Li* - *L. innocua* ATCC 33090, *"Nk"* - negative control, *M* - MassRuler^{TM} DNA Ladder.

This microbiota is usually referred to as “house flora”. If the microbiota isolated from traditional sausages is well described, the resident microbiota in the environment of the processing unit is still poorly known. The presence of aerobic bacteria,, enterobacteria, enterococci and presence of *L. monocytogenes* in fillings A and B surely resulted from cross contamination either with workings surfaces or after the meat mincing and mixing with spices, that is as a consequence of the specific filling preparation technique by manual mixing on the wooden table for ca. 15-30 min (Ikonić et al., 2010). Generally, *L. monocytogenes* and *S. aureus* were detected in 2.77%, while *E. coli* was enumerated in 6.7%. Sausage samples at the end of the production cycle (270.day) were safe with presence of bacteria populations from the working environment, such as: aerobic bacteria, *Micrococcaceae, Lactic acid bacteria* and *Enterococcus*. *L. monocytogenes* was sometimes present in environmental swabs and not detected by the end of the drying process. The results are in accordance with the results obtained by Lebert et al. (2007). Pathogen microorganisms were detected in 56.64% of the samples. Several critical points were identified such as the drain, saws, workers’ hands, mincing and stuffing machines. The current study revealed that the majority of the sampling sites (control point) tested were (2 to 6 log cfu/cm^2) contaminated by spoilage flora (*Enterobacteriaceae*) with knives and saws, water for rinsing the equipment (*E. coli*), mincing machines (*Listeria monocytogenes*), workers’ hands (*Staph. aureus, E. coli*), a table, which surely indicates to an inappropriate slaughtering process, and to a low level of personal hygiene. Detection of nonpathogenic *Listeria* spp. can be considered as a useful indicator of a deterioration in hygiene or process conditions during food production, leading to an increased risk of contamination with pathogenic *Listeria* spp. Therefore, the detection of all *Listeria* spp. is necessary when testing food and environmental samples. In fact, unclean, insufficiently or inadequately cleaned pieces of equipment have often been identified as a source of pathogens. The results are unique and crucial for the improvement of hygiene control systems in traditional meat processing units. The data suggested that the improved sanitary practices on food contact surfaces and during the handling of product had reduced the risk of *Listeria* spp. and other pathogens studied.

Conclusion

Based on the test results during the production of the *Petrovac sausage* in the traditional manner, it was found out that the hygienic status of the processing environ-

ment, equipment and raw materials, plays an essential role in the microbial stability and safety of the final products. Therefore, traditional households and the *Petrovac sausage*, at the end of the drying process after 270^{th} day, did not present sanitary risk as no pathogens were not found.

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