Microbial Contamination after Sanitation of Food Contact Surfaces in Dairy and Meat Processing Plants

Jarmila SCHLEGELOVÁ[1], Vladimir BABÁK[1], Martina HOLASOVÁ[1], Lucie KONSTANTINOVÁ[1], Lenka NECIDOVÁ[2], Frantisek ŠIŠÁK[1], Hana VLKOVÁ[1], Petr ROUBAL[3] and Zoran JAGLIC[1]

[1]Veterinary Research Institute, Brno, Czech Republic; [2]Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; [3]MILCOM a.s., Dairy Research Institute, Prague, Czech Republic

Abstract

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The occurrence of *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, *Staphylococcus* spp., *Enterococcus* spp., and *Escherichia coli* in raw food materials, food products, and on food contact surfaces after sanitation was investigated during the period of 2005–2006 in three dairy cattle farms (120 samples), one dairy (124 samples), and two meat processing plants (160 samples). A total of 1409 isolates were identified. The epidemiological characterisation and determination of the virulence factors and antimicrobial resistance were performed on selected isolates. The level of bacterial contamination generally decreased during the production process (the contamination of food products was lower than that of raw material). However, the contamination of food contact surfaces was relatively high even after sanitation. Moreover, specific microbiological profiles were found on the inside equipment surfaces in dairy facilities, where genetically closely related multi-resistant strains persisting in biofilm communities may occur as demonstrated for staphylococci. Although the occurrence of potentially significant pathogens was not high, the microorganisms such as *L. monocytogenes*, *Salmonella* spp., and shiga-toxin positive *E. coli* principally contaminated the meat processing plants. *B. cereus* isolates, among which 76% were positive for diarrhogenic enterotoxin, typically occurred on the inside equipment surfaces and in the heat-treated products.

Keywords: food safety; microbial contamination; bacterial biofilm; resistance; sanitation efficiency

Surviving in the form of microbial communities, known as biofilms, the microorganisms adhering to food contact surfaces of technological equipment can become potential sources of food contamination (Zottola & Sasahara 1994). On the open technological equipment surfaces, the biofilm occurrence on special sites is highly probable. These places can be identified by observation or by examination of surface swabs. However, the identification is difficult on the inside surfaces of the technological equipment (e.g., in dairies; Verran *et al.* 2008). Nevertheless, fitting out the

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equipment with removable stainless steel chips for testing is one of the possible ways to control the inside surfaces (Gunduz & Tuncel 2006).

The biofilm formation in food processing plants is mainly associated with damp surfaces, on which the microorganisms can easily aggregate (Chmielewski & Frank 2003). Some bacteria (such as in genera *Klebsiella, Pseudomonas* and *Staphylococcus*) produce exopolymers that can fix additional microorganisms. Firmly attached to the surface, these can survive in the form of mixed biofilms (Sasahara & Zottola 1993). Both pathogenic and food spoilage microorganisms have been isolated from such bacterial communities. It was also found that *Listeria monocytogenes* and *Enterobacter aerogenes* or bacteria of the genera *Bacillus*, *Streptococcus*, *Staphylococcus*, *Shigella*, *Escherichia*, and *Klebsiella* survived cleaning and disinfection (Austin & Bergeron 1995; Sharma & Anand 2002; Gunduz & Tuncel 2006).

Bacteria which survive in the biofilms on surfaces are much more resistant to biocidal agents than planktonic cells of the same species (Carpentier & Cerf 1993; Campanac *et al.* 2002). Due to this fact, the conventional sanitation and disinfectant agents may fail to kill bacteria under certain conditions (Hodd & Zottola 1997). Moreover, it was found that the cell-to-cell DNA transmission occurs in a micro-community, and the biofilm development can be stimulated by their conjugation mechanism (Molin & Tolker-Nielsen 2003). Biofilms can also provide a place for potential transmission of determinants of virulence and resistance to biocidal agents between microorganisms (Ehlers & Bouwer 1999; Hausner & Wuertz 1999).

Even though the above-mentioned information can be considered serious from the hygienic aspect, there is paucity of the data on microbiology of food contact surfaces on the technological equipment after sanitation in the food processing plants, and on the selection of microorganisms commonly occurring in the food production process. The present study was performed: (1) to describe the ecology of the selected pathogenic and potentially pathogenic microorganisms (*Bacillus cereus*, *Enterococcus* spp., *L. monocytogenes*, *Staphylococcus* spp., *Escherichia coli* and *Salmonella* spp.) in three dairy farms, in one milk processing plant, and in two meat processing plants; (2) to give an estimation of persisting bacterial communities, on the basis of microbiological testing of swabs, repeatedly obtained from the food contact surfaces of technological equipment after sanitation.

MATERIAL AND METHODS

Samples. The samples collected in 2005–2006, their types (raw material, swab, and product), the collection sites (inside or open surfaces of technological equipment) and samples specification details are presented in Table 1. During the monitored period, the samples were collected four times, 4–6 months apart. Four groups of samples were examined: (1) from three dairy cattle farms with average bulk tank milk somatic cell counts of 250–400 thousand cells/ml (group 1); (2) from a dairy plant that processed daily 200 thousand liters of milk per day (group 2); (3) from a meat processing plant that also slaughtered pigs and cattle (group 3); and (4) from a meat processing plant that also slaughtered poultry (group 4). A Hazard Analysis and Critical Control Point (HACCP) system of the production control, including sanitation procedures, was adopted in the dairy and meat processing plants. A sample from the meat processing plants consisted of three concurrently collected raw material pieces or three swabs from carcass. The surface swabs from the food equipment were always taken from the same sites, directly exposed to the processed raw material, and were collected within 2 h after standard sanitation of the equipment. In the poultry processing plant, the swabs were collected on two days: the first sampling performed 1 h after standard sanitation was followed by two additional samplings carried out 10 h and 54 h after sanitation.

The sampling and sample preparation of milk and milk products were in accordance with EN ISO 707:1997 and EN ISO 8261:2002. The sampling and sample preparation of meat and meat products were performed according to ISO 17604:2003 and EN ISO 6887-2:2003. The samples collected using abrasive swabs were immediately shaken in 10 ml of saline containing 0.1% peptone (w/v) and processed so as to obtain analytical samples according to EN ISO 6887-1:1999.

Isolation, identification and typing of microorganisms. A total of 404 analytical samples were processed for the isolation of bacteria belonging to 6 genera: *Bacillus*, *Enterococcus*, *Listeria*, *Staphylococcus*, *Escherichia*, and *Salmonella*. In the respective samples, no more than 5 suspect morphologically different colonies in various genera were examined. Table 2 refers to the media used for the selective cultivations and to the methods and commercial kits used for the identification,

Table 1. Samples collected from three dairy cattle farms, one dairy plant and two different meat processing plants during 2005 to 2006

Group of samples	*n* samples (positive)[a]	Sample specification		
1. Dairy farms[b]	12 (12)	raw milk		bulk tank milk samples
	108 (76)	swab	inside	liners, milk tube, claws, milkline, receiver, delivery line, outflow from filter above heat-exchanger in the milk room and heat-exchanger walls
2. Dairy plant	24 (23)	raw material		raw milk from container and tank, and other devices before pasteurisation in the dairy and non-pasteurized cream
	32 (15)	swab	inside	pasteurisation station, heat-exchanger after pasteurisation, pasteurised milk tank, connecting board placed before the filler, milk filler (tube), filling pipe for pasteurised cream, butter-maker, heat-exchanger in the cheese room and cheese-maker-stirrer
	20 (10)	swab	open	cheese-cutting boards, cutting devices, conveyor belt in the cottage cheese room
	48 (33)	pasteurised products		sterilised milk, acidophilic milk, sour milk products, cream, cottage cheese, butter, cheese before salt addition and cheeses
3. Pig and cattle slaughter house	20 (20)	raw material		carcass swabs, emulsion of comminuted meat for production of sausages
	40 (35)	swab	open	hanger, saw, carcass cutting tables, scalding vat, bowel cutter, sausage stuffers, conveyor belt and transportation cart
	20 (15)	meat products		raw chopped pork intended for sale, heat-treated sausages, frankfurters and bratwurst
4. Poultry slaughter house	16 (16)	raw material		liver, neck skin, emulsion of comminuted meat for poultry sausages
	40 (27)	swab	open	hanger, evisceration device, cutting tables, knives for cutting and deboning, conveyor belts, transportation and side vats
	24 (24)	meat products		sliced raw poultry meat intended for sale, heat-treated poultry sausages, frankfurters and bratwurst
Total	404 (306)			

[a]numbers of samples positive for at least one of the monitored microorganisms are shown in parenthesis; [b]three dairy farms were studied

confirmation, and typing of the respective microorganisms. The isolates were kept at –80°C in tryptose-soya broth (TSB; Oxoid, Cambridge, UK) supplemented with 20% glycerol.

To evaluate whether or not particular microorganisms may persist and spread within the food processing plant, *Staphylococcus aureus* and *S. epidermidis* isolated from food equipment surfaces and milk products in dairy technologies were used as model microorganisms in pulsed-field gel electrophoresis (PFGE) analysis. For PFGE, DNA was digested with *Apa*I (New England BioLabs, Ipswich, USA) and the restriction fragments were separated using the CHEF-DR III System (Bio-Rad, Hercules, USA) at 6 V/cm for 22 h with an initial switch time of 1 s, increasing to 30 seconds. Restriction endonuclease patterns were analysed with the GelCompar software (Applied Maths, Sint-Martens-Latem, Belgium).

The resistance to ampicillin (AMP), ampicillin-sulbactam (AMS), cephalothin (CLT), cephotaxime (CTX), chloramphenicol (CMP), clindamycin (CLI), co-trimoxazole (COT), erythromycin (ERY), gentamicin (GEN), neomycin (NEO), norfloxacin (NOR), oxacillin (OXA), penicillin (PEN), streptomycin (STR), tetracycline (TET), teikoplanin (TEI), and vancomycin (VAN) was determined using standard procedures according to Clinical and Laboratory Standards Institute documents (CLSI 2006a,b), including the recommended reference strains. For the resistance testing using the microdilution method, commercial tests Gram Positive and Gram Negative, intended for veterinary isolates (Trios, Prague, Czech Republic) were applied. Oxoid discs

Table 2. Procedures and methods used for isolation, identification, confirmation a typing of Gram-positive and Gram-negative isolates

Genus (No. of isolates)	Isolation and identification			Typing		
	selective media	identification	confirmation	virulence factors	resistance	epidemiological typing
Bacillus (25)	MYP agar (Oxoid, UK)		*B. cereus gyr*B gen (Yamada *et al.* 1999)	Diarrhogenic enterotoxin (BCET-RPLA KIT; Oxoid, UK)	NT	NT
Enterococcus (770)	Slanetz-Bartley agar (HiMedia, India)	*Enterococcus tuf* gen (Ke *et al.* 1999)	*E. faecalis* and *E. faecium sod*A gen (Jackson *et al.* 2004)	NT	NT	NT
Listeria (9)	EN ISO 11290-1 1/2 Fraser, complete: Fraser, ALOA, Rapid'L. Mono (BIO-RAD, France)	API Listeria (bioMérieux, France)		NT	NT	Listeria Antisera (Denka Seiken, Japan) multiplex PCR (Doumith *et al.* 2004)
Staphylococcus (322)	Baird-Parker and Kranep agar (Merck, Germany)	Staphy 24 test (Pliva-Lachema, Czech Republic)	*S. aureus* SA442 sequence (Martineau *et al.* 1998)	*S. aureus in vitro* biofilm (Arciola *et al.* 2001; Cucarella *et al.* 2001)	microdilution method (CLSI 2006a,b) iMLS (Fiebelkorn *et al.* 2003) *mec*A gene (Sauer *et al.* 2008) *erm*C, *msr*A genes (Lüthje & Schwarz 2006)	PFGE[a] (Pantůček *et al.* 1996)
			S. epidermidis SE705 sequence (Martineau *et al.* 1996)	*S. epidermidis in vitro* biofilm (see above) *ica*AB genes (Frebourg *et al.* 2000)		
Escherichia (281)	Rapid'*E. coli* 2 (BIO-RAD, France)	API 20 E (bioMérieux, France)		*stx*1, *stx*2, *eae*A, *hly*A (Paton & Paton 1998)	microdilution method (CLSI 2006a,b)	O serogroup (Salajka *et al.* 1992)
Salmonella (2)	EN ISO 6579 PPV-MKKTn medium/RVS medium-XLD/ BGA (Oxoid, UK)	API 20 E (bioMérieux, France)	Salmonella LA (Denka Seiken, Japan)	NT	disk diffusion method CLSI 2006a,b)	Salmonella Antisera (Denka Seiken, Japan) phagotyping (HPA Colindale, UK)

[a]performed in *S. aureus* and *S. epidermidis* originating from food equipment surfaces and milk products in dairy technologies; NT – non-tested

(Oxoid, Cambridge, UK) were used for the disc diffusion method.

Total count of microorganisms in surface swabs. The standard plate count (performed according to EN ISO 4833) was an estimate of the total number of aerobic mesophilic microorganisms (TCM) present in an analytical sample that were capable of growing on Plate Count Agar (Merck, Germany) when incubated at 30°C for 48 hours. The counted colonies were expressed as the CFU/100 cm^2 of the food contact surface or as the CFU per equipment if the surface was smaller.

Statistical methods. The two-sided Fisher's test was performed to analyse the results (GraphPrism 5 for Windows; GraphPad Software Inc., San Diego, USA).

RESULTS

Table 3 summarises the prevalence of the monitored microorganisms in various types of samples examined in this study.

Dairy cattle farms (group 1)

Significant pathogens *L. monocytogenes* and *Salmonella* spp. were not found in the dairy cattle farms. The most frequently isolated bacteria were enterococci and staphylococci, which contaminated 100% of the raw milk samples and approximately 40% to 50% of the swab samples. Among staphylococci, *S. epidermidis* was the most prevalent (n = 43), however, a relatively frequent occurrence of *S. aureus* (n = 17) was also recorded (Table 4). Eight biofilm-positive isolates of *S. epidermidis* were identified on the inside surfaces of instrumentation and six of them were of the same PFGE profile. These six isolates (obtained from six different sites of the milking appliance in one farm) were identically multi-resistant carrying both *mec*A and *erm*C genes and expressing iMLS$_B$-phenotype. *E. coli* was less frequently detected and was found mainly in raw milk (75% samples; three isolates were multi-resistant to 4 to 5 antimicrobial agents), whereas the contamination of the inside surfaces was markedly lower (8% swab samples) compared to enterococci and staphylococci. However, the occurrence of *E. coli* was significantly more frequent ($P < 0.01$) on the surface sites with TCM $\geq 10^5$ CFU.

The dairy (group 2)

Unlike the dairy cattle farms, *L. monocytogenes* (serovar 1/2a; PCR group I.1) was found in one sample of raw milk. However, *L. monocytogenes* was not isolated from the surfaces and pasteurised milk products. *Salmonella* spp. was not detected. Similarly to the dairy cattle farms, enterococci and staphylococci dominated (44% and 42% positive samples, respectively) followed by *E. coli* (20% positive samples; two isolates from raw milk were

Table 3. Prevalence of samples positive for the investigated Gram-positive and Gram-negative bacteria

Group	Sample type	Surface	Positive samples for genera											
			Bacillus[a]		*Enterococcus*[b]		*Listeria*		*Staphylococcus*		*Escherichia*		*Salmonella*	
			No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
1. Dairy farms	raw milk		0	0	12	100	0	0	12	100	9	75	0	0
	swab	inside	1(0)	1(0)	55	51	0	0	44	41	9	8	0	0
2. Dairy plant	raw material		0	0	20	83	1	4	16	67	12	50	0	0
	swab	inside	3(2)	9(6)	1	3	0	0	13	41	0	0	0	0
	swab	open	0	0	10	50	0	0	5	25	4	20	0	0
	pasteurised products		7(6)	15(13)	23	48	0	0	18	38	9	19	0	0
3. Pig and cattle slaughter house	raw material		0	0	15	75	4	20	10	50	15	75	1	5
	swab	open	0	0	31	78	2	5	22	55	18	45	0	0
	meat products		4(3)	20(15)	7	35	0	0	11	55	6	0	0	0
4. Poultry slaughter house	raw material		0	0	15	94	0	0	10	63	15	94	0	0
	swab[c]	open	0/0	0/0	19/8	48/20	1/0	3/0	20/14	50/35	26/6	65/15	1/0	3/0
	meat products		10(8)	42(33)	9	38	1	4	20	83	9	38	0	0

[a]in parenthesis, the prevalence of samples positive for diarrhogenic enterotoxin producing *B. cereus* are shown; [b]*E. faecalis* and *E. faecium* contaminated 45% and 7% of the total samples, respectively; [c]numbers shown on the right of the slash sign express the mean frequency of positive samples collected 10 h and 54 h after completing the sanitation, during a production break in the processing plant

Table 4. *Staphylococcus* species in raw material samples (S), swabs from contact surfaces of the technological equipment (T) and final products (F) collected on farms and in food processing plants with different conditions of production

Species of genus *Staphylococcus*	*n* isolates	Processing plants										
		dairy farms		dairy plant			pigs and cattle[a]			poultry[b]		
		S[c]	T	S	T	F	S	T	F	S	T	F
S. auricularis	10	1	1	2	0	0	0	5	1	0	0	0
S. aureus subsp. *aureus*	53	11	6	11	4	5	2	3	0	5	2	4
S. cohnii subsp. *cohnii*	2	0	2	0	0	0	0	0	0	0	0	0
S. caprae	4	1	0	0	0	0	1	1	1	0	0	0
S. epidermidis	91	11	32	0	14	11	2	4	4	0	2	11
S. felis	2	2	0	0	0	0	0	0	0	0	0	0
S. haemolyticus	19	10	1	0	1	2	1	1	1	0	0	0
S. hominis subsp. *hominis*	1	0	1	0	0	0	0	0	0	0	0	0
S. chromogenes	4	1	3	0	0	0	0	0	0	0	0	0
S. lentus	1	1	0	0	0	0	0	0	0	0	0	0
S. piscifermentans	4	3	0	0	0	0	1	0	0	0	0	0
S. saprophyticus subsp. *sapr.*	18	0	7	0	3	0	1	6	1	0	0	0
S. sciuri subsp. *sciuri*	1	0	1	0	0	0	0	0	0	0	0	0
S. simulans	2	0	0	1	0	0	1	0	0	0	0	0
S. capitis subsp. *urealyticus*	3	1	2	0	0	0	0	0	0	0	0	0
S. warneri	44	10	13	1	7	3	2	2	6	0	0	0
S. xylosus	16	3	7	0	0	2	3	1	0	0	0	0
Coagulase-negative	47	5	6	1	0	1	1	0	0	8	16	9
Total isolates	322	12[d]	12	4	5	5	9	8	6	1	2	2

[a]pig and cattle slaughter house; [b]poultry slaughter house; [c]numbers of isolates from specified samples S, T, F; [d]*n* – numbers of species, excepting non-identified coagulase-negative staphylococci

multi-resistant to four antimicrobial agents). Overall, these bacteria were most prevalent in raw milk followed by open surfaces and pasteurised products. Their occurrence in pasteurised products correlated with that on surfaces indicating a secondary contamination during the processing of pasteurised milk. We found that the conveyor belt surface in the cottage cheese processing manufacture was highly contaminated with bacteria (10^3–10^6 CFU) after cleaning. In general, the profile of microorganisms on the inside surfaces differed from that found on the farms. While Gram-positive cocci (mainly staphylococci) were also detected on the inside surfaces, *E. coli* was not isolated from these sites. Furthermore, the contamination with enterococci markedly decreased and TCM values were also lower (on average 10 CFU in the dairy compared to 10^4 CFU on the farms). In contrast, an increased occurrence of *B. cereus* was observed, on the inside surfaces of the plant instruments, which may explain its relatively frequent finding in the pasteurised products (15% of products). Moreover, the majority of *B. cereus* isolates found in the dairy were positive for diarrhogenic enterotoxin (Table 3).

Comparably to the dairy farms, *S. epidermidis* and *S. aureus* belonged to the most prevalent staphylococci (Table 4). Unlike *S. aureus* which prevailed in raw milk, *S. epidermidis* was typically isolated from surfaces, which was also in accordance with the findings on the farms. This indicates that *S. epidermidis* tends to be part of the environmental microflora and a secondary contaminant of the milk products (11 *S. epidermidis* isolates originated from pasteurised products). In three *S. epidermidis* isolates from the inside surfaces, the ability of biofilm formation was confirmed. Two of them, which had been obtained from dif-

ferent sites (the cheese-maker-stirrer and the milk filler tube), and at different time points (five months apart), were found to be closely related as demonstrated by PFGE (94% genetic similarity). Both isolates were identically ERY-resistant (*erm*C gene; $iMLS_B$-resistance) and OXA-resistant (*mec*A gene) but differed in tetracycline resistance (PEN, TET, COT, CLI versus PEN, COT, CLI). In one biofilm-negative *S. epidermidis* isolate from the inside surfaces, multi-resistance to PEN, COT, and ERY ($iMLS_B$; *erm*C gene) was also detected. The genetic relatedness was also recorded in two *S. aureus* isolates found on the inside surfaces of the pasteurisation station and the milk filler tube, respectively. These isolates were of the same PFGE type and showed 96% similarity with the PFGE profile of one isolate from pasteurised milk filled with the device. This demonstrates the persistence of particular subpopulations of *Staphylococcus* spp. and their potential to spread in the dairy plant environment as well as dairy farms.

Pig and cattle slaughter house and meat processing plant (group 3)

Compared to the dairy farms and dairy plant, the occurrence of significant pathogens *L. monocytogenes* and *Salmonella* spp. was higher (Table 3). Four samples of raw materials (20% samples), collected at different times, were contaminated with *L. monocytogenes* of identical serovar and PCR group (4ab; II.1). From the raw material, *Salmonella* Typhimurium phage type DT104 exhibiting the ACSSuT pentaresistance was isolated as well. *L. monocytogenes* (two isolates) was also found on the surfaces (the work-table in the cutting room and the bowel-cutter equipment). These two isolates differed in serovar and PCR group: the first one was 4ab and II.1, which was in agreement with the isolates obtained from raw material, and the second was 1/2a and I.1. Neither *L. monocytogenes* nor *Salmonella* spp. was found in the meat products.

In general, no marked differences were observed between the occurrence of the most prevalent bacteria, *Enterococcus* spp., *Staphylococcus* spp., and *E. coli*, which contaminated 66%, 54%, and 49% of the samples, respectively. However, except for staphylococci, the microbial profile of the meat products was inconsistent with the contamination of the raw materials and surfaces (Table 3). The meat products were less frequently contaminated with enterococci and *E. coli* than the raw materials and surfaces (the work-table and the cutter belonged to the most contaminated pieces of equipment with a TCM of 10^6–10^7 CFU). This can be explained by the heat treatment of particular products, in which enterococci (with the exception of one isolate from a sausage) and *E. coli* were not found. However, one isolate of *E. coli* positive for the shiga-toxin gene *stx*2 was identified in raw meat intended for sale. On the other hand, *B. cereus*, which produces heat stable spores, was isolated only from heat treated products (n = 4; 20% samples). Similarly to the dairy plant results, diarrhogenic enterotoxin positive isolates dominated (n = 3; 15% samples).

Ten species of staphylococci, without significant dominance of any of them, were identified (Table 4). The resistance to PEN and/or COT was observed among three *S. aureus* isolates (two of them biofilm positive) collected from various surfaces. No *S. aureus* isolates were found in the meat products. *S. epidermidis* was isolated from 10% of the surface samples as compared to 30% and 27% of the surface samples in the dairy farms and dairy plant, respectively. Neither biofilm production nor multi-resistance was detected in *S. epidermidis* isolates. On the other hand, multi-resistance (to three antimicrobial agents at least) was observed in 14 *E. coli* isolates from raw meat (n = 7) and surfaces (n = 7).

Poultry slaughterhouse and sausages processing plant (group 4)

Unlike with the pig and cattle meat processing plant (group 3), *L. monocytogenes* and *Salmonella* spp. were not found in raw material. However, both these pathogens (*L. monocytogenes*, serovar 1/2a, PCR group I.1 and *Salmonella* Enteritidis, phage type PT21b) contaminated one of the surface samples obtained 1 h after sanitation (Table 3). *L. monocytogenes* (serovar 1/2b; PCR group II.2) was also found in one sample of the raw meat intended for sale. In general, the prevalence of enterococci, staphylococci, and *E. coli*, which contaminated 54%, 63%, and 63% of the samples, respectively, was similar to that observed in group 3 (one *E. coli* isolate from raw meat intended for sale and two from surfaces belonged to the epidemiologically significant O103 serovar). Moreover, a decreased contamination of meat products as compared to raw material and surfaces was again observed with enterococci and

E. coli. On the other hand, staphylococci contaminated meat products even more frequently than raw material (83% vs 63% samples), from which enterococci and *E. coli* were often isolated (94% samples). Similarly to group 3, the occurrence of enterococci and *E. coli* in the heat-treated products was sporadic: with the exception of two isolates, all originated from frozen meats intended for sale. On the contrary, *B. cereus* was only found in the heat-treated products (n = 10; 42% samples) as also observed in group 3. Moreover, the poultry meat products were more frequently contaminated with diarrhogenic enterotoxin positive *B. cereus* as opposed to the other types of food products examined in this study (Table 3).

Among staphylococci, *S. aureus*, *S. epidermidis*, and a prevailing group of coagulase-negative staphylococci (CoNS) were isolated (Table 4). However, the species type of CoNS could not be determined indicating a profile of staphylococci different from those found in large farm animals. While *S. aureus* occurred similarly in all types of samples, *S. epidermidis* unambiguously prevailed in the meat products and was not found in the raw material. This indicates that these isolates could originate from the working personnel since human skin is a natural ecological niche for this microorganism. All but one of *S. epidermidis* isolates were biofilm negative, however, 85% of them were resistant to at least one antimicrobial agent, mainly to PEN (six isolates), ERY and COT (four isolates). Two isolates were also resistant to OXA (*mec*A gene), one of them exhibiting multi-resistance against seven antimicrobial agents. Multi-resistance to at least three antimicrobial agents was also observed in 15 *E. coli* isolates from raw meat (n = 7), surface samples (n = 2) and meat products (n = 6). None of them were of serovar O103.

One hour after sanitation, microbiological contamination of the technological equipment was higher (0; 48; 2; 50; 65, and 2% of positive swabs, respectively, Table 3) in comparison to that found 10 h and 54 h after completing the sanitation procedures (0; 20; 0; 35; 15, and 0% of positive swabs, respectively). The level of the surface contamination decreased most markedly with enterococci and *E. coli* ($P < 0.01$) with the TCM of 10^1 CFU to 10^2 CFU. However, it did not concern the surfaces on which chickens were transported from the cold storage room. The TCM on these surfaces remained high even 54 h after sanitation (10^6–10^7 CFU), which was apparent at the first sight.

DISCUSSION

Microbiological safety of food is closely associated with the quality of raw materials and hygienic practices on farms and in the food processing plants (Verran *et al.* 2008). The results of the present study showed that both raw milk and raw meat material were frequently contaminated with particular bacteria, in some cases up to 100% of the collected samples. These bacteria can directly penetrate into food products or can persist in the food processing environment as secondary contaminants (Ray & Bhunia 2007). Therefore, it is important to identify potential sources of food contamination in order to develop effective sanitation and food processing methods which should prevent the presence of microorganisms in food. An effective cleaning procedure may lead to a significant reduction (of up to 99.8%) of bacteria occurring on the food processing equipment (Dunsmore *et al.* 1981), however, in this study, a relatively high number of the examined surfaces remained contaminated after sanitation. The fact that raw milk is pasteurised prior to its processing indicates that other factors (e.g. personnel) may also play an important role in the contamination of the dairy plant environment (Jaglic *et al.* 2010).

The outbreaks of foodborne listeriosis in humans have been attributed to the consumption of various types of both dairy and meat products (Churchill *et al.* 2006; Ray & Bhunia 2007). However, with the exception of one isolate (*L. monocytogenes* from raw milk) found in this study, the contamination mainly occurred in the meat processing plants (n = 8). This is in agreement with the general findings that raw meat and its products are most frequently contaminated with this pathogen (Farber & Peterkin 1991; Chen *et al.* 2009). Furthermore, *L. monocytogenes* normally occurs in raw material and raw food products while its occurrence in heated products is a consequence of either inadequate heat treatment or re-contamination after heating (Kathariou 2002). In this study, none of the examined heat-treated products was positive for *L. monocytogenes* and none of the *L. monocytogenes* isolates belonged to serovar 4b, which was described as the most frequent causative agent of the invasive form of listeriosis before June, 2007, in Europe (Goulet *et al.* 2008). As well as listeriosis, human salmonellosis belongs to the major foodborne diseases and is mainly associated with the consumption of contaminated meat products followed by dairy products (Ray & Bhunia 2007). Both of the

salmonella isolates detected in this study originated from the meat processing plants and were identified as the serovars Enteritidis and Typhimurium, which had been noted as the most prevalent causative agents of human salmonellosis (Kingsley & Baumler 2002). Furthermore, the phage type DT104 identified in the serovar Typhimurium has also been recognised as a dominant phage type in the inhabitants of the European Union (EFSA 2006).

Frequent occurrence of enterococci and *E. coli* in raw milk, which could be explained by the hygiene practices applied during raw milk handling on farms, was in agreement with our previous study (Schlegelová *et al.* 2002) as well as the results of other authors (Giraffa *et al.* 1997). However, high numbers of raw milk samples positive for staphylococci exceeded our previous findings 2 to 3 times (Schlegelová *et al.* 2002). This could be associated with subclinical mastitis, as indicated by the increased bulk tank milk somatic cell counts on the selected farms. Nevertheless, two hours after sanitation, *E. coli* was rarely found on the inside surfaces of the technological equipment used in dairy technologies, when compared to enterococci and staphylococci. Due to the fact that the inside surfaces remained damp after sanitation, we could rule out the selective loss of viability of *E. coli* cells due to drying (Leistner & Rödel 1975). Accordingly, we could consider this finding as a consequence of selective sanitation, i.e. the used sanitation procedures were more efficient against Gram-negative *E. coli* than against the Gram-positive bacteria investigated. The effect of selective sanitation was reported for acid sanitisers, which decrease pH and negatively influence Gram-negative bacteria (Marriott & Gravani 2006). Such acid sanitisers are still commonly used for the cleaning and disinfection of the enclosed dairy systems in the Czech Republic.

The enclosed systems, which are typical of dairy processing technologies and where organic material providing protection and nutrients to microorganisms can accumulate, pose a specific risk to the production of safe food. The contaminated sites which may occur in such systems are often hard to access and identify (Verran *et al.* 2008). In this study, we noticed that the microbiological profile on the inside surfaces of the equipment used in the dairy processing technologies generally differed from those observed in raw milk and on open surfaces. For example, *B. cereus*, which was not found in raw milk and on open surfaces, was detected in the samples taken from the inside surfaces, which reflects its occurrence in the milk products. Similarly, staphylococci (including *S. aureus*) were also frequently present on these sites. This indicates that specific bacterial subpopulations may occur, persist and spread within the systems as demonstrated for staphylococci by PFGE. Such observations indicate the possibility that these bacteria may survive in biofilm communities (Sharma & Anand 2002), in which increased levels of resistance to disinfectants have been recorded (Brooks & Flint 2008), and where the disinfectant efficacy could be adversely affected by the cell population density (Srinivasan *et al.* 1995). *E. coli*, which was rarely found on the inside surfaces, originated mainly from such highly contaminated surface sites. It also should be mentioned that two of the three *B. cereus* isolates from the inside surfaces were positive for diarrhogenic enterotoxin. Both *B. cereus* and *S. aureus* have been described as causative agents of severe alimentary intoxications (Ray & Bhunia 2007). A multiple human intoxication caused by a toxigenic strain of *S. aureus,* persisting on the inside surfaces of the dairy plant equipment, has been reported in Japan (Asao *et al.* 2003).

Multi-resistance was more frequently observed in staphylococci isolated from the dairy facilities (eight methicillin and $iMLS_B$-resistant *S. epidermidis* isolates were collected from the inside surfaces) compared to the meat processing plants (two methicillin-resistant *S. epidermidis* isolates were collected from poultry meat products). Moreover, all of the biofilm positive isolates from the inside surfaces were multi-resistant. de Araujo *et al.* (2006) already reported an association between the multi-resistance and biofilm production in *S. epidermidis* and speculated that the increased genetic exchange in the biofilm environment may contribute to the multi-resistance phenotype. On the contrary, multi-resistance was more often detected in *E. coli* from the meat processing plants (29 isolates) in comparison to the dairy facilities (5 isolates).

The open surfaces of dairy and meat processing plants were frequently contaminated with enterococci, staphylococci and *E. coli*. However, we cannot unequivocally conclude that the microorganisms detected on the open surfaces after sanitation originated from the biofilm communities. Depending on the hygiene standards applied at the processing facility, there is a high probability of the recontamination of the food equipment by microorganisms

from various sources such as bioaerosol, floors, and waste (Mettler & Carpentier 1998; Verran *et al.* 2008). In regard to the stipulations above, the PFGE analysis did not reveal any significant clonality among staphylococci isolated from the open surfaces in the dairy. We also observed that particular microorganisms survived on the open surfaces for up to 54 h after sanitation. However, their incidence decreased over time which indicates that allowing the surfaces to dry during the production break (> 10 h) may be more efficient for the devitalisation of bacteria than sanitation per se. A rapid reduction in water content negatively affects the growth of microorganisms and their lifespan (McMeekin & Ross 1996). Therefore sanitation, followed by ventilation, could effectively help to minimise the number of bacteria.

Finally, the main purpose of hygiene in the food production is to ensure the safety of the food products. Although in this study the substantial contamination of food products with potentially significant pathogens was not observed, meat products seemed to be more hazardous than milk products. In addition to one isolate of *L. monocytogenes*, one isolate of *stx*2 positive *E. coli* and one isolate of O103 *E. coli* found in raw meat intended for sale, the meat products were generally more frequently (Table 3) contaminated with diarrhogenic enterotoxin positive *B. cereus*, which was typically isolated from the heat-treated products. It is known that heat treatment, which eliminates the other species of microflora, can facilitate spore germination and cell multiplication of *B. cereus* (Lin *et al.* 1998; Ray & Bhunia 2007).

CONCLUSIONS

Although the level of microbial contamination generally decreased during the production process, the contamination of food equipment surfaces remained relatively high even after sanitation. Moreover, the inside surfaces in dairies constitute a specific environment for the survival of particular microorganisms in biofilm communities. Thus, this study reveals that more efficient sanitation programs should be adopted. For example, microbial biofilms should be treated in the early stages of development (at least several times during the food-processing operation) and with disinfectants enriched with suitable hydrolysing enzymes. Allowing the surfaces of open equipment to dry showed to be more efficient for the devitalisation of microorganisms than sanitation *per se*.

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Corresponding author:

Dr. vet. Med. Zoran Jaglic, Ph.D., Výzkumný ústav veterinárního lékařství, v.v.i., Hudcova 70, 621 00 Brno, Česká republika
tel.: + 420 533 331 619, e-mail: jaglic@vri.cz