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Antimicrobial Resistance of Coagulase-Negative Staphylococci Isolated from Spontaneously Fermented Sausages

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Summary

The antimicrobial susceptibility of coagulase-negative staphylococci isolated from spontaneously fermented sausages was assessed using both traditional and molecular methods. Isolates were tested for sensitivity to vancomycin, ampicillin, erythromycin, tetracycline, gentamycin and oxacillin by the disk diffusion method and quantitative-qualitative epsilometer test. PCR was used for the detection of resistance genes *mec*A, *erm*B, *tet*K and *tet*M. The identified coagulase-negative staphylococci were *Staphylococcus epidermidis* (69 %), *S. capitis* (5 %) and *S. warneri* (2.5 %). *S. epidermidis* showed a high rate of phenotypical resistance to tetracycline and erythromycin (44.4 % of strains). Molecular evaluation of resistance determinants revealed *tet*K or *tet*M genes in eight *S. epidermidis* strains. Although *S. epidermidis* is not classical food poisoning bacteria, its presence in food could be of public health significance due to the possible spread of antimicrobial resistance determinants. Our findings implicate that spontaneous meat fermentation could result in products with a potential hazard to consumers.

Key words: fermented sausages, *Staphylococcus epidermidis*, resistance genes

Introduction

Coagulase-negative staphylococci (CNS) are the most important microbial group in meat fermentation, alongside lactic acid bacteria (LAB). Their favourable activities are expressed in colour and aroma development, proteolytic and lipolytic succession, which all contribute to the sensory properties of the final product. Traditional meat products are a rich source of CNS/LAB with potential use in meat processing as (functional) starter cultures (*1–3*). However, the selection criteria for choosing appropriate strains, in addition to technological properties, extend to potential health hazards such as the production of biogenic amines or resistance to antibacterial agents (*4*). The most commonly used CNS in meat starter cultures are *Staphylococcus xylosus* and *Staphylococcus carnosus*, which occupy a significant part of the intrinsic population of naturally fermented meat products (*5*). In addition, several studies reported the presence of other species of staphylococci in fermented sausages, such as *Staphylococcus epidermidis*, *Staphylococcus warneri*, *Staphylococcus equorum* or *Staphylococcus saprophyticus* (*6–9*). These

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staphylococcal species are frequently isolated from different specimens in meat chain production, including final meat products (*10*). The pathogenic potential of staphylococci in general depends on the toxigenic component, which is rarely present in food-related CNS (*11*). On the other hand, recent research has shown that resistance to antibiotics, including resistance to some antibiotics of therapeutic importance, also occurs in strains of the important starter cultures *S. xylosus*/*S. carnosus* as well as in opportunistic pathogens isolated from fermented sausages such as *S. epidermidis*, *S. warneri* and *S. saprophyticus* (*7*). The most frequent reports were of resistance to ampicillin, erythromycin, penicillin, tetracycline or lincomycin (*7,10–12*). There is only limited information on the antimicrobial resistance of CNS isolated from autochthonous Croatian dry sausages. Babić *et al.* (*13*) reported that the majority of CNS isolated from the Croatian brand product Slavonski kulen are sensitive to most antimicrobials, which is a relevant result for the selection of potential autochthonous starter cultures. Authors reported that only *Staphylococcus lentus* was resistant to erythromycin and gentamycin, while *Staphylococcus auricularis* exhibited resistance to neomycin. Recently, Zdolec *et al.* (*14*) found that 64 % of CNS isolates from spontaneously fermented wild boar sausages were resistant to erythromycin, tetracycline or lincomycin.

The prudent use of antimicrobial agents in food-producing animals is of great importance, not only for emerging foodborne pathogen resistance, but also for commensals (such as LAB, CNS, enterococci) which enter the food chain *via* raw material (*15,16*). This is of particular importance in the agri-food chain, especially in the production of traditional fermented products where specific hurdle technologies are not employed. Thus, the aim of this study is to collect autochthonous strains of CNS from Croatian traditional fermented sausages to test their susceptibility to selected antimicrobials and to evaluate the presence of resistance genes.

Material and Methods

Biochemical identification of isolates

A total of 70 isolates were collected from two Croatian traditional fermented meat products: kulen and homemade Slavonian sausage produced in an artisanal facility. For microbiological analysis, 25 g of product were diluted in 225 mL of salt peptone water and homogenized for 2 min (Stomacher 400 Circulator, Seward Ltd., Worthing, UK). Further serial decimal dilutions were prepared, and 0.1 mL of appropriate dilution was spread on manitol salt agar (MSA; bioMérieux, Marcy l'Etoile, France) and incubated at 30 °C for 48 h. Isolates were subjected to Gram staining, catalase activity (Bactident catalase, Merck, Darmstadt, Germany) and the coagulase test (Bactident coagulase, Merck). Gram-positive, catalase-positive and coagulase-negative bacteria were presumptively identified as staphylococci (N=39), and were selected for further biochemical identification. Prior to analysis, isolates were stored in brain heart infusion broth (BHI; bioMérieux) supplemented with 20 % glycerol at –20 °C. The isolates were grown in BHI broth at 37 °C for 24–48 h, streaked on MSA and incubated at 37 °C for 24 h. One isolated colony was retransferred to BHI and reincubated under the same conditions. Then the culture was streaked densely by sterile swab on MSA for ID32 Staph identification (bioMérieux). The test was performed following the manufacturer's instructions and determination was done using api*web*TM v. 1.2.1. software (bioMérieux).

Molecular identification of isolates to the genus level

Separated MSA colonies were resuspended in 200 μL of distilled water (UltraPure™ DNase/RNase-free distilled water, Invitrogen, Paisley, UK), then heated for 20 min at 95 °C and centrifuged for 1 min at 14 000×*g*. The supernatant was used as DNA template for the PCR. For the identification of isolates to the genus level, part of the 16S rRNA *Staphylococcus* gene was amplified according to Mason *et al.* (*17*). *Staphylococcus aureus* ATCC 25923 was used as the positive control. The PCR reaction mixture of 20 μL contained 10 μL of HotStarTaq Master Mix (Qiagen, Hilden, Germany), 6 μL of water (RNase-free water, Qiagen), 1 μL of primer Staph 16S-1 (CCT ATA AGA CTG GGA TAA CTT CGG G), 1 μL of primer Staph 16S-2 (CTT TGA GTT TCA ACC TTG CGG TCG) and 2 μL of DNA template. The final concentration of each primer (Invitrogen) in the reaction mixture was 0.5 μM. Amplification was carried out in a Veriti 96-well thermal cycler (Applied Biosystems, Life Technologies, Grand Island, NY, USA) with a polymerase activation step (95 °C/15 min), followed by 35 cycles of denaturation (95 °C/1.5 min), annealing (55 °C/1 min) and extension (72 °C/1 min), and a final extension step (72 °C/7 min). The amplification product size (expected, 791 bp) was determined by QIAxcel and the computer program QIAxcel BioCalculator (Qiagen).

Antimicrobial susceptibility test

Qualitative antimicrobial susceptibility testing was performed on Mueller-Hinton agar (MH; bioMérieux) using the disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines (*18*). The isolates were tested for their susceptibility to ampicillin (10 μg), erythromycin (15 μg), tetracycline (30 μg), gentamycin (10 μg) and oxacillin (1 μg) using antimicrobial disks (Bio-Rad, Marnes-la-Coquette, France). Plates were incubated at 35 °C for 20–24 h and the results were interpreted according to the CLSI document M100-S20 (*18*). Strains which were found to be intermediate or resistant by disk diffusion method (according to the CLSI breakpoints) were subjected to qualitative and quantitative epsilometer test (Etest; AB BIODISK, bioMérieux) for the detection of minimal inhibitory concentrations (MIC). Further, all strains resistant to any of the tested antimicrobials in disk diffusion test were additionally tested for sensitivity to vancomycin and oxacillin (MH agar with 2 % NaCl) using the Etest, since disk diffusion method is not recommended for these two agents (*18*).

Molecular identification of mecA, ermB and tet genes

Primers used to detect *mec*A, *erm*B and *tet* genes are listed in Table 1 (*19–22*).

Table 1. Primers used in this study

Gene	Primer	Primer sequence	Amplicon size/bp	Reference
*erm*B	ermB-F	CTA TCT GAT TGT TGA AGA AGG ATT	142	(*19*)
	ermB-R	GTT TAC TCT TGG TTT AGG ATG AAA		
*tet*K	tetK-F	TTA GGT GAA GGG TTA GGT CC	718	(*20*)
	tetK-R	GCA AAC TCA TTC CAG AAG CA		
*tet*M	tetM-F	ACA GAA AGC TTA TTA TAT AAC	171	(*21*)
	tetM-R	TGG CGT GTC TAT GAT GTT CAC		
*mec*A	MecA-1	GGG ATC ATA GCG TCA TTA TTC	527	(*22*)
	MecA-2	AAC GAT TGT GAC ACG ATA GCC		

Amplification of DNA for *erm*B and *tet* genes was performed in a 20-μL reaction mixture containing 10 μL of HotStarTaq Master Mix (Qiagen), 6 μL of water (RNase-free water, Qiagen), 1 μL of $MgCl_2$ (25 mM, Qiagen), 0.5 μL of each primer (20 μM, Sigma) and 2 μL of genomic DNA. The mixture was processed in a thermocycler (Veriti 96-well thermal cycler, Applied Biosystems) with a polymerase activation step (95 °C/15 min), followed by 40 cycles of denaturation (95 °C/30 s), annealing (50 °C/30 s) and extension (72 °C/1 min), and final extension step (72 °C/7 min). Amplicons were analyzed using the QIAxcel system and QIAxcel BioCalculator software (Qiagen). The positive control for resistance gene determination was *Staphylococcus pseudintermedius* (*23*). The volume of the PCR reaction mixture for the amplification of *mec*A gene was 20 μL. The reaction mixture contained 10 μL of HotStarTaq Master Mix (Qiagen), 7 μL of water (RNase-free water, Qiagen), 0.5 μL of each primer and 2 μL of DNA template. The final concentration of each primer (Invitrogen) in the reaction mixture was 0.5 μM. Amplification was carried out in a Veriti 96-well thermal cycler (Applied Biosystems) with a polymerase activation step (95 °C/15 min), followed by 35 cycles of denaturation (95 °C/30 s), annealing (55 °C/30 s) and extension (72 °C/1 min) with a final extension step (72 °C/10 min). The amplification products were analyzed using the QIAxcel system (Qiagen).

Results and Discussion

Coagulase-negative staphylococci have significant technological importance in the production of fermented meat products. Due to their natural presence in traditional fermented meat, they contribute to the succession of favourable sensory properties of the final products. Thus, indigenous food microbiota seem to be the best starter culture candidates for specified products due to their good adaptation to the meat substrate. In addition to the technological advantages, CNS have intensively been investigated for their antimicrobial and probiotic properties that could contribute to consumer health. Generally, meat fermentation microbiota is recognized as safe, although antimicrobial resistance is a potential reason for concern (*4*).

In the present study of 39 coagulase-negative staphylococci, the following species were identified using ID32 Staph: *Staphylococcus epidermidis* (*N*=27; 69 %), *Staphylococcus capitis* (*N*=2; 5 %) and *Staphylococcus warneri* (*N*=1; 2.5 %), while 9 isolates (23.07 %) were identified as *Staphylococcus* spp. The identification to the genus level by amplification of specific part of the 16S rRNA gene confirmed that all isolates (*N*=39) belong to the genus *Staphylococcus* (Fig. 1).

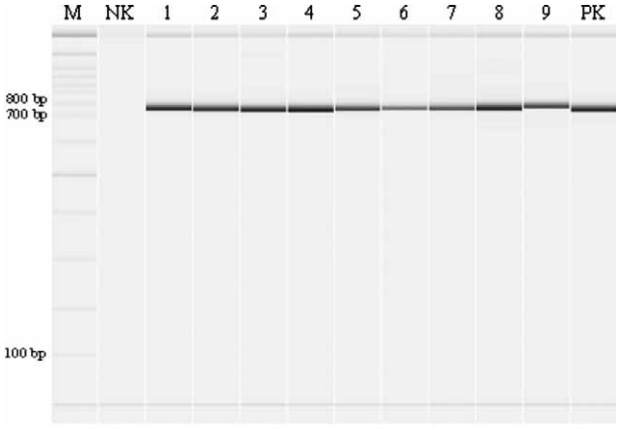

Fig. 1. PCR identification of isolates to the genus level (*Staphylococcus*). Lane M=marker with amplification products of sizes 15, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000 and 3000 bp; lane NK=negative control; lane PK=positive control, ATCC 25923; lanes 1–9=isolates

Biochemical characteristics of the strains are presented in Table 2. According to the determined biochemical profiles, the predominant species was *S. epidermidis* followed by *S. capitis* and *S. warneri*. These species were found in different kinds of fermented sausages, although usually in low numbers (*7*,*8*,*24*–*26*). On the other hand, Marty *et al.* (*9*) reported a high incidence of *S. epidermidis* and *S. saprophyticus* in spontaneously fermented Swiss sausages. Our results support the latter findings indicating that spontaneous meat fermentation could result in products potentially risky to consumers. *Staphylococcus epidermidis* is widely distributed in the environment and belongs to the group of opportunistic pathogens (*12*). Schlegelová *et al.* (*27*) reported finding meat and milk products significantly more contaminated with *S. epidermidis* than raw materials and reported its regular presence on food contact surfaces.

Table 2. Biochemical profiles of coagulase-negative staphylococci isolated from traditional fermented sausages

Parameter	*S. epidermidis* (*N*=27)	*S. capitis* (*N*=2)	*S. warneri* (*N*=1)	*Staphylococcus* spp. (*N*=9)
urease	9 (33.3)	0	1 (100)	0
arginine dehydrolase	19 (70.3)	2 (100)	1 (100)	9 (100)
lysine decarboxylase	18 (66.6)	0	0	1 (11)
esculin hydrolase	0	0	0	0
fermentation of:				
D-glucose	27 (100)	2 (100)	1 (100)	9 (100)
D-fructose	27 (100)	2 (100)	1 (100)	8 (88.9)
D-mannose	13 (48.1)	1 (50)	0	2 (22.2)
D-maltose	27 (100)	2 (100)	1 (100)	8 (88.9)
D-lactose	26 (96.2)	2 (100)	1 (100)	7 (77.8)
D-trehalose	0	0	0	2 (22.2)
D-mannitol	0	0	0	1 (11)
D-raffinose	0	0	0	0
D-ribose	0	0	0	0
D-cellobiose	0	0	0	0
D-saccharose	27 (100)	2 (100)	1 (100)	8 (88.9)
N-acetyl-glucosamine	0	0	0	2 (22.2)
D-turanose	11 (40.7)	1 (50)	0	2 (22.2)
L-arabinose	0	0	0	0
nitrate reduction	25 (92.6)	2 (100)	1 (100)	9 (100)
acetoin	14 (51.8)	0	0	6 (66.7)
β-galactosidase	1 (3.7)	0	0	0
arginine arylamidase	0	0	0	0
alkaline phosphatase	4 (14.8)	0	0	2 (22.2)
pyrrolidonyl arylamidase	0	0	0	2 (22.2)
novobiocin resistance	0	0	0	0
β-glucuronidase	0	0	1 (100)	1 (11)
coagulase	0	0	0	0

The numbers indicate the positive strains regarding each parameter with the percentage of positive strains in brackets
Identity level of biochemical identification by ID32 Staph: *S. epidermidis* 74.9–99.0 %, *S. capitis* 82.5–95.6 %, *S. warneri* 97.8 %

Antimicrobial resistance of CNS isolated from food had previously been reported, including *S. epidermidis* (*11*,*27*). Among 39 tested isolates, 21 intermediate or resistant staphylococci were found based on the CLSI breakpoints for selected antimicrobials used in the disk diffusion test (Table 3).

The majority of strains harboured resistance to erythromycin, tetracycline and ampicillin. This is partially presented by Martin *et al.* (*7*) in the highest rate of resistance of *S. epidermidis* and *S. warneri* strains to ampicillin and erythromycin, detected by the disk diffusion method. Further testing of our strains by Etest confirmed resistance to erythromycin and tetracycline, but not to ampicillin. Based on the Etest breakpoints for staphylococci, all suspected strains (intermediate or resistant in disk diffusion test) were sensitive to ampicillin and gentamycin. All strains were also sensitive to oxacillin and vancomycin. Using Etest, a lower number of multiresistant strains was found, compared to the results of disk diffusion test (Tables 4 and 5).

In total, the Etest revealed 13 resistant strains (33.3 %) that were subjected to molecular determination of resistance genes *tet*K, *tet*M and *erm*B. Although phenotypic resistance to oxacillin/methicillin was not detected, all strains (*N*=39) were tested for the presence of the *mec*A gene. Molecular testing showed the presence of *tet*K or *tet*M genes in 8 strains of *S. epidermidis* (61.5 %), with one strain positive for both genes (Figs. 2 and 3). The *mec*A and *erm*B genes were not detected in any of the tested strains.

Staphylococcus epidermidis, *S. equorum* and *S. saprophyticus* show the highest rate of antibiotic resistance among staphylococci isolated from food (*28*). Our results are in agreement with Even *et al.* (*11*) who found that 69 % of multiresistant strains isolated from food/clinical specimens belonged to *S. epidermidis* species with dominant resistance to erythromycin, tetracycline and penicillin that were traced back (for the last two agents) to the presence of *tet*K and *bla*Z genes. The *tet*K and *tet*M genes

Table 3. Number of sensitive (S), intermediate (I) and resistant (R) strains

Antimicrobial agent	Agar disk diffusion test			Etest		
	S	I	R	S	I	R
vancomycin**	–	–	–	13	0	0
ampicillin*	32	0	7	7	0	0
erythromycin*	26	1	12	1	0	12
tetracycline*	23	4	12	10	0	6
gentamycin*	37	0	2	2	0	0
oxacillin**	–	–	–	13	0	0

*only strains which were intermediate or resistant in the disk diffusion test were subjected to the Etest (ampicillin, *N*=7; erythromycin, *N*=13; tetracycline, *N*=16; gentamycin, *N*=2)
**all strains which were resistant in the disk diffusion test to ampicillin, erythromycin, tetracycline or gentamycin were subjected to the Etest for vancomycin and oxacillin (*N*=13)
– not tested

Table 4. Relation of strain origin and (multi)resistance to antimicrobials (agar diffusion method)

Strain origin	*N* of resistant strains (%)	*N* of resistant strains to specific agent (%)				*N* of multiresistant strains (%)			
		Amp	Ery	Tet	Gm	1	2	3	4
dry fermented sausage kulen (*N*=25)	15 (60)	7 (47)	8 (53)	9 (60)	1 (7)	8 (53)	5 (33)	1 (7)	1 (7)
Slavonian fermented sausage (*N*=14)	4 (28.5)	0	4 (100)	3 (75)	1 (25)	0	4 (100)	0	0

Amp=ampicillin, Ery=erythromycin, Tet=tetracycline, Gm=gentamycin

Table 5. Relation of strain origin and (multi)resistance to antimicrobials (Etest)

Strain origin	*N* of resistant strains (%)	*N* of resistant strains to specific agent (%)				*N* of multiresistant strains (%)			
		Amp	Ery	Tet	Gm	1	2	3	4
dry fermented sausage kulen (*N*=16*)	9 (56)	0	8 (88)	4 (44)	0	6 (67)	3 (33)	0	0
Slavonian fermented sausage (*N*=5*)	4 (80)	0	4 (100)	2 (50)	0	2 (50)	2 (50)	0	0

*strains which were intermediate or resistant by the agar diffusion test
Amp=ampicillin, Ery=erythromycin, Tet=tetracycline, Gm=gentamycin

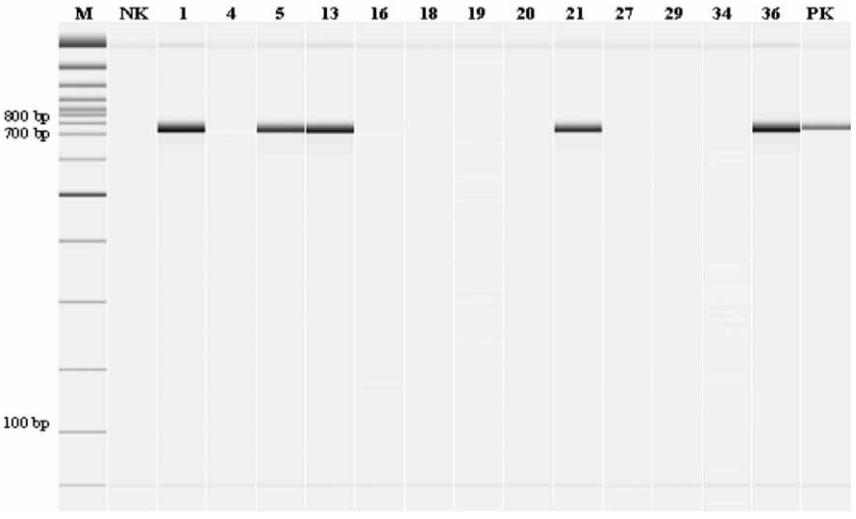

Fig. 2. Identification of the *tet*K gene. Lane M=size marker (15, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000 and 3000 bp); lane NK=negative control; lane PK=positive control (*S. pseudintermedius tet*K and *erm*B positive); lanes 1, 4, 5, 13, 16, 18, 19, 20, 21, 27, 29, 34, 36=isolates

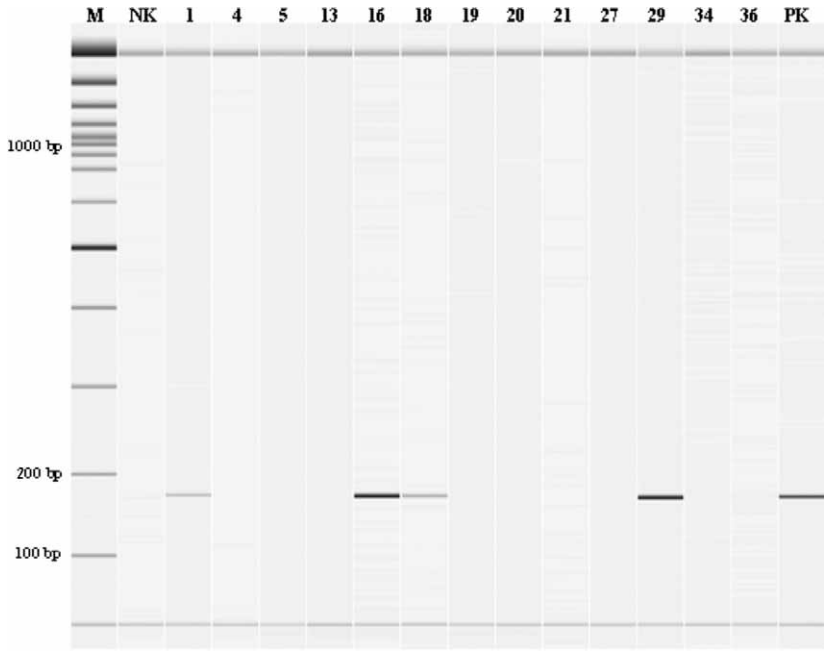

Fig. 3. Identification of the *tet*M gene. Lane M=size marker (15, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000 and 3000 bp); lane NK=negative control; lane PK=positive control (*S. pseudintermedius tet*M positive); lanes 1, 4, 5, 13, 16, 18, 19, 20, 21, 27, 29, 34, 36=isolates

were also reported as most prevalent antibiotic resistance genes in CNS from the production chain of swine meat commodities, where *S. epidermidis* was dominant species (*10*). Many studies have shown a comparably low correlation between phenotypic and genotypic detection of antibiotic resistance. In this respect, Resch *et al.* (*12*) found *tet*K in 93 % of CNS that were phenotypically resistant to tetracycline, but *tet*L and *tet*M could not be detected; *lnu*A gene in only 3 % of 120 strains phenotypically resistant to lincomycin; and finally the absence of *mec*A gene in strains which showed phenotypic resistance to oxacillin/methicillin. The results presented in this study are partially in agreement with this finding since *tet*K or *tet*M were detected in 8 out of 12 phenotypically resistant strains, while the *erm*B gene was not found in the erythromycin-resistant strains. Since the highest number of strains were resistant to erythromycin in the susceptibility tests, it would be necessary to determine the presence of other genes encoding erythromycin resistance, as shown by Schlegelová *et al.* (*29*). They reported a relatively low occurrence of erythromycin-resistant staphylococci in the food chain, but isolates were carriers of the *erm*, *msr* and *mph* genes.

Conclusions

The indigenous microbiota of fermented sausages is a rich source of potential autochthonous starter cultures. However, traditional production could promote the growth of undesirable bacterial species due to hygienic or technological omissions. In this study, this is presented by the domination of the opportunistic pathogen *Staphylococcus epidermidis* in spontaneously fermented sausages produced in an artisanal facility. Although *S. epidermidis* is not a factor in food poisoning, its presence in food could be of public health importance. This study showed that 30 % of *S. epidermidis* strains were carriers of resistance genes that could be transferred to other bacteria, including pathogens. However, a more detailed and comprehensive study is needed to detect the routes of transfer of resistant strains through the agri-food chain. Preventing the occurrence or reducing the number of resistant commensal or pathogenic bacteria in animals and the environment (food production facilities) should be a prerequisite for the production of safe spontaneously fermented food. Another approach is the implementation of competitive protective starter cultures.

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References

1. N. Zdolec, M. Hadžiosmanović, L. Kozačinski, Ž. Cvrtila, I. Filipović, M. Škrivanko, K. Leskovar, Microbial and physicochemical succession in fermented sausages produced with bacteriocinogenic culture of *Lactobacillus sakei* and semi-purified bacteriocin mesenterocin Y, *Meat Sci. 80* (2008) 480–487.
2. M.G. Bonomo, A. Ricciardi, G. Salzano, Influence of autochthonous starter cultures on microbial dynamics and chemical-physical features of traditional fermented sausages of Basilicata region, *World J. Microbiol. Biotechnol. 27* (2011) 137–146.
3. N. Zdolec: Lactobacilli – Functional Starter Cultures for Meat Applications. In: *Lactobacillus: Classification, Uses and Health Implications*, A.I. Peres Campos, A.L. Mena (Eds.), Nova Science Publishers, Hauppauge, NY, USA (2012).

4. R. Talon, S. Leroy, Diversity and safety hazards of bacteria involved in meat fermentations, *Meat Sci. 89* (2011) 303–309.
5. M. Seiiter (née Resch), C. Nerz, R. Rosenstein, F. Götz, C. Hertel, DNA microarray based detection of genes involved in safety and technologically relevant properties of food associated coagulase-negative staphylococci, *Int. J. Food Microbiol. 145* (2011) 449–458.
6. G. Blaiotta, D. Ercolini, C. Pennacchia, V. Fusco, A. Casaburi, O. Pepe, F. Villani, PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of *se*G and *se*I in *S. aureus* AB-8802, *J. Appl. Microbiol. 97* (2004) 719–730.
7. B. Martin, M. Garriga, M. Hugas, S. Bover-Cid, M.T. Veciana-Nogués, T. Aymerich, Molecular, technological and safety characterization of Gram-positive catalase-positive cocci from slightly fermented sausages, *Int. J. Food Microbiol. 107* (2006) 148–158.
8. M.G. Bonomo, A. Ricciardi, T. Zotta, M.A. Sico, G. Salzano, Technological and safety characterization of coagulase--negative staphylococci from traditionally fermented sausages of Basilicata region (Southern Italy), *Meat Sci. 83* (2009) 15–23.
9. E. Marty, J. Buchs, E. Eugster-Meier, C. Lacroix, L. Meile, Identification of staphylococci and dominant lactic acid bacteria in spontaneously fermented Swiss meat products using PCR-RFLP, *Food Microbiol. 29* (2012) 157–166.
10. D. Simeoni, L. Rizzotti, P. Cocconcelli, S. Gazzola, F. Dellaglio, S. Torriani, Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities, *Food Microbiol. 25* (2008) 196–201.
11. S. Even, S. Leroy, C. Charlier, N.B. Zakour, J.P. Chacornac, I. Lebert *et al.*, Low occurrence of safety hazards in coagulase negative staphylococci isolated from fermented foodstuffs, *Int. J. Food Microbiol. 139* (2010) 87–95.
12. M. Resch, V. Nagel, C. Hertel, Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. *Int. J. Food Microbiol. 127* (2008) 99–104.
13. I. Babić, K. Markov, D. Kovačević, A. Trontel, A. Slavica, J. Đugum *et al.*, Identification and characterization of potential autochthonous starter cultures from a Croatian 'brand' product 'Slavonski kulen', *Meat Sci. 88* (2011) 517–524.
14. N. Zdolec, V. Dobranić, I. Filipović, D. Marcincakova, Antimicrobial resistance of coagulase-negative staphylococci isolated from spontaneously fermented wild boar sausages, *Proceedings of Lectures and Posters, Hygiena Alimentorum XXXIII*, Strbske Pleso, Slovakia (2012) pp. 283–287.
15. N. Zdolec, I. Filipović, Ž. Cvrtila Fleck, A. Marić, D. Jankuloski, L. Kozačinski, B. Njari, Antimicrobial susceptibility of lactic acid bacteria isolated from fermented sausages and raw cheese, *Vet. Arhiv, 81* (2011) 133–141.
16. S. Gazzola, C. Fontana, D. Bassi, P.S. Cocconcelli, Assessment of tetracycline and erythromycin resistance transfer during sausage fermentation by culture-dependent and -independent methods, *Food Microbiol. 30* (2012) 348–354.
17. W.J. Mason, J.S. Blevins, K. Beenken, N. Wibowo, N. Ojha, M.S. Smeltzer, Multiplex PCR protocol for the diagnosis of staphylococcal infection, *J. Clin. Microbiol. 39* (2001) 3332–3338.
18. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement, CLSI Document M100–S20, Clinical and Laboratory Standards Institute, Wayne, PA, USA (2010).
19. F. Martineau, J.F. Picard, N. Lansac, C. Menard, H.P. Roy, M. Ouellette, G.M. Bergeron, Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Antimicrob. Agents. Chemother. 44* (2000) 231–238.
20. F.M. Aarestrup, Y. Agerso, P. Gerner–Smidt, M. Madsen, L.B. Jensen, Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark, *Diagn. Microbiol. Infect. Dis. 37* (2000) 127–137.
21. R.I. Aminov, N. Garrigues-Jeanjean, R.I. Mackie, Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins, *Appl. Environ. Microbiol. 67* (2001) 22–32.
22. Multiplex PCR for the Detection of the *mec*A Gene and the Identification of *Staphylococcus aureus,* National Food Institute, Technical University of Denmark, Lyngby, Denmark (2009) (*http://www.crl-ar.eu/data/images/tc_april-2009/who%20meca-pcr_protocol%209-06-11.pdf*).
23. K. Matanović, Genotyping and detection of resistance genes in methicillin-resistant *Staphylococcus pseudintermedius, PhD* Thesis, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia (2011).
24. G. Mauriello, A. Casaburi, G. Blaiotta, F. Villani, Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy, *Meat Sci. 67* (2004) 149–158.
25. L. Kozačinski, N. Zdolec, M. Hadžiosmanović, Ž. Cvrtila, I. Filipović, T. Majić, Microbial flora of the Croatian traditionally fermented sausage, *Arch. Lebensmittelhyg. 57* (2006) 141–147.
26. E. Coton, M.H. Desmonts, S. Leroy, M. Coton, E. Jamet, S. Christieans *et al.*, Biodiversity of coagulase-negative staphylococci in French cheeses, dry fermented sausages, processing environments and clinical samples, *Int. J. Food Microbiol. 137* (2010) 221–229.
27. J. Schlegelová, V. Babák, M. Holasová, M. Dendis, The biofilm-positive *Staphylococcus epidermidis* isolates in raw materials, foodstuffs and on contact surfaces in processing plants, *Folia Microbiol. 53* (2008) 500–504.
28. J.C. Soares, M.R. Marques, F.K. Tavaria, J.O. Pereira, F.X. Malcata, M.M. Pintado, Biodiversity and characterization of *Staphylococcus* species isolated from a small manufacturing dairy plant in Portugal, *Int. J. Food Microbiol. 146* (2011) 123–129.
29. J. Schlegelová, H. Vlková, V. Babák, M. Holasová, Z. Jaglic, T. Stosová, P. Sauer, Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain, *Veterinarni medicina, 53* (2008) 307–314.