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Dissemination and characteristics of *Klebsiella* spp. at the processed cheese plant

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Abstract: The genus *Klebsiella* is not generally considered as a major foodborne pathogen and with regard to food production it represents also a hygiene indicator. This study was focused on the monitoring of *Klebsiella* spp. dissemination at the processed cheese plant and determination of virulence determinants and antibiotic resistance of obtained strains. *Klebsiella* spp. were detected in 43% (37/87) of samples: swabs from the cheese processing environment (34/69), personnel (2/3) and raw material (milk powder) from an opened packaging (1/7). All tested samples of final processed cheeses were negative for the presence of *Klebsiella* spp. Obtained strains were identified as *K. oxytoca* (n = 32) and *K. pneumoniae* (n = 23). Typing results enabled to reveal the presence of two predominant PFGE clusters of *K. pneumoniae* and *K. oxytoca* suggesting the occurrence of suspect persistent strains, and spread of *Klebsiella* spp. between the production environment and the personnel. The melting process of processed cheese production was able to eliminate *Klebsiella* spp. in the final products, although some *K. oxytoca* and *K. pneumoniae* strains carried genes of the locus of heat resistance 1 (LHR1), which may lead to their increased heat resistance. *K. pneumoniae* and *K. oxytoca* isolated from the processed cheese plant did not represent any hypervirulent or multidrug-resistant strains which could be a potential threat to public health.

Keywords: *Klebsiella* genus; cheese; hygiene; heat resistance; virulence; antibiotic resistance

Processed cheeses are made in a discontinuous way by heating a mixture of natural cheeses (using the melting temperatures from 90 to 100 °C) with emulsifying salts under lower pressure and constant stirring until a homogeneous mass of desired properties is formed (Buňka et al. 2009). With respect to the safety of processed cheeses and public health, it is necessary to suppress the development of contaminating microflora. Microbiological changes in processed cheeses depend on several factors, including the cheese type used, pH, dry matter, fat, sodium chloride content, concentration and type of emulsifying salts and heating temperature. Other factors affecting the shelf-life and quality of processed cheese are mainly the microbiological quality of the raw materials used, strict hygienic conditions during the manufacturing process as well as the type of packaging materials and storage conditions (Buňková and Buňka 2017).

Coliforms, members of the family *Enterobacteriaceae*, are used as indicator reflecting the general microbiological conditions of food or food processing environments. The genus *Klebsiella* represents coliforms originating not only from faecal contamination, but also from other environmental sources (Martin et al. 2016). Moreover, *Klebsiella* spp. represent also a significant public health problem worldwide, being among the most common causes of both hospital and community-associated infections due to their virulence factors and/or multiresistance to antibiotics. *K. pneumoniae* and, to a lesser extent, *K. oxytoca* are responsible for urinary tract infections, cystitis, pneumonia, surgical wound infections, endocarditis and septicaemia (Podschun and Ullmann

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1998). With regard to antibiotic resistance, *Klebsiella* species show natural resistance to penicillins and some cephalosporins due to the production of chromosomally encoded β-lactamases including penicillinase (Arakawa 2020) and the incidence of *Klebsiella* spp. strains isolated from humans, resistant to extended-spectrum beta-lactamases (ESBL) is on the rise (Dunn et al. 2019).

In general, *K. pneumoniae* can be divided into classical and hypervirulent strains based on their properties. Classical strains of *K. pneumoniae* (c-KP) are commonly associated with infections in immunocompromised patients (Effah et al. 2020). Hypervirulent *K. pneumoniae* (hv-KP) strains were first described in Asia in connection with the occurrence of liver abscesses and meningitis and the importance of this variant is linked to their ability to cause life-threatening infection among young and healthy individuals (Wang et al. 1998). Currently, hv-KP strains have spread to various parts of the world (Bialek-Davenet et al. 2013). The high virulence of hv-KP strains is mainly associated with increased capsule formation and mucoviscosity. In addition to c-KP and hv-KP, a third type of *K. pneumoniae* has been detected in recent years and it has been characterised by a combination of multidrug resistance (MDR) and also hypervirulence (Zhang et al. 2015; Surgers et al. 2016). These MDR-hv-KP are recognised as a significant threat to public health, however characterisation of these strains has not been systematically investigated yet.

K. pneumoniae and *K. oxytoca* are not generally considered as major foodborne pathogens and data on the prevalence of virulent and/or antibiotic resistant strains originating from food and food processing environment are limited. Multidrug-resistant and virulent *Klebsiella* species have been detected in meat, seafood, milk and dairy products (El-Sukhon 2003; Gundogan et al. 2011; Guo et al. 2016). Therefore, it is necessary to describe the occurrence of *Klebsiella* spp. in food and related environment, not only as a hygiene indicator, but also with respect to their virulence properties and antibiotic resistance. The monitored processed cheese plant had a long-term problem with the recurrence of *Klebsiella* spp. on the equipment surfaces. Due to this fact, this study was focused on the presence of the genus *Klebsiella* among other hygienic indicators of *Enterobacteriaceae*, which were sporadically detected during regular environmental hygiene checks in the food processing plant. The aim of this study was to monitor the dissemination of *Klebsiella* spp. in the environment of a cheese production plant and determine the virulence and antibiotic resistance of obtained strains.

MATERIAL AND METHODS

Sample collection. In this study, a total of 87 samples from one processed cheese plant in the Czech Republic were collected on five sampling dates from March to July 2018. Swabs of the processing environment from the whole production process (n = 69), raw material: milk powder (n = 7), final products: processed cheeses of different batches (n = 8) and rinses from hands of the personnel (n = 3) were analysed on the cheese processing plant level (Table 1). Swabs of the processing environment were collected by EZ-PUR polyurethane sponges with handle in 10 mL of buffered peptone water (BioIng, s.r.o., Czech Republic). One subunit of 25 g

Table 1. Examined and *Klebsiella* spp. positive samples from the cheese processing plant (n)

Sample type	Description	Examined	Positive
Raw material	milk powder	7	1
	containers for raw material (cheese, butter)	7	4
	cheese melting machine	1	0
	processed cheese wrapping machines	46	21
Environmental swabs	floors	11	7
	drains	2	2
	ventilation system	1	0
	conveyor	1	0
Personnel	rinses from hands	3	2
Products	final cheeses	8	0
Total		87	37

n – number of samples

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from samples of milk powder and processed cheeses was examined. The rinses from hands of the personnel were performed in disposable gloves with 10 mL of buffered peptone water (Oxoid, UK).

Detection and identification of *Klebsiella* spp. The samples were enriched in buffered peptone water (Oxoid, UK) at 37 °C overnight. The overnight-enriched cultures (a 10 µL loopful) were inoculated onto HiCrome *Klebsiella* Selective Agar (Himedia, India) and incubated at 37 °C overnight. Suspect *Klebsiella* spp. isolates (one to three colonies per sample) were identified using the matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS/MS). Mass spectra were processed by Biotyper (software 3.1; Bruker Daltonic GmbH, Germany) standard procedure with a score above 2.0.

Pulsed field gel electrophoresis (PFGE). The diversity of *K. oxytoca* and *K. pneumoniae* isolates was assessed by a macrorestriction analysis using *Xba*I endonuclease with subsequent pulsed-field gel electrophoresis (PFGE) according to the PulseNet Europe (2017) protocol suitable for *Enterobacteriaceae*. To construct a dendrogram by the unweighted pair group method with arithmetic mean (UPGMA), BioNumerics 5.1 (AppliedMaths, Belgium) was used with the following settings: Dice (Opt. 1.10%) (Tol. 1.0%–1.0%) (H > 0.0% S > 0.0%) [0.0%–100.0%]. Strains (n = 30) selected on the basis of PFGE results representing individual pulsotypes were used for further testing. When the PFGE cluster contained more strains detected in different sampling periods, more than one strain of the same pulsotype was selected for further analysis.

PCR detection of the locus of heat resistance (LHR). Selected strains (n = 30) were screened for the presence of the locus of heat resistance 1 (LHR1) on the basis of PCR detection of three separate regions (Mercer et al. 2015). Screening for the presence of LHR2 was performed by the detection of the *clpK2* gene (Boll et al. 2017). Primer sequences, product sizes and temperatures of annealing are mentioned in Table 2.

Detection of selected capsular serotypes and virulence genes. In *Klebsiella* spp. strains (n = 30), detection of selected capsular serotype-specific genes *magA* and *K2A* (Remya et al. 2018) as markers for K1 and K2 capsular serotypes, virulence genes *rpmA* (Remya et al. 2018), *mrkD* (Boll et al. 2017) and *clbB* gene as a marker of *pks* genomic island (Shimpoh et al. 2017) was carried out by individual polymerase chain reaction (Table 2).

Table 2. Primers used for PCR detection of the locus of heat resistance and selected virulence determinants in *K. pneumoniae* and *K. oxytoca*

PCR	Primer	Sequence (5' → 3')	Size	Ta (°C)	Encoding (region/gene)
1	HR-F1 HS-R1	TTAGGTACCGCTGTCCATTGCCTGA AGACCAATCAGGAAATGCTCTGGACC	~ 1.8 kb	65.2	LHR1 (region A)
2	HR-F2.2 HR-R2	GAGGTACCTGTCTTGCCTGACAACGTTG TATCTAGAATGTCATTTCTATGGAGGCATGAATCG	~ 2.8 kb	62.6	LHR1 (region B)
3	HS-F1 HR-R3	GCAATCCTTTGCCGCAGCTATT GTCAAGCTTCTAGGGCTCGTAGTTCG	~ 2.8 kb	62.6	LHR1 (region C)
4	clpK2_F clpK2_R	ACGATCACTATCGCCAACTG AGTATTTATCCAGCTCGGGCGTG	711 bp	60.0	LHR2 (*clpK2* gene)
5	magA-F magA-R	GGTGCTCTTACATCATTGC GCAATGGCCATTTGCGTTAG	1 283 bp	60.0	K1 serotype (*magA* gene)
6	K2A-F K2A-R	CAACCATGGTGGTCGATTAG TGGTAGCCATATCCCTTTGG	531 bp	60.0	K2 serotype (*K2A* gene)
7	rpmA-F rpmA-R	CATTAGAGTATTGGTTGACAG CTTGCATGAGCCATCTTTCA	461 bp	60.0	mucosity (*rmpA* gene)
8	mrkD_F mrkF_R	TCGAAGGGTCGCGCTTTACG CATGGTAGCGGTAGTGCTGGTGG	365 bp	60.0	adhesin (*mrkD* gene)
9	clbB_F clbB_R	GCGCATCCTCAAGAGTAAATA GCGCTCTATGCTCATCAACC	283 bp	55.0	*pks* island (*clbB* gene)

Ta – temperature of annealing; LHR1 – locus of heat resistance 1; LHR2 – locus of heat resistance 2

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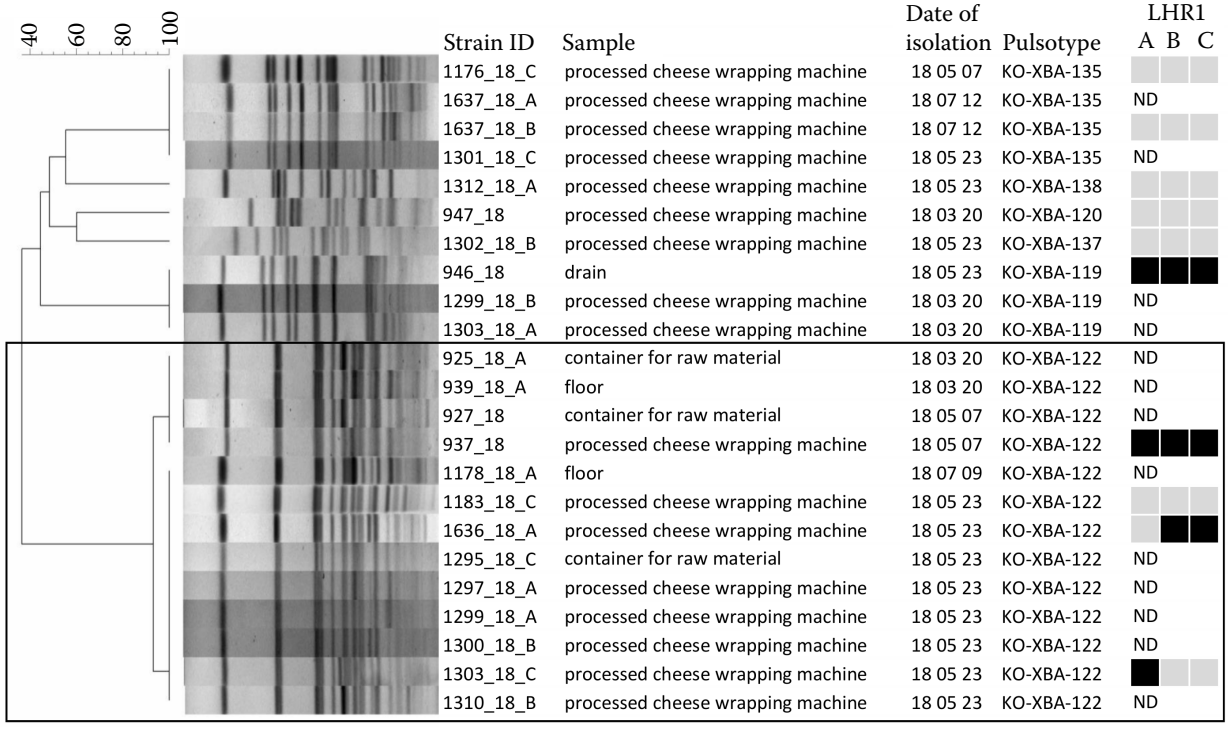
Antimicrobial susceptibility testing. Antibiotic susceptibility of *Klebsiella* spp. strains (n = 30) was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI 2017). *E. coli* strain ATCC 25922 was used as a control. Tested antibiotics included amoxicillin/clavulanic acid (20/10 μg), cefotaxime (30 μg), meropenem (10 μg), chloramphenicol (30 μg), streptomycin (10 μg), kanamycin (30 μg), gentamicin (10 μg), sulphamethoxazol/trimethoprim (1.25/23.75 μg), trimethoprim (5 μg), tetracycline (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and aztreonam (30 μg). Antibiotic disks were obtained from Oxoid Ltd (UK). Strains showing resistance to one or more antimicrobial agents were considered resistant, and strains resistant to three or more groups of antimicrobials were considered multidrug-resistant.

RESULTS AND DISCUSSION

The presence of specific strains of the genus *Klebsiella* in a clean operation during the packaging of processed cheeses could indicate an insufficient level of good hygienic practices, an inefficient separation of workplaces into unclean and clean zones, and an uncontrolled movement of the staff. On the other hand, despite the use of effective disinfectants, it is often difficult to eliminate present bacteria in practice especially from the equipment with difficult-to-access and cleanable surfaces. In this study, the presence of *Klebsiella* spp. was detected in 43% (37/87) of samples. Almost half of the swabs (49%, 34/69) collected from the cheese processing environment was positive for *Klebsiella* spp. In the monitored cheese processing plant, there were several processed cheese wrapping machines for packaging of the processed cheese mass (into sealed aluminium foil). During the whole investigated period, bacteria of *Klebsiella* spp. were detected on each of these machines, especially on the areas under the rotating device, but also on the pistons which ensured the passage of the melt.

Hands of the personnel were reported together with the gastrointestinal tract as principal reservoirs of *Klebsiella* spp. (Podschun and Ullmann 1998). In workers from this cheese processing plant, rinses from three hands of the personnel were also examined for the presence of *Klebsiella* spp. and two of them were found to be positive. Also one positive sample of raw material (milk powder) was detected. This sample was collected from an opened packaging, and thus the cheese processing environment was the prob-



Strain ID	Sample	Date of isolation	Pulsotype	LHR1 A	LHR1 B	LHR1 C
1176_18_C	processed cheese wrapping machine	18 05 07	KO-XBA-135			
1637_18_A	processed cheese wrapping machine	18 07 12	KO-XBA-135	ND		
1637_18_B	processed cheese wrapping machine	18 07 12	KO-XBA-135			
1301_18_C	processed cheese wrapping machine	18 05 23	KO-XBA-135	ND		
1312_18_A	processed cheese wrapping machine	18 05 23	KO-XBA-138			
947_18	processed cheese wrapping machine	18 03 20	KO-XBA-120			
1302_18_B	processed cheese wrapping machine	18 05 23	KO-XBA-137			
946_18	drain	18 05 23	KO-XBA-119	■	■	■
1299_18_B	processed cheese wrapping machine	18 03 20	KO-XBA-119	ND		
1303_18_A	processed cheese wrapping machine	18 03 20	KO-XBA-119	ND		
925_18_A	container for raw material	18 03 20	KO-XBA-122	ND		
939_18_A	floor	18 03 20	KO-XBA-122	ND		
927_18	container for raw material	18 05 07	KO-XBA-122	ND		
937_18	processed cheese wrapping machine	18 05 07	KO-XBA-122	■	■	■
1178_18_A	floor	18 07 09	KO-XBA-122	ND		
1183_18_C	processed cheese wrapping machine	18 05 23	KO-XBA-122			
1636_18_A	processed cheese wrapping machine	18 05 23	KO-XBA-122		■	■
1295_18_C	container for raw material	18 05 23	KO-XBA-122	ND		
1297_18_A	processed cheese wrapping machine	18 05 23	KO-XBA-122	ND		
1299_18_A	processed cheese wrapping machine	18 05 23	KO-XBA-122	ND		
1300_18_B	processed cheese wrapping machine	18 05 23	KO-XBA-122	ND		
1303_18_C	processed cheese wrapping machine	18 05 23	KO-XBA-122	■		
1310_18_B	processed cheese wrapping machine	18 05 23	KO-XBA-122	ND		

Figure 1. Cluster analysis of the pulsed field gel electrophoresis of *K. pneumoniae* XbaI pulsotypes and the presence of the locus of heat resistance 1 (LHR1)

A, B, C – designation of the regions of LHR1; grey square – negative; black square – positive; ND – not detected; black frame – PFGE cluster of suspect persistent strains

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able source of milk powder contamination. *Klebsiella* was not detected in final processed cheeses in any of the samples tested (Table 1). The production process did not contribute to the contamination of the final products with *Klebsiella* spp. It is consistent with the fact that the majority of vegetative forms of microorganisms, including bacteria of the family *Enterobacteriaceae,* are inactivated during the cheese melting process (Buňková and Buňka 2017).

Obtained strains (n = 55) were identified as *K. oxytoca* (n = 32) and *K. pneumoniae* (n = 23) by MALDI-TOF MS/MS. Results of PFGE showed high heterogeneity of obtained strains of *K. oxytoca* (17 different pulsotypes) and *K. pneumoniae* (6 pulsotypes). However, the occurrence of two predominant PFGE clusters was determined in both of these species, which included strains detected at different sampling points of the production environment during the whole monitored period. Most of the *K. pneumoniae* strains (57%, 13/23) belonged to pulsotype designated as KO-XBA-122 (Figure 1). The predominant PFGE cluster of *K. oxytoca* included strains (31%, 10/32) of pulsotypes designated as KO-XBA-132, KO-XBA-132a, KO-XBA-132b and KO-XBA-132c (Figure 2). This fact may indicate the presence of suspect persistent *K. pneumoniae* and *K. oxytoca* strains at the monitored cheese production plant. *K. pneumoniae* and *K. oxytoca* strains of these two dominant PFGE clusters were detected across the whole production environment: from the swabs of processed cheese wrapping machines, on the floor under them, but also in swabs from containers after cleaning or with food raw materials (butter and cheese) (Figure 3). At the same time, the dissemination of some other pulsotypes (*K. pneumoniae*: KO-XBA-119, KO-XBA-135, and *K. oxytoca*: KO-XBA-121, KO-XBA-123, KO-XBA-126, KO-XBA-134) was found out, which indicates the possibility of cross-contamination in the production plant (Figures 1 and 2).

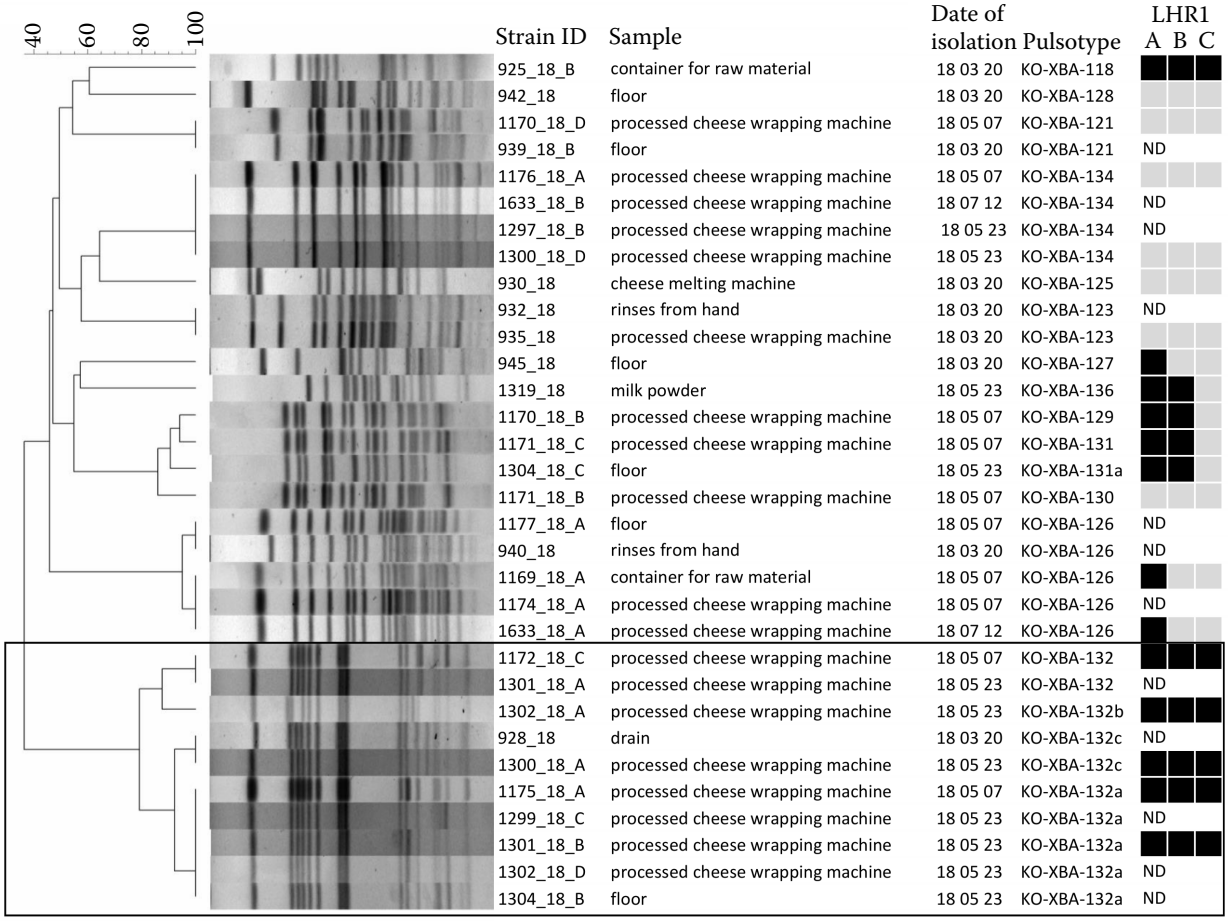

Figure 2. Cluster analysis of the pulsed field gel electrophoresis of *K. oxytoca* XbaI pulsotypes and the presence of the locus of heat resistance 1 (LHR1)

A, B, C – designation of the regions of LHR1; grey square – negative; black square – positive; ND – not detected; black frame – PFGE cluster of suspect persistent strains

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K. oxytoca strains (n = 2) isolated from rinses of hands carried indistinguishable pulsotypes with strains from the environment (Figure 2). The strain of pulsotype KO-XBA-123 was also detected on the piston of one of the processed cheese wrapping machines. *K. oxytoca* strains of pulsotype KO-XBA-126 were detected from the hands of the personnel and at the same time from the swab of the processed cheese wrapping machines, the floor under them, and the container with raw materials (butter and cheese). PFGE results confirmed the spread of *Klebsiella* spp. in the cheese processing plant between the production environment and the staff.

More than half (n = 13, 65%) of the 20 selected strains of *K. oxytoca* representing individual pulsotypes carried at least part of the locus of heat resistance 1 (LHR1). All of them were positive for the region A of LHR1 that carried the *clpK* gene (Figure 2). The *clpK* gene carried on a conjugative plasmid was at first described in heat-resistant *K. pneumoniae* (Bojer et al. 2010). In *Klebsiella* spp. strains the variability of the genetic region surrounding the *clpK* gene or truncated version of the locus decrease heat-resistance in strains (Bojer et al. 2013). In *E. coli*, the presence of truncated LHR1 does not increase heat resistance of strains (Mercer et al. 2015). This fact could lead to different heat resistance phenotypes of the strains carrying truncated LHR1 detected in the processed cheese plant. All tested suspect persistent strains of *K. oxytoca* belonging to PFGE cluster KO-XBA-132 carried a complete LHR1, which supports the hypothesis about their heat resistance.

On the other hand, tested *K. pneumoniae* strains carried genes of LHR1 to a lesser extent (40%, 4/10). Moreover, the variability in the presence of LHR1 was revealed in suspect persistent strains of *K. pneumoniae* pulsotype KO-XBA-122. Only one tested *K. pneumoniae* strain of pulsotype KO-XBA-122 carried the complete genomic island LHR1. The rest of these tested strains were LHR1 negative or positive only partially (Figure 1). This genomic island from diverse *Enterobacteriaceae* exhibits > 99% sequence identity and there is a hypothesis that the species of this family acquired LHR1 by horizontal gene transfer (Mercer et al. 2015), which could explain the diversity of this locus in *K. pneumoniae* belonging to the same PFGE cluster. None of the *K. oxytoca* and *K. pneumoniae* strains carried the *clpK2* gene, marker of a 19-kb genomic island designated as the heat resistance locus LHR2, which was described for the first time on a plasmid located in *E. coli* (Boll et al. 2017). The genotyping results of these strains should be verified in the future study by phenotypes expressed by D- and z-values, which would enable to discuss their resistance to tempera-

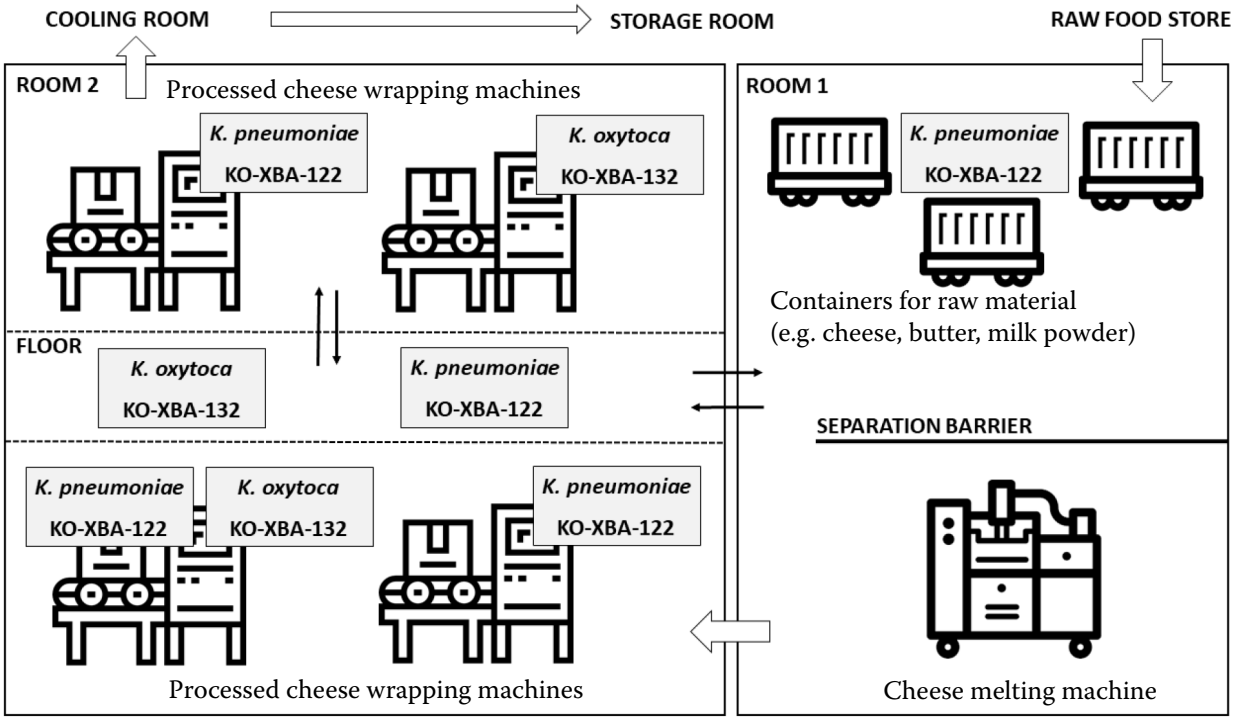

Figure 3. Scheme of the dissemination of suspect persistent *K. pneumoniae* and *K. oxytoca* strains in the processed cheese plant

Open arrow – direction of production; solid arrow – spread of *Klebsiella* spp. at the processed cheese plant

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tures used, for example, during the cleaning process of the technological equipment.

Although some *K. oxytoca* and *K. pneumoniae* strains (Figures 1 and 2) carried a complete LHR1, temperature of about 70 °C during the forming and packaging phase of processed cheese was sufficient to eliminate *Klebsiella* spp. in final products as expected. Only spore-forming bacteria and heat-resistant enterococci survived the cheese-making process and in a small proportion these bacteria caused defects of final products (unpublished data). In *E. coli*, the contribution of LHR1 to resistance to chlorine and oxidative stress was described (Wang et al. 2020). These properties could constitute an advantage for dissemination and persistence of LHR1-positive *Klebsiella* spp. strains in the cheese processing environment.

Out of the 30 tested *K. pneumoniae* and *K. oxytoca* strains representing individual pulsotypes detected in the processed cheese plant all belonged to non-K1 and non-K2 capsular serotypes. Capsule is an important virulence factor protecting bacteria from phagocytosis and inhibition of the host immune response (Ko 2017). Genes of capsular serotypes K1 and K2 have been identified in high frequency in hypervirulent *K. pneumoniae* isolates and co-occur with other virulence encoding genes (Choby et al. 2020). All tested strains of *K. pneumoniae* and *K. oxytoca* were also negative for the presence of *pks* (polyketide synthase) gene cluster necessary for colibactin synthesis and *rpmA* gene, which are associated with the hypervirulence of *K. pneumoniae* (Guo et al. 2017; Choby et al. 2020). Although the studies dealing with *Klebsiella* spp. virulence from cheese production are limited, the siderophore production in *K. pneumoniae* and *K. oxytoca* strains originating from milk and dairy products (ice cream, cheese) has been described (El-Sukhon 2003). The MrkD protein belongs to the type 3 fimbrial adhesin and mediates binding to extracellular matrix proteins such as collagen molecules and thus can facilitate the development of infection (Jagnow and Clegg 2003). Among human clinical strains of *K. pneumoniae* the *mrkD* gene was described as the common virulence factor (Guo et al. 2017; Rastegar et al. 2019). The *mrkD* gene was detected in seven *K. pneumoniae* strains of pulsotypes KO-XBA-122 (n = 4), KO-XBA-135 (n = 2) and KO-XBA-137 (n = 1).

Resistance to antibiotics was found out only in *K. oxytoca* species (Table 3). Resistance to kanamycin was detected in five *K. oxytoca* strains assigned to PFGE cluster KO-XBA-132 and in one strain of pulsotype KO-XBA-118. Two *K. oxytoca* strains of pulsotype KO-XBA-134 were resistant to streptomycin. None of the *Klebsiella* strains was resistant to cefotaxime or meropenem (ESBL and carbanemase producers), no multidrug-resistant strain was found (Table 3). Data concerning antimicrobial resistance in *Klebsiella* spp. originated from cheeses or cheese processing plants are scarce. However, in Turkey ESBL producing

Table 3. Antibiotic agents used and results of susceptibility testing in *K. pneumoniae* (n = 10) and *K. oxytoca* (n = 20)

Antibiotics	Number of strains			
	K. pneumoniae		*K. oxytoca*	
	S	R	S	R
Amoxicillin/clavulanic acid	10	0	20	0
Cefotaxime	10	0	20	0
Meropenem	10	0	20	0
Chloramphenicol	10	0	20	0
Streptomycin	10	0	18	2
Kanamycin	10	0	14	6
Gentamicin	10	0	20	0
Sulphamethoxazol/trimethoprim	10	0	20	0
Trimethoprim	10	0	20	0
Tetracycline	10	0	20	0
Nalidixic acid	10	0	20	0
Ciprofloxacin	10	0	20	0
Aztreonam	10	0	20	0

S – sensitive; R – resistant

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K. pneumoniae (26.3%) and *K. oxytoca* (10.8%) were detected in retail cheeses (Husan and Çadirci 2019). The ESBL producing strains of *Klebsiella* spp. were also described from chicken meat (Gundogan et al. 2011; Guo et al. 2016). Comparison of virulence determinants and antibiotic resistance of *K. pneumoniae* from retail meats and human urinary tract infections suggests that the barriers for transmission between these two sources are low (Davis et al. 2015). In this regard, it is important to monitor the contribution of *Klebsiella* spp. originated from different kinds of foods for human colonisation and infection.

CONCLUSION

Despite the heterogeneity of tested *Klebsiella* spp., the results revealed the occurrence of identical clusters of *K. pneumoniae* and *K. oxytoca* on equipment that either comes or does not come into direct contact with the raw material and final products. The presence of two dominant PFGE clusters of *K. pneumoniae* and *K. oxytoca* collected on different sampling dates indicates the occurrence of suspect persistent strains in the processed cheese plant. Results of this study showed the spread of *K. pneumoniae* and *K. oxytoca* between the production environment and the personnel. This fact could indicate an insufficient level of good hygiene practices, the separation of workplaces or the occurrence of difficult-to-access and cleanable surfaces of processed cheese wrapping machines in the monitored processing plant. Although some *K. oxytoca* and *K. pneumoniae* strains carried the complete locus of heat resistance (LHR1), temperature of about 70 °C during the forming and packaging phase of processed cheese was sufficient to eliminate *Klebsiella* spp. in the final products. *K. pneumoniae* and *K. oxytoca* isolated from the processed cheese plant did not represent any hypervirulent or multidrug-resistant strains which could be a potential threat to public health.

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