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Original article

Antimicrobial resistance of bacterial isolates from sheep and goat cheeses in eastern Slovakia

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Abstract

The aim of this study was to determine prevalence of undesirable bacteria and their antimicrobial profile in samples obtained from a productive farm situated in border region Slanské vrchy (Slovakia), involved in keeping sheep and goats for the purpose of processing raw milk to special products (cheeses). Genus and species identification was carried out by PCR method and MALDI –TOF MS. Isolates thus identified were detected for antimicrobial resistance using the Agar Dilution Method.

Bacteria of *Staphylococcus* spp. exhibited the highest resistance to penicillin (98% isolates). Isolates from the family *Enterobacteriaceae* showed the highest resistance to azithromycin (90%). At the same time, in isolates of *Enterococcus* spp. we detected high resistance to linezolid (100%). Our investigation showed that all tested strains were resistant to more than one antibiotic used in this study.

Key words: antimicrobial resistance, sheep cheese, goat cheese, Staphylococcaceae, Enterobacteriaceae

Introduction

As antibiotic resistance is a constantly increasing problem, collection of information about resistance of the pathogens is very important from the epidemiological point of view. Especially serious risk is associated

with multiresistant micro-organisms and their resistance to more than one antibiotic (Regecová et al. 2014).

Thus, contamination of milk by agents found in common housing environment can be highly relevant. Activity of such micro-organisms can affect health safety of milk and its technological processing. Unde-

sirable microbiota of milk include frequently present enterobacteria of genera *Escherichia* and *Enterobacter* (*Escherichia coli*, *Enterobacter aerogenes*). From among other micro-organisms one should mention in this respect for example *Pseudomonas* spp., *Enterococcus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp. and other (Dudriková 2011).

Enterococci are ubiquitous microorganisms and belong to the part of the normal gastrointestinal microbiota of mammals and other warm-blooded animals, and we can also find them in soil, plants and water. By intestinal or environmental contamination, these microorganisms colonize raw food such as milk and meat, throughout any fermentation process (Ribeiro et al. 2011). As enterococci in food are not always present due to faecal contamination, the legislation in force (European Commission, 2007) sets no limit for enterococcal presence in food. In fact in some kinds of food such as cheeses and fermented meats, enterococci are added during the production process, both to extend their shelf life and to improve their organoleptic properties (Cocolin et al. 2007).

The degree of antibiotic resistance varies among bacteria of the family *Enterobacteriaceae* *Staphylococcaceae*, so their identification at the genus and species level is important.

The available methods of their identification involve polymerase chain reaction and mass spectroscopy MALDI-TOF based on obtaining protein profiles of bacteria (Singhal et al. 2015).

On the basis of the above mentioned arguments the aim of our study was to determine proportional representation – abundance – of micro-organisms using the PCR method and MALDI – TOF mass spectroscopy to identify bacteria isolated from sheep and goat cheese produced on a farm located in border area and, subsequently, determine antimicrobial resistance of bacteria found in these products. The cheeses tested by us belong to the group of so-called RTE (ready-to-eat). We also include bryndza among such products. Bryndza is a typical Slovak cheese made from raw milk with no special starter culture (Vrabec et al. 2015). According to our knowledge, the studies dealing with antimicrobial susceptibility of bacteria isolated from products made from raw milk are scarce and thus our study will contribute to the body of knowledge in this area.

Materials and Methods

Isolation of strains

From May to September 2019, we isolated staphylococci and strains from samples of sheep (n=10) and goat (n=10) cheeses. The cheeses were made from

non-pasteurised milk without adding a cheese starter culture, and were ripened for 30 days. Subsequently, basic suspension and decimal dilutions were prepared from all tested samples according to the STN EN ISO 6887-5 (2010).

Isolates of staphylococci from the examined samples were obtained according to the STN EN ISO 6888-1 (1999), isolates of *Enterobacteriaceae* according to the STN ISO 21528-1 (2019) and enterococci according to Čanigová et al. (2012). Based on their characteristic appearance, typical colonies of individual genera of bacteria were further identified. Plates containing 15 to 150 colonies were used for this purpose. Collected typical colonies were inoculated with a sterile bacterial loop on the surface of Columbia blood agar (Oxoid, United Kingdom) and incubated at 37°C for 24 to 48 hours. After incubation, individual strains were used for identification by PCR and MALDI TOF MS.

Genus identification by PCR method

The total genomic DNA was isolated from staphylococcal strains as described by Hein et al. (2005). The PCR method was used to identify the genera of *Staphylococcus* *Enterococcus* and family *Enterobacteriaceae* according to KE et al. (1999), Nakano et al. (2003) and Strommenger et al. (2003). Each primer was synthesized by Generi Biotech s.r.o. Czech Republic (Table 1).

Reaction mixture in total a volume of 20 µl contained 1 µl genomic DNA, 10 pmol.l⁻¹ of each primer and HotFirepol® Mastermix (Ecoli s.r.o., Slovakia). The amplification was terminated by cooling to 6°C. The PCR protocol was as follows: initial denaturation at 95°C for 12 min, 30 cycles consisting of denaturation at 95°C 20 s, annealing at different temperatures depending on the type of primer used (Table 1), and extension at 72°C for 2 min. Final extension at 72°C for 10 min followed the last cycle. PCR products were separated in a 2% agarose gel stained with Goldview™Nucleic acid stain (Beijing SBS Genetech Co. LTD, China) and visualized using the DNR Bio Imaging system (MiniBIS Pro®, Israel).

The sequences obtained from the studied strains used in this work were submitted to the GenBank-EMBL database. The DNA sequences obtained from fungal strains were searched for homology to those available at the GenBank-EMBL database using the BLAST program (NCBI software package).

Species identification by MALDI-TOF

The species identification of bacteria was subsequently provided with the help of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Table 1. DNA sequences and descriptions of primers used in this study.

Primer name	Primer sequence (5'-3')	Tm (°C)	PCR product	Reference
<i>Staphylococcus</i> spp				
16S1	CAGCTCGTGTCTGAGATGT	55 – 420 bp		Strommenger et al. 2003
16S2	AATCAATTTGTCCCACCTTCG			
<i>Enterococcus</i> spp.				
Ent1	TACTGACAAACCATTTCATGATG	55 – 112 bp		Ke et al. 1999
Ent2	AACTTCGTCACCAACGCGAAC			
Inko1 (internal control)	GGAGGAAGGTGGGGATGACG	55 – 241 bp		Martineau et al. 1996
Inko2 (internal control)	ATGGTGTGACGGGGCGGTGTG			
<i>Enterobacteriaceae</i>				
Ent3	GTTGTAAAGCACTTTCAGTGGTGAGGAAGG	60 – 425 bp		Nakano et al. 2003
Ent4	GCCTCAAGGGCACAACCTCCAAG			

(MALDI-TOF MS; Bruker Daltonics, Fremont, CA, USA) according to the standard sample preparation protocol of manual BRUKER DALTONICS, (2007). The samples were analysed in collaboration with the Institute of Animal Physiology of the Slovak Academy of Sciences in Košice.

Phenotypic assessment of antibiotic sensitivity

The susceptibility of isolated bacterial strains to selected antibiotics was determined by the agar dilution method (ADM) according to the procedure described by CLSI document M100-S28 (2018). ADM is performed on Petri dishes with Müller-Hinton agar (Hi-Media, India) in duplicate. Test plates containing different concentrations of antibiotics were used for the determination of minimum inhibitory concentrations (MICs). Drops of 24-hour bacterial suspensions adjusted to the 0.5 McFarland turbidity standard were placed in parallel on the surface of each test plate. Inoculated plates were then incubated at 37°C for 24 hours. After incubation, the lowest concentrations of antibiotics that inhibited the visible growth of staphylococcal strains were determined. Results were evaluated according to CLSI document M100-S28 (2018).

In determining the MICs plates with final concentration of antibiotics were used on *Staphylococcus* spp.: Penicillin (P) 0.12; 0.25; 0.5 mg l⁻¹; Cefaroline (KF) 0.5; 1.0; 2.0; 4.0; 8.0 mg l⁻¹; Teicoplanin (TEC) 4.0; 8.0; 16.0; 32.0; 64.0 mg l⁻¹; Gentamicin (GN) 2.0; 4.0; 8.0; 16.0; 32.0; 64.0 mg l⁻¹; Erythromycin (E) 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 mg l⁻¹; Tetracycline (TE) 2.0; 4.0; 8.0; 16.0; 32.0 mg l⁻¹; Ofloxacin (OFX) 0.5; 1.0; 2.0; 4.0; 8.0 mg l⁻¹. For *Enterobacteriaceae*: Azithromycin (AZI) 8.0; 16.0; 32.0; 64.0 mg l⁻¹; Doxycycline (DO) 2.0; 4.0; 8.0; 16.0; 32.0 mg l⁻¹; Minocycline (MH) 2.0; 4.0; 8.0; 16.0; 32.0 mg l⁻¹; Ciprofloxacin (CIP) 0.5; 1.0; 2.0;

4.0; 8.0 mg l⁻¹; Levofloxacin (L) 1.0; 2.0; 4.0; 8.0; 16.0 mg l⁻¹; Nitrofurantoin (F) 16.0; 32.0; 64.0; 128.0; 256.0 mg l⁻¹; Fosfomycin (FOT) 32.0; 64.0; 128.0; 256.0 mg l⁻¹; Chloramphenicol (C) 8.0; 16.0; 32.0 mg l⁻¹; Kanamycin (K) 8.0; 16.0; 32.0; 64.0; 128.0 mg l⁻¹. For *Enterococcus* spp.: Vankomycin (VAN) 2.0; 4.0; 8.0; 16.0; 32.0; 64.0 mg l⁻¹; Teicoplanin (TEC) 4.0; 8.0; 16.0; 32.0; 64.0 mg l⁻¹; Erythromycin (E) 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 mg l⁻¹; Doxycycline (DO) 2.0; 4.0; 8.0; 16.0; 32.0 mg l⁻¹; Minocycline (MH) 2.0; 4.0; 8.0; 16.0; 32.0 mg l⁻¹; Ciprofloxacin (CIP) 0.5; 1.0; 2.0; 4.0; 8.0 mg l⁻¹; Levofloxacin (L) 1.0; 2.0; 4.0; 8.0; 16.0 mg l⁻¹; Nitrofurantoin (F) 16.0; 32.0; 64.0; 128.0; 256.0 mg l⁻¹; Rifampicin (RD) 0.5; 1.0; 2.0; 4.0; 8.0 mg l⁻¹; Fosfomycin (FOT) 32.0; 64.0; 128.0; 256.0 mg l⁻¹; Linezolid (LZD) 1.0; 2.0; 4.0; 8.0; 16.0 mg l⁻¹.

Statistical analyses

Statistical analysis was performed using software GraphPad Prism 5.0 (2007). Chi square test (χ^2 test) was used to compare the individual proportions. The dependence of the individual signs was tested at a significance level $\alpha = 0.05$, with critical values = 2.388 for *Staphylococcus* spp., 12.529 for *Enterobacteriaceae* strains and 5.326 for *Enterococcus* spp.

Reference strains

Reference strains of *S. aureus* ATCC 29213, *Enterococcus* (*E.*) *faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 (Czech Collection of Microorganisms, Brno, Czech Republic) were used in this study as a positive control for the agar dilution method and the polymerase chain reaction.

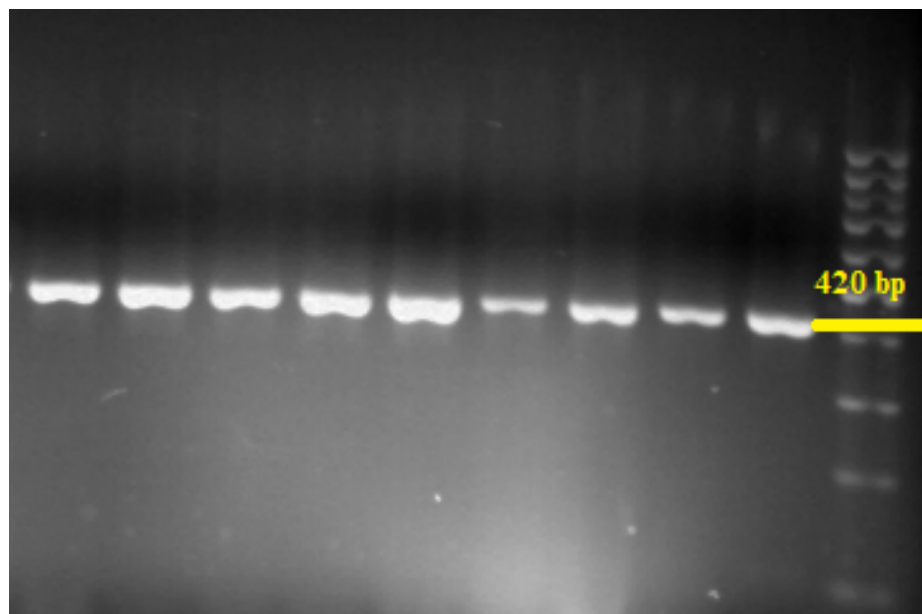


Fig. 1. Identification of isolates of *Staphylococcus* spp. by PCR method.
 L – 100 bp ladder; Lines 1,2,3,4,5,6,7,8,9 – isolates of *Staphylococcus* spp. (420 bp).

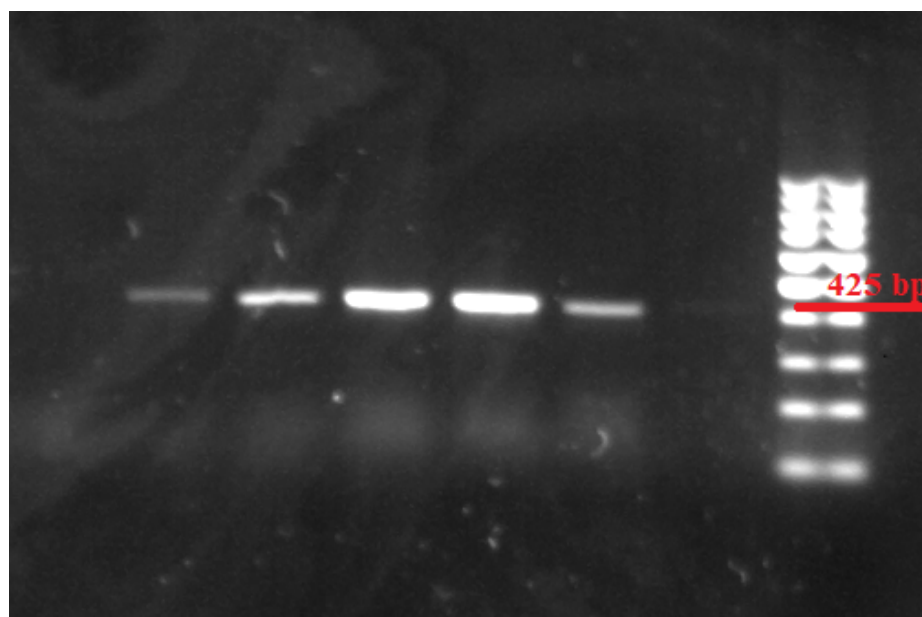


Fig. 2. Identification isolates of the family *Enterobacteriaceae* by PCR method.
 L – 100 bp ladder; Lines 1,7 – unidentified isolates, Lines 2,3,4,5,6 – isolates of the family *Enterobacteriaceae* (425 bp).

Results

Microbiological cultivation and examination of individual samples of cheeses and subsequently identification of isolates by PCR method we detected 115 isolates: 41 isolates of *Staphylococcus* spp. (Fig. 1), 61 isolates of the family *Enterobacteriaceae* (Fig. 2) and 13 isolates of *Enterococcus* spp. (Fig. 3).

Subsequently, we carried out species identification of the 115 investigated isolates by the MALDI –TOF MS method, and were able to identify 10 bacterial

species (Fig. 4). The score value of the identified strains ranged from 2.096 to 2.268 in *S. aureus* 1.976-2.105 in *S. chromogenes* and from 1.992 to 2.224 in *S. simulans*. For isolates identified as *Ent. hormaechei*, the score value ranged 1.982-2.124, in *Ent. cloacae* 1.989-2.161, in *Ent. asburiae* 1.897-2.035, in *R. ornitholytica* 1.994-2.301 and in isolates identified as *K. oxytoca* the score value was 1.972-2.237. For *E. faecium* isolates the score value ranged from 2.062 to 2.183, for it ranged 1.992-2.101 and for *E. durans* the score value ranged 2.000-2.120.

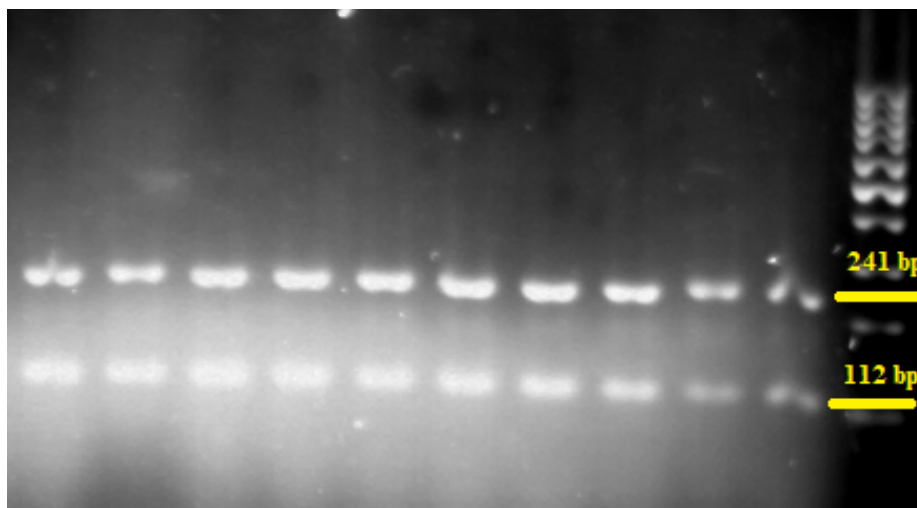


Fig. 3. Identification isolates of *Enterococcus* spp. by PCR method

L – 100 bp ladder; Lines 1,2,3,4,5,6,7,8,9,10 – isolates of *Enterococcus* spp. (112 bp) and internal control (241 bp).

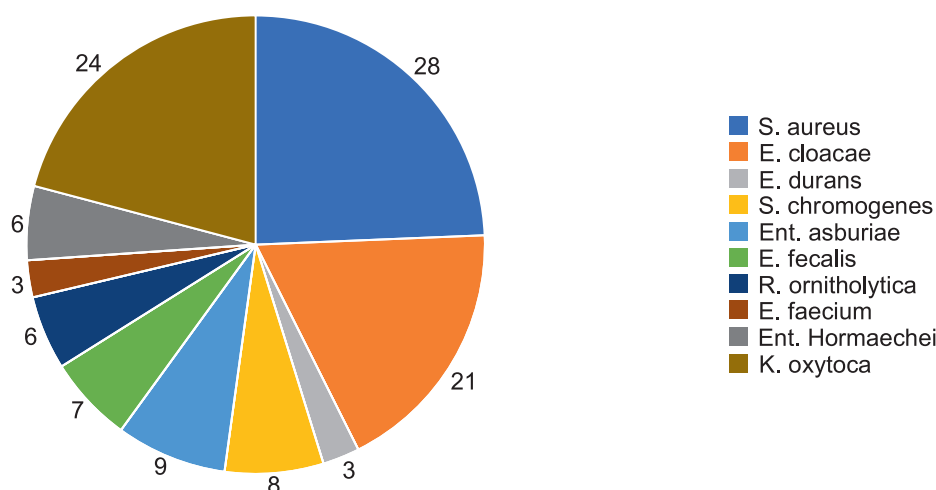


Fig. 4. Identification of strains using MALDI-TOF.

Because goat and sheep milk products are good substrate for the growth of resistant bacteria of *Enterococcus* spp. as well as the families *Staphylococcaceae* and *Enterobacteriaceae*, we also examined the identified isolates for their resistance to selected antibiotics.

Bacteria of *Staphylococcus* spp. (41 isolates) showed highest resistance to penicillin (98%; 40 isolates) (Table 2). The agar dilution method showed that isolates of the family *Enterobacteriaceae* (Table 3) were most resistant to azithromycin (90%; 55 isolates), similar to isolates of the *Staphylococcus* spp. when resistance to macrolides (erythromycin) was higher (75%; 30 isolates; Table 2). Specifically, in isolates of the genus *Enterobacter* (31 isolates) we also observed high resistance to azithromycin (28 isolates; Table 3). Species *Ent. asburiae* showed this resistance in all strains (9 isolates). This species was also resistant to ciprofloxacin (2 isolates), chloramphenicol (3 isolates), levofloxacin (1 isolate), nitrofurantoin (1 isolate) and

fosfomycin (1 isolate). All strains of this species were sensitive to doxycycline, minocycline and kanamycin. Results obtained by ADM method showed also for the genus *Enterococcus* (13 isolates) high resistance to linezolid (100%, 13 isolates; Table 4).

Therefore, it is very important to investigate prevalence and antibiotic resistance of this species. Results of our study indicate (Table 3) that these isolates were most resistant to azithromycin (4 isolates).

Another species identified by MALDI – TOF MS was *K. oxytoca* (24 isolates), an important member of the genus *Klebsiella*. Isolates examined in this study showed considerable resistance to azithromycin and somewhat lower resistance to kanamycin and minocycline. They exhibited considerable susceptibility to ciprofloxacin, levofloxacin and fosfomycin (Table 3).

Isolates of the family *Enterobacteriaceae* showed also intermediary resistance, particularly to minocycline (31%; 19 strains) in species *Ent. cloacae*

Table 2. Number of resistant (R), intermediately susceptible (IS) and susceptible (S) species of *Staphylococcus spp.*

Antibiotics	Tested of staphylococcal strains									Test*
	<i>S. aureus</i> (n=28)			<i>S. simulans</i> (n=5)			<i>S. chromogenes</i> (n=8)			
	S	IS	R	S	IS	R	S	IS	R	
PEN	1	0	27	0	0	5	0	0	8	12.757 ¹
KF	20	5	3	2	1	2	4	2	2	2.354
TEC	21	5	2	5	0	0	3	5	0	0.482
GN	11	12	5	2 2	2	1	5	2	1	1.431
E	3	5	20	0 0	0	5	1	2	5	9.326 ¹
TE	22	1	5	5	0	0	2	0	6	3.612 ¹
OFX	24	1	3	5	0	0	5	1	2	2.316

* Chi-quadrat test (significance level $\alpha=0.05$; critical value $\chi^2=2.388$; G – testing value); the independence of the individual characters at the significance level $\alpha=0.05$ has not been rejected, i.e., statistical independence was confirmed when $G < \chi^2$; ¹ – the independence of individual characters (S; IS, R) at the significance level $\alpha=0.05$ was rejected when $G > \chi^2$, i.e., the statistically confirmed dependence of the observed traits in the test group of *S. aureus* (n = 28); *S. simulans* (n=5) and *S. chromogenes* (n=8) against antibiotic substances with PEN, E and TE.

Table 3. Number of resistant (R), intermediately susceptible (IS) and susceptible (S) species of family *Enterobacteriaceae*.

Antibiotics	Tested of <i>Enterobacteriaceae</i> strains												Test*
	<i>Ent. hormaechei</i> (n = 21)			<i>Ent. cloacae</i> (n = 10)			<i>R. ornitholytica</i> (n = 6)			<i>K. oxytoca</i> (n = 24)			
	R	IS	S	R	IS	S	R	IS	S	R	IS	S	
AZI	18	0	3	10	0	0	4	0	2	23	1	0	29.079 ¹
DO	0	5	16	0	2	8	0	0	6	0	10	14	5.066
MH	3	12	6	0	7	3	1	0	5	3	0	21	10.555
CIP	0	4	17	2	0	8	0	0	6	0	0	24	5.435
L	0	0	21	1	0	9	0	0	6	0	0	24	8.613
F	7	6	8	1	3	6	1	0	5	2	1	21	15.046 ¹
FOT	0	0	21	1	0	9	0	0	6	0	0	24	5.325
C	0	0	21	3	3	4	0	0	6	2	2	20	9.151
K	0	4	17	0	3	7	1	1	4	4	10	10	10.410

* Chi-quadrat test (significance level $\alpha=0.05$; critical value $\chi^2=12.592$; G – testing value); the independence of the individual characters at the significance level $\alpha=0.05$ has not been rejected, i.e., statistical independence was confirmed when $G < \chi^2$; ¹ – the independence of individual characters (S; IS, R) at the significance level $\alpha=0.05$ was rejected when $G > \chi^2$, i.e., the statistically confirmed dependence of the observed traits in the test group of *Ent. hormaechei* (n=21); *Ent. cloacae* (n=10); *R. ornitholytica* (n=6) and *K. oxytoca* (n=24) against antibiotic substances with **AZI** and **F**

(12 strains). At the same time, we observed higher degree of intermediary susceptibility to doxycycline (28%; 17 strains), particularly in species *K. oxytoca* (10 strains). However, according to phenotype manifestations of isolates of the family *Enterobacteriaceae*, the highest resistance was observed concomitantly with fosfomycin and levofloxacin (98%; 60 isolates; Table 5). Particularly the species *K. oxytoca* exhibited high resistance to up to 8 antibiotics (Table 5).

Examination by ADM showed that all tested strains (100%; 115 isolates) were resistant to more than one selected antibiotic (Table 5).

Discussion

The study by Alves et al. (2018) points out that from 64 evaluated samples, 33 were contaminated with *S. aureus* as identified with phenotypic tests and confirmed by 16S rRNA, which is in accordance with the findings of our study. *S. aureus* was most frequently isolated from ready-to-eat cheese (82%), followed by raw material (50%) (Alves et al. 2018).

Over the course of the last decade, Matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) has enabled rapid and accurate identification

Antimicrobial resistance of bacterial isolates from sheep ...

Table 4. Number of resistant (R), intermediately susceptible (IS) and susceptible (S) species of *Enterococcus* spp.

Antibiotics	Tested of enterococcal strains									Test*
	<i>E. faecium</i> (n=3)			<i>E. faecalis</i> (n=7)			<i>E. durans</i> (n=3)			
	S	IS	R	S	IS	R	S	IS	R	
VA	0	0	3	1	0	6	1	0	2	10.244 ¹
TEC	0	0	3	2	0	5	0	0	3	11.047 ¹
E	0	0	3	0	2	5	1	0	2	9.488 ¹
DO	3	0	0	5 2	2	0	3	0	0	2.013
MH	3	0	0	1 0	2	4	0	2	1	1.299
CIP	3	0	0	4	2	1	3	0	0	3.322
L	3	0	0	6	0	1	3	0	0	0.923
F	2	1	0	2	1	4	0	1	2	2.013
RD	3	0	0	0	0	7	0	0	3	4.556

* Chi-quadrat test (significance level $\alpha = 0.05$ (5%); critical value $\chi^2=5.326$; G – testing value); the independence of the individual characters at the significance level $\alpha = 0.05$ has not been rejected, i.e., statistical independence was confirmed when $G < \chi^2$;

¹ – the independence of individual characters (S; IS, R) at the significance level $\alpha=0.05$ was rejected when $G > \chi^2$, i.e., the statistically confirmed dependence of the observed traits in the test group of *E. faecium* (n=3); *E. faecalis* (n=7) and *E. durans* (n=3) against antibiotic substances with VA, TEC and E.

Table 5. Numerous of multiresistant species of bacteria from sheep and goat cheeses.

	2 ATB	3 ATB	4 ATB	5 ATB	6 ATB	8 ATB
<i>S. aureus</i>	9	4	10	3	1	0
<i>S. simulans</i>	0	0	3	2	0	0
<i>S. chromogenes</i>	0	2	0	2	4	0
<i>Ent. hormaechei</i>	0	0	0	1	0	0
<i>Ent. cloacae</i>	0	3	7	10	1	0
<i>Ent. asburiae</i>	0	0	0	4	3	1
<i>R. ornitholytica</i>	1	0	4	0	1	0
<i>K. oxytoca</i>	0	0	2	0	17	5
<i>E. durans</i>	0	1	0	0	2	0
<i>E. faecalis</i>	0	1	1	2	2	1
<i>E. faecium</i>	0	0	0	3	0	0

ATB – antibiotics

of many microorganisms. MALDI-TOF MS was first adapted for the identification of bacteria, and the technique was later adapted for yeast (Cassagne et al. 2016 Böhme et al. 2016).

According to the study by Renye et al. (2008) the isolates most commonly identified in raw milk by MALDI – TOF MS were *Klebsiella* (*K.*) *pneumonia*, *K. ornithinolytica*, *K. oxytoca* and *Raoultella* (*R.*) *spp.*, species-related to *K. oxytoca*. Other isolates included *Enterobacter spp.*, *Citrobacter spp.*, *Escherichia coli* and *E. hormaechei*, which have also been identified in our study.

In the study by Seng et al. (2016), identification by MALDI-TOF was regarded as a rapid method of deter-

mination, particularly of pathogen *R. ornithinolytica* isolated from milk, causing infection in humans. Pukančíková et al. (2016) used MALDI-TOF MS for examination of raw cow milk and isolated and identified bacterium *Raoultella ornithinolytica* in milk from a productive unit in Slovakia (17% occurrence) (Lee et al. 2016, Lin et al. 2016).

Beyene et al. (2017) reported high effectiveness of gentamycin against staphylococci. However, our study indicated only intermediary susceptibility of isolates to the mentioned antibiotics although the diameters of inhibition zones corresponded to border values of intermediary susceptibility (34% isolates). Jamali et al. (2015) reported that *S. aureus* isolated from raw

milk and milk products was highly resistant to tetracycline and penicillin, but susceptible to oxacillin, lincomycin, klindamycin, erythromycin, streptomycin, cefoxitin, kanamycin, gentamycin and to chloramphenicol as mentioned in our study. Kraemer et al. (2017) confirmed more frequent resistance to erythromycin compared to other antimicrobial agents.

It is well known that increasing resistance to antimicrobials is associated with the extensive use of these drugs in veterinary practice (Chrobak et al. 2011).

From the investigated goat and sheep milk products we isolated and identified also bacteria of the family *Enterobacteriaceae*, which are considered a reservoir of antibiotic resistance genes in animal husbandry (Nováková et al. 2009).

The study by Parlapani et al. (2020) also points to the resistance of this species to macrolides and the high sensitivity to tetracyclines.

Klare et al. (2015) C. O'Driscoll et al. (2015) described increasing prevalence of linezolid resistance in *E. faecium*. This study also mentioned that increasing resistance in this species could be related to increased administration of linezolid in hospitals.

Sheep and goat milk products frequently contain species *Raoultella ornithinolytica* (6 isolates). Clinical signs as well as antimicrobial resistance of this pathogen have been poorly explained (Seng et al. 2016). However, in the study by Seng et al. (2016) the isolates of *R. ornithinolytica* were classified according to their phenotype manifestations as resistant to amoxicillin, ceftriaxone, ciprofloxacin and ofloxacin (Pukančíková et al. 2016).

Gaglio et al. (2016) in their study confirmed antimicrobial resistance in strains of *Enterococcus* spp. The frequency of resistance was as follows: 4 strains resistant to ciprofloxacin, one strain resistant to chloramphenicol. No resistance was observed for vancomycin, levofloxacin, linezolid. Similarly, in our study we detected antimicrobial resistance in 13 enterococci strains: one strain from (*E. faecalis*) was resistant to ciprofloxacin and chloramphenicol. *Enterococcus faecium* and *E. faecalis* might represent a public health issue due to their resistance to cephalosporins, lincosamides, penicillins, and low levels of aminoglycosides (Hammad et al. 2015).

Considering the innovativeness of the study, there are scarce data to evaluate the resistance of isolated bacteria from regional sheep and goat cheeses. At this level, papers are published that focus on the evaluation of resistance of bacteria from only one sheep product Bryndza (Drahovská et al. 2004, Vrabec et al. 2015). Other studies are focused on the evaluation of resistance of bacteria from raw sheep's or goat's milk.

In the paper of Drahovská et al. 2004, antibiotic

resistance was tested in bacteria isolated from traditional Slovak sheep cheese (bryndza). The majority of strains were identified as *E. faecium* (76 %) and *E. faecalis* (23 %). Several strains of *E. durans* and 1 strain of *E. hirae* were also present. The resistance to 6 antimicrobial substances (ampicillin, ciprofloxacin, higher concentration of gentamicin, nitrofurantoin, tetracycline and vancomycin) was tested in enterococci isolated from food and clinical samples. A higher level of resistance was found in clinical than in food samples strains and *E. faecium* had a higher resistance than *E. faecalis*; no resistance to vancomycin was detected which was also confirmed in our study.

Our results are supported by the study by Seng et al. (2016) who reported as high as 80% resistance to several types of antibiotics in bacteria of the family *Enterobacteriaceae* and in staphylococci isolates. The study by Hleba et al. (2015) also reported presence of multiresistant bacteria in milk and milk products.

Conclusion

Investigation of selected milk products brought new information about prevalence of resistant and multi-resistant bacteria in sheep and goat milk and milk products produced in the selected border area. We also confirmed the presence of little investigated species *Raoultella ornithinolytica*, which by its activity participates in development of undesirable flaws of milk and milk products. Our study also indicated the necessity of rapid and effective identification of individual bacterial species because of differences in antimicrobial profiles of individual species and their relevance from the point of view of protection of health of humans and animals.

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References

- Alves VF, Niño-Arias FC, Pitondo-Silva A, Araújo Frazílio D, Oliveira Gonçalves L, Toubas LC, Torres IMS, Oxaran V, Dittmann K, De Martinis ECP (2018) Molecular characterisation of *Staphylococcus aureus* from some artisanal Brazilian dairies. *Int Dairy J* 85: 247-253.
- Beyene T, Hayishe H, Gizaw F, Beyi AF, Abunna F, Mammo B, Ayana D, Waktole H, Abdi RD (2017) Prevalence and antimicrobial resistance profile of *Staphylococcus* in dairy

- farms, abattoir and humans in Addis Ababa, Ethiopia. *BMC Res Notes* 10: 171.
- Bruker Daltonics, MALDI Biotyper 2.0. (2007) Software for microorganism identification and classification user manual. United Kingdom.
- Böhme K, Antelo SC, Fernández-No IC, Quintela-Baluja M, Barros-Velázquez J, Cañas B, Calo-Mata P (2016) Chapter 15 - Detection of Foodborne Pathogens Using MALDI-TOF Mass Spectrometry. *Antimicrobial Food Packaging* 2016, pp 203-214.
- Cassagne C, Normand A C, L'Ollivier C, Ranque S, Piarroux R (2016) Performance of MALDI-TOF MS platforms for fungal identification. *Mycoses* 59: 678-690.
- Chrobak D, Kizerwetter-Świda M, Rzewuska M, Binek M (2011) Antibiotic resistance of canine *Staphylococcus intermedius* group (SIG)-practical implications. *Pol J Vet Sci* 14: 213-218.
- Clinical and Laboratory Standards Institute - CLSI (2018) In: Performance Standards for Antimicrobial Disk Susceptibility T; Approved Standards-M100-S28. Clinical and Laboratory Standards Institute. Wayne, PA, USA, p 296.
- Cocolin L, Foschino R, Comi G, Fortina M G (2007) Description of the bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk. *Food Microbiology* 24: 752-758.
- Čanigová M, Ducková V, Kročko M, Račková J, Bezeková J (2012) *Enterococci* and their ability to form a biofilm. *Slovak J Food Sci* 6: 15-18.
- Drahovská H, Slobodníková L, Kocíncová D, Seman M, Končeková R, Trupl J, Turňa J (2004) Antibiotic resistance and virulence factors among clinical and food enterococci isolated in Slovakia. *Folia Microbiol* 49: 763.
- Dudriková E (2011) Hygienic and technological aspects of the collection and processing of sheep's milk in the mountain conditions in Slovakia. University of Veterinary Medicine and Pharmacy in Košice, Slovakia.
- European Commission (2007). Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, 332: 12-29. <http://data.europa.eu/eli/reg/2007/1441/oj>
- Gaglio R, Couto N, Marques C, Lopes MD, Moschetti G, Pomba C, Settanni L (2016) Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials, and traditional cheeses. *Int J Food Microbiol* 236: 107-114.
- Hammad AM, Hassan HA, Shimamoto T (2015) Prevalence, antibiotic resistance and virulence of *Enterococcus* spp. in Egyptian fresh raw milk cheese. *Food Control* 50: 815-820.
- Hein I, Jorgensen HJ, Loncarevic S, Wagner M (2005) Quantification of *Staphylococcus aureus* in unpasteurised bovine and caprine milk by real-time PCR. *Res Microbiol* 156: 554-563.
- Hleba L, Petrová J, Kántor A, Cubon J, Kačaniová M (2015) Antibiotic resistance in enterobacteriaceae strains isolated from chicken and milk samples. *J Microbiol* 4: 19-22.
- Jamali H, Paydar M, Radmehr B, Ismail S, Dadrasnia A (2015) Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control* 54: 383-388.
- Ke D, Picard F, Martineau F, Ménard C, Roy PH, Ouellette M, Bergeron M (1999) Development of a PCR assay for rapid detection of enterococci. *Journal of Clinical Microbiol* 3497-3503.
- Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, Werner G (2015) Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. *J Glob Antimicrob Res* 3: 128-131.
- Kraemer JG, Pires J, Kueffer M, Semaani E, Endimiani A, Hilty M, Oppliger A (2017) Prevalence of extended-spectrum β -lactamase-producing Enterobacteriaceae and Methicillin-Resistant *Staphylococcus aureus* in pig farms in Switzerland. *Sci Total Environ* 603: 401-405.
- Lee YC, Chen YF, Huang YL, Kung HF, Chen TY, Tsai YH (2016) Hygienic quality, adulteration of pork and histamine production by *Raoultella ornithinolytica* in milk fish dumplings. *J Food Drug Anal* 24: 762-770.
- Lin CS, Kung HF, Lin CM, Tsai HC, Tsai YH (2016) Histamine production by *Raoultella ornithinolytica* in mahi-mahi meat at various storage temperatures. *J Food Drug Anal* 24: 305-310.
- Moradigaravand D, Martin V, Peacock S J, Parkhill J, (2017) Population structure of multidrug-resistant *Klebsiella oxytoca* within hospitals across the United Kingdom and Ireland identifies sharing of virulence and resistance genes with *K. pneumoniae*. *Genome Biol Evol* 9: 574-584.
- Nakano S, Kobayashi T, Funabiki K, Matsumura A, Nagao Y, Yamada T (2003) Development of a PCR Assay for Detection of Enterobacteriaceae in Foods. *J Food Prot* 66: 1798-1804.
- Nováková I, Kačaniová M, Haščík P, Pavličová S, Hleba L (2009) The resistance to antibiotics in strains of *E. coli* and *Enterococcus* sp. isolated from rectal swabs of lambs and calves. *Zootehnie și Biotehnologii* 42: 322-326.
- O'Driscoll C, Murphy V, Doyle O, Wrenn C, Flynn A, O'Flaherty N, Fenelon L, Schaffer K FitzGerald SF (2015) First outbreak of linezolid-resistant vancomycin-resistant *Enterococcus faecium* in an Irish hospital, February to September 2014. *J Hosp Infect* 91: 367-370.
- Parlapani FF, Kyritsi M, Sakka M, Chatziniolaou K, Donos S, Boziaris IS, Hadjichristodoulou Ch, Athanassiou Ch (2020) Matrix-assisted laser desorption ionization-time of flight mass spectrometry reveals *Enterococcus* and *Enterobacter* spp. in major insect species involved in food security with resistance to common antibiotics. *J Pest Sci* 93: 159-170.
- Pukančíková L, Lipničanová S, Kačaniová M, Chmelová D, Ondrejovič M (2016) Natural microflora of raw cow milk and their enzymatic spoilage potential. *Nova Biotechnol Chem* 15: 142-155.
- Regecová I, Pipová M, Jevinová P, Kmet' V, Výrostková J, Sopková D (2014) Antimicrobial Resistance of Coagulase-negative Species of Staphylococci Isolated from the Meat of Wild Pheasants (*Phasianus Colchicus*). *Ital J Anim Sci* 13: 3476.
- Renye JN., Somkuti GA, Vallejo-Cordoba DL, Van Hekken DL, Gonzalez-Cordova AF (2008) Characterization of the microflora isolated from queso fresco made from raw and pasteurized milk. *J Food Saf* 28: 59-75.
- Ribeiro T, Oliveira M, Fraqueza M J, Lauková A, Elias M, Tenreiro R, Barreto A S, Semedo-Lemsaddek T (2011) Antibiotic resistance and virulence factors among entero-

- cocci isolated from Chouriço, a traditional Portuguese dry fermented sausage. *J Food Prot* 74: 465-469.
- Seng P, Boushab B M, Romain F, Gouriet F, Bruder N, Martin C, Paganelli F, Emmanuelle B, Treut Y, Thomas P, Papazian L, Raoult D, Stein A (2016) Emerging role of *Raoultella ornithinolytica* in human infections: a series of cases and review of the literature. *Int J Infect Dis* 45: 65-71.
- Singhal N, Kumar M, Kanaujia P K, Viridi JS (2015) MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol* 6: 791.
- STN EN ISO 6888-1 (1999) Microbiology of food and animal feeding stuffs. Horizontal 247 method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium, SÚTN, Bratislava, Slovakia.
- STN EN 21528-1 (2019) Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* Part 1: Detection of *Enterobacteriaceae*. SÚTN, Bratislava, Slovakia.
- STN EN 6887-5 (2010) Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 5: Specific rules for the preparation of milk and milk products. SÚTN, Bratislava, Slovakia.
- Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J Clin Microbiol* 41: 4089-4094.
- Vrabec M, Lovayová V, Dudriková K, Gallo J, Dudriková E. (2015) Antibiotic resistance and prevalence of *Enterococcus* spp. and *Escherichia coli* isolated from bryndza cheese. *Italian Journal of Animal Science* 14(4): 3968.