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REP-PCR TYPING OF STAPHYLOCOCCUS SPP. STRAINS IN MEAT PASTE PRODUCTION LINE AND IDENTIFICATION OF THEIR ORIGIN

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ABSTRACT

A meat paste production line and its microbial parameters have been evaluated in single Czech company. The raw meat paste samples before heat treatment were tested positively for the presence of three staphylococci species: *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*. Subsequent microbial analysis of meat paste components and ingredients (fresh meat, water, spices, equipment) identified only the spices used as positive for *S. aureus* (coriander, cinnamon, badian, mustard – (10 – 40 cfu/g)) and *S. haemolyticus* strains (juniper, ginger). The collection of sixteen collected strains (*S. aureus* (n = 4), *S. haemolyticus* (n = 4), *S. epidermidis* (n = 8)) has been typed with the rep-PCR method utilising (GTG)5 primer. Analysis of the fingerprints using the unweighted pair-group method using arithmetic averages (UPGMA) clustering method revealed presence of eleven strain clusters with similarity lower than 90%: two fingerprint clusters of *S. aureus*, three individual clusters characteristic for *S. haemolyticus* and six different *S. epidermidis* specific clusters. The *S. aureus* strains from different types of spice were identical, resp. very similar. Molecular tracking composed from the rep-PCR analysis of acquired isolates and comparison among all collected fingerprints confirmed the spices to be the source of both *S. aureus* and *S. haemolyticus* strains identified in raw meat paste. The additional rep-PCR analysis of the *S. epidermidis* collection confirmed usability and performance of this method. The antibiotic susceptibility to fourteen individual antibiotics has been examined among the collected staphylococci strains. The predominant erythromycin resistance (68.8%) was followed with the resistance to amoxicillin/clavulanic acid (56.2%). Other resistances observed were less frequent (clindamycin – 12.5%, oxacillin – 6.3%, tetracycline – 6.3%, sulphamethoxazole-trimethoprim – 6.3%, chloramphenicol – 6.3%, novobiocin – 6.3%). As shown by our experimental results, rep-PCR with the (GTG)5 primer is an applicable tool for typing of bacterial strains and may be used for identifying the source of contamination.

Keywords: rep-PCR; typing; meat; staphylococci; spice

INTRODUCTION

Molecular typing of bacteria is frequently used for estimation genetic relationship of the strains. Moreover, reliable identification and selection of individual bacterial strains provides a very valuable tool for further research of their dissemination and evolution. The possibility of identifying concrete pathogenic strain is very important and helpful also for effective control and monitoring of target pathogens. This can be practically utilised in protection of the food chain or for improvement of the food production technology. There are a number of methods applicable for typing bacterial strains in clinical microbiology **(Gherardy et al., 2015; Collins et al., 2015)**. They work on different principles like PCR-based fingerprinting, pulsed-field gel electrophoresis, ribotyping, multilocus variable number tandem repeat analysis, multilocus sequence typing, whole genome sequencing with the whole genome SNPs analysis or proteomic-based mass spectrometry. However, only some of those methods are suitable for realising of powerful, time and cost-effective typing experiment. Repetitive element sequence-based PCR (rep-PCR) represents an easy-to-perform technique utilising primers targeting repetitive sequence fragments dispersed in bacterial genomes **(Versalovic et al., 1998).** It may be practically utilised also for typing organism possessing genome of higher size, e.g. fungi **(Abdollahzadeh and Zolfaghari, 2014)**. Moreover, an automated system for typing bacteria working on the rep-PCR basis has been already developed and employed by several research teams for *S. aureus* and MRSA typing **(te Witt et al., 2009; Grisold et al., 2010).**

In our work, we investigated microbial parameters in meat paste production line at single Czech manufacturer. The *S. aureus* and *S. haemolyticus* strains were detected in raw heat-untreated meat paste. The rep-PCR method was applied for typing acquired staphylococcal isolates with the aim to identify the source of their spreading and bring knowledge about their dissemination.

MATERIAL AND METHODOLOGY

Samples

The primary group of analysed samples consisted of raw meat paste samples acquired before heat treatment and

from final meat paste samples at selected single Czech manufacturer. Based on the positive identification of pathogenic *S. aureus* strains in the raw meat paste, we decided to analyse the main meat paste components and ingredients: fresh meat, water, spices, and equipment swab samples. The swab samples were transported to the laboratory for further procedure in a cooling box. The following spices utilised during technological process of meat paste production were tested: black pepper, allspice, coriander, juniper, cumin, cinnamon, badian, white mustard, bay leaf, sweet paprika, rosemary, garlic, ginger, thyme, cardamom. The mentioned spices were not treated with ionizing irradiation and originated from different countries.

The representative *S. aureus* and *S. haemolyticus* strains of raw meat paste origin were included in the rep-PCR analysis to confirm the source of their spreading.

Microbiological analysis

The selective cultivation on the Baird Parker Agar (HiMedia, India) was used for detection of staphylococci (ČSN EN ISO 6888-1). Plates were incubated at 37 ±1 °C for 48 hours and colonies with zones of precipitation were submitted to the tube for free coagulase test. The suspected colonies were then inoculated onto Blood agar (Oxoid, Basingstoke, UK), cultivated at 37 °C for 24 h and subsequently identified biochemically using the STAPHYtest with the identification programs TNW Pro 7.5 (Erba Lachema, s .r.o., Brno, Czech Republic) to species level. Isolates identified as *S. aureus* were confirmed by the multiplex PCR targeting specific fragment SA442 according to **Martineau et al., (1998)**. MRSA and MR-CNS (methicillin resistant coagulase-negative staphylococci) were tested for presence of the *mecA* gene encoding the methicillin resistance phenotype as described by **Poulsen et al., (2003)**. The total count of *S. aureus* was determined in cfu per 1 g of prepared spice samples. The starting samples were prepared as follow: 5 g of spices (milled or powder) was dissolved in 45 g of sterile distilled water (the whole spices were crushed in the bowl before). The solution was filtered and the supernatant was used for analyses.

The antibiotic susceptibility testing to oxacillin, tetracycline, erythromycin, chloramphenicol, sulphamethoxazole-trimethoprim, amoxicillin-clavulanic acid, clindamycin, gentamicin, ciprofloxacin, vancomycin, teicoplanin, rifampicin, cefoxitin and novobiocin was determined by disc diffusion method following the recommendations of CLSI **(CLSI, 2012).**

Rep-PCR analysis

A loopful of individual colonies cultivated on the blood agar were transferred into clean tubes, resuspended in 180 µl solution of 20 mM Tris/HCl, 2 mM EDTA, 1% TRITON X-100 (pH 8), supplemented with 20 mg/ml lysozyme and incubated for 60 min at 37 °C. The genomic DNA was purified using the NucleoSpin Tissue kit (Macherey Nagel Inc., France); concentration of individual samples was measured on the Nanodrop 2000 instrument (Thermo Fisher Scientific Inc., DE, USA). The samples were diluted to obtain identical concentration 200 ng/µl. Rep-PCR analysis was realised with the (GTG)5 primer (5´-GTGGTGGTGGTGGTG- 3´) in 25 µl reaction mixture as follows: 1 µm primer; 1 x PPP PCR buffer containing Taq DNA polymerase (TopBio Ltd., CZE) and 1 µl of template DNA. Identical quantity of template DNA was added to each rep-PCR run to ensure maximal reproducibility of the results. The rep-PCR time and temperature profile comprised of initial denaturation 94 °C / 7 min; 30 cycles of: 94 °C / 1 min; 40 °C/1 min and 65 °C/8 min. The last cycle was followed by the final single extension step 65 °C / 16 min. Electrophoretic separation of rep-PCR products was realised in 1.5% agarose gel containing ethidium bromide (0.5 µg ml^{-1}) at 1.6 Vcm^{-1} for 12 hours. Molecular weight marker (GeneRuler 100 bp Plus, Thermo Fisher Scientific Inc., DE, USA) was positioned in lateral lines of every gel to allow later normalisation of gel images. The obtained image data with fingerprints were processed using the BioNumerics v. 6.6 software (Applied Maths NV, Belgium). The unweighted pair-group method using arithmetic averages (UPGMA) clustering method with the Dice correlation coefficient was utilized for interpretation of the results (BioNumerics, Applied Maths NV, Belgium). The fingerprints with similarity ≤90% have been considered to represent unique strain complexes. A single control *S. epidermidis* strain was used for artificial contamination of heat-treated meat paste, to evaluate the process of cultivation and rep-PCR analysis.

RESULTS AND DISCUSSION

1 Microbial contamination

Analysis of meat paste components and ingredients revealed, only spices used were positive for *S. aureus.* It was identified in whole coriander, cinnamon, badian and mustard; no SA was identified in final meat paste product. The observed numbers were low (30, 40, 20 and 10 $cfu \cdot g^{-1}$ respectively). The *S. haemolyticus* strains were cultivated also from spices (juniper, ginger) and a single *S. haemolyticus* strain was identified in final meat paste. The results indicated that the meat paste production technology involving heat treatment was able to successfully eliminate contaminants present in input ingredients. The overview of investigated spices with the results of microbial analysis is shown in Table 1. Based on the primary microbial analysis of samples in our study, we collected sixteen staphylococcal strains for further investigation (*S. aureus* (n = 4), *S. haemolyticus* (n = 4) and *S. epidermidis* (n = 8)). This was supplemented with the *S. aureus* and *S. haemolyticus* strain previously identified in raw meat paste. The overview of analysed strains and their origin is shown in Table 2.

As compared to our results, **Shamsuddeen (2009)** found out high *Staphylococci* occurrence in the mixture of spice composed from ginger, cloves, black pepper, groundnut, salt and seasoning. The geometric mean was $1.73 \cdot 10^{9}$ $cfu \cdot g^{-1}$ but they didn't specify the staphylococcal species. **Tulu et al., (2014)** detected low numbers (4 ±0.8 $cfu \cdot g^{-1}$) of *Staphylococci* in Red chilies (*Capsicum spp*.) and Turmeric (*Curcuma longa*) samples in Ethiopia. **Sospedra et al., (2010)** analysed thirty types of different spice in Spain, 7% of the samples was *S. aureus* positive. Positive detection of *S. aureus* in spice was observed also in study from Brazil **(Moreira et al., 2009).**

Table 1 Microbiological analysis investigating *Staphylococcus spp.* in spices used in meat paste production .

species	form	name	origin	species/ strain number	(cfu/g)	rep-PCR fingerprint type
allspice	whole	*Pimenta dioica*	Mexico	neg		
allspice	ground		Mexico	neg		
badian	whole	*Illicium verum*	Vietnam	SA (621)	20	B
bay leaf	ground	*Laurus nobilis*	Turkey	neg		
black pepper	whole	*Piper nigrum*	Vietnam	neg		
black pepper	ground		Vietnam	neg		
cardamon	ground	*Elettaria cardamomum*	Guatemala	neg		
cinnamon	whole	*Cinnamomum*	Czech Republic	SA (620)	40	A
coriander	whole	*Coriandrum sativum*	Ukraine	SA (619)	30	A
coriander	ground		Ukraine	neg		
cumin	ground	*Carum carvi*	Czech Republic	neg		
garlic	powder	*Allium*	China	neg		
ginger	ground	*Zingiber officinale*	Nigeria	SH (625)	/	C
juniper	whole	*Juniperus communis*	Bosna-Hercegovina	SH (626)	/	D
juniper	ground		Macedonia	SH (624)	/	C
rosemary	ground	*Rosmarinus officinalis*	Morocco	neg		
sweet paprika	ground	*Capsicum*	Hungary	neg		
thyme	ground	*Thymus vulgaris*	Poland	neg		
white mustard	whole	*Sinapis alba*	India	SA (623)	10	A

$cfu \cdot g^{-1}$ = colony forming units per gram

Table 2 Overview of investigated *Staphylococcus spp.* strains and its origin.

strain number	origin	sampling	species
603	raw meat paste	Apr. 2014	SA
605	raw meat paste	Apr. 2014	SH
619	coriander whole	May 2014	SA
620	cinnamon whole	May 2014	SA
621	badian whole	May 2014	SA
623	white mustard whole	May 2014	SA
624	juniper ground	May 2014	SH
625	ginger ground	May 2014	SH
626	juniper whole	May 2014	SH
644	final liver meat paste	May 2014	SH
613	final meat paste	Dez. 2013	SE
614	final meat paste	Dez. 2013	SE
635	final roe meat paste	May 2014	SE
637	hog fresh meat	May 2014	SE
638	hog fresh meat	May 2014	SE
641	final liver meat paste	May 2014	SE
642	final liver meat paste	May 2014	SE
645	lamb liver	May 2014	SE

2 Antibiotic susceptibility

The profiles of ATB susceptibility in tested staphylococci strains bring relatively positive observation. Although 68.8%, resp. 56.2% of collected staphylococci isolates possessed resistance to erythromycin, resp. amoxicillin/clavulanic, other resistances observed were

minimally frequent: clindamycin – 12.5%, oxacillin – 6.3%, tetracycline – 6.3%, sulphamethoxazole-trimethoprim – 6.3%, chloramphenicol – 6.3%, novobiocin – 6.3% (Table 3). No *S. aureus* strain was confirmed as MRSA. Among the twelve *S. haemolyticus* and *S. epidermidis* strains, only single *S. haemolyticus* isolate was positive for the *mecA* gene and thus represented the MR-CNS (Methicillin Resistant Coagulase-Negative Staphylococci). In works of other authors and also in our previous study, the occurrence of MR-CNS among CNS of animal or human origin was more frequent **(Manga and Vyletělová, 2011; Vyletělová et al., 2011)**. **Huber et al., (2011)** detected MR-CNS in 48.2% of samples from livestock and chicken carcasses, in 46.4% of samples from bulk tank milk and minced meat, and in 49.3% of human samples. The *S. epidermidis* together with *S. haemolyticus* and other CNS (Coagulase-Negative Staphylococci) represent a species with common occurrence on skin or mucous membranes of live animals including human. The both species are human opportunistic pathogens. However, the CNS may be also donors of genetic elements encoding antibiotic resistance to other bacterial species including *S. aureus*. A numbers of works suggested, the MR-CNS have significant impact on evolution and spreading of the MRSA strains **(Berglund and Söderquist, 2008; Bloemendaal et al., 2010)**. The clinical CNS isolates of human origin often displays multiresistance, MR-CNS are widely frequent in hospitals and represent important carriers of methicillin resistance **(Barros et al., 2012)**. Therefore, monitoring of antibiotic resistances in CNS of both animal and human origin has its own importance and CNS are being explored by many scientists.

3 Rep-PCR analysis

The rep-PCR analysis of *S. aureus*, *S. haemolyticus* and *S. epidermidis* isolates obtained from spices, fresh meat and meat paste gave rep-PCR fragments ranged from 180 to almost 3000 bp (Figure 1A, 1B). As regards to species affiliation, all investigated strains have been successfully distinguished. The rep-PCR analysis of *S. aureus* (Figure 2) showed that the four *S. aureus* strains isolated from different types of spices belong to only two strain clusters (A, B). The strains in cluster type A showed 100% homology. Moreover, the high similarity (90%) between these two clusters indicates high relatedness among all four strains. With regard to different origin of spices positive for *S. aureus*, this finding is surprising. However, all spice products with positive *S. aureus* detection were derived from the same manufacturer. Therefore, we suppose the technology of final processing or packaging may be the source of contaminants. The high performance of rep-PCR method for typing bacterial strains from different species and origins was repeatedly confirmed. The *Staphylococcus spp.* rep-PCR typing experiments were successfully realised also by other authors **(Reinoso et al., 2008; Nordin et al., 2011).** With regard to this, we have to consider the *S. aureus* strains analysed in our work as very similar (resp. identical in one of the observed clusters). Other theoretic explanation of our findings may be a secondary contamination during sample analysis. Since we received the same results when repeated whole analysis of identical spice samples, this possibility seems to be unlikely (data not shown). Rep-PCR typing of four *S. haemolyticus* isolates coming from spices and final meat paste identified three unique strain clusters (C, D, E) (Figure 2). The two *S. haemolyticus* strains isolated from juniper and ginger (n. 624, 625) merged to the same strain cluster (C) with indicated 100% similarity. This refers to similar situation as described at *S. aureus* rep-PCR results. With other words, the presence of *S. haemolyticus* in juniper and ginger is connected in some way. In addition, the *S. haemolyticus* strain n. 644 from final meat paste representing unique fingerprint (E) featured with high homology (90%) to C type cluster. Third cluster (D type) representing with single *S. haemolyticus* strain n. 626 differed markedly from the others. Rep-PCR typing of

Table 3 Antimicrobial resistances in sixteen *Staphylococcus spp.* strains isolated in meat paste production line.

antimicrobial agents / species	SA	SA	SA	SA	SH	SH	SH	SH	SE	SE	SE	SE	SE	SE	SE	SE
strain n.	619	620	621	623	624	625	626	644	613	614	635	637	638	641	642	645
OX	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
TE	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
E	-	-	+	+	-	-	+	-	-	-	+	-	+	-	-	-
C	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
SXT	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
AMC	-	-	-	-	+	+	+	+	-	-	+	-	-	-	+	+
DA	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
NV	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+

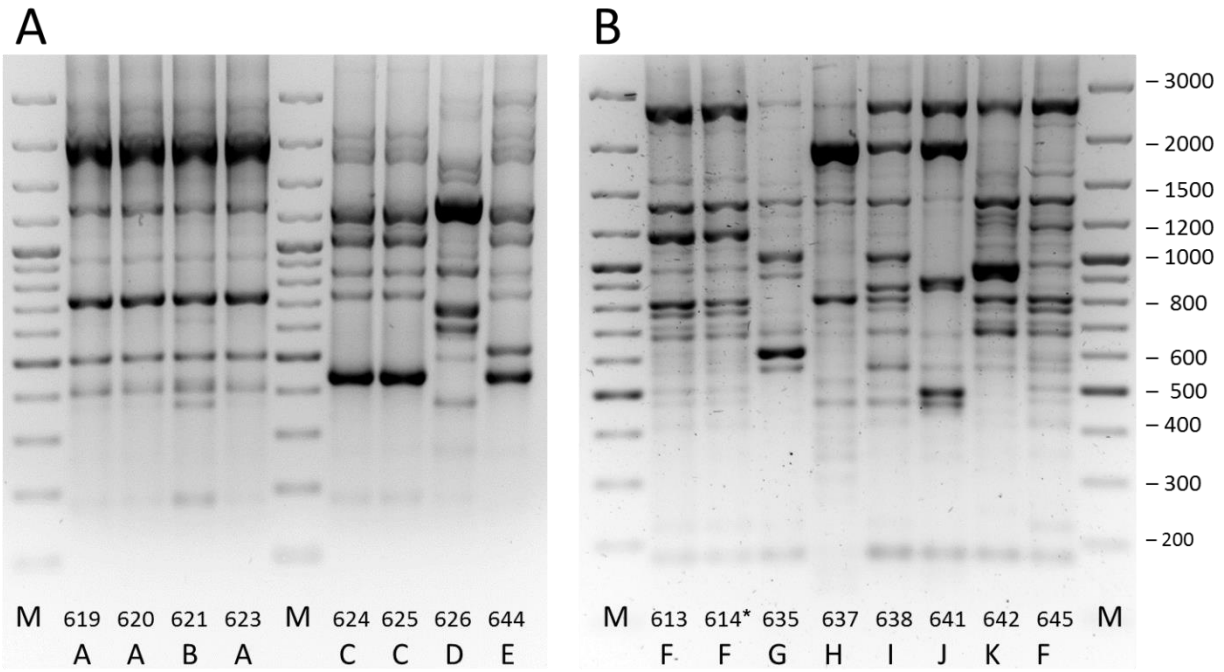
SA = *S. aureus*, SH = *S. haemolyticus*, SE = *S. epidermidis*, n = number, OX = oxacillin, TE = tetracycline, E = erythromycin, C = chloramphenicol, SXT = sulphamethoxazole-trimethoprim, AMC = amoxicillin-clavulanic acid, DA = clindamycin, NV = novobiocin; no resistance to gentamicin ciprofloxacin, vancomycin, teicoplanin, rifampicin and cefoxitin was observed

S. epidermidis isolated from meat paste and fresh meat supplemented the whole rep-PCR analysis and demonstrated discrimination power of the method for typing experiments. Among the eight *S. epidermidis* strains, six unique strain complexes were found (Figure 2). Comparison of all acquired fingerprints among sixteen isolates belonging to three staphylococcal species indicated eleven unique clusters of strains with similarity lover than 90%.

Comparative analysis of rep-PCR fingerprints among *S. aureus* strains from spices and *S. aureus* strain from raw meat paste revealed the strains were identical and belong to the same unique strain cluster (Figure 3). This provided evidence, that *S. aureus* cultivated from raw meat paste was originated in used spice. The both C and D fingerprint types characteristic for *S. haemolyticus* isolates from spices were detected also in *S. haemolyticus* isolates from raw meat paste; a representative rep-PCR analysis is indicated in Figure 3. This confirmed that the source of selected *S. haemolyticus* strains was the spice.

The control *S. epidermidis* strain (n. 614) used for artificial contamination of heat-treated meat paste was successfully cultivated and the rep-PCR analysis provided consistent result with expected fingerprint type (Figure 1 and 2). The whole process of DNA isolation and rep-PCR analysis of collected *S. aureus* and *S. haemolyticus* strains was performed twice to validate the reproducibility of the results. The acquired rep-PCR fingerprints in both independently repeated runs contained all strain-specific selective rep-PCR fragments and thus provided the identical results (data not shown). In some cases, the individual profiles of ATB susceptibility among rep-PCR-determined strain clusters differed a little. This is most probably due to existence of horizontal gene transfer, significantly affecting the antimicrobial phenotype of individual strains at given time **(Juhas, 2015)**.

As regards to primary strain processing and DNA isolation in rep-PCR analysis, the alkaline lysis of cultivated strains followed with direct rep-PCR analysis is an optional alternative because of rapid sample processing and DNA isolation **(Švec et al., 2008)**. However, the unevaluated or ignored heterogeneity in template DNA quality and quantity may negatively affect the amplification efficiency as well as reproducibility of the rep-PCR analysis. In particular the rep-PCR strain typing experiments require precise laboratory work. Applying of purified DNA samples with equivalent quantity increases the reproducibility and the discrimination power of the rep-PCR **(Manga and Vyletělová, 2012)**. The reliable rep-PCR identification of bacterial species seems to be easier to perform **(Gevers et al., 2001; Švec et al., 2010)**. The performance of rep-PCR method is affected also with the type of primer used for analysis. Besides the (GTG)5 primer **(Koreňová et al., 2009; Švec et al., 2010; Manga and Vyletělová, 2012)**, the REP1/ REP2 **(Zhong et al., 2009)**, ERIC **(Reinoso et al., 2008)**, RW3A **(Zee et al., 1999)** or BOX primers **(Begović et al., 2013)** have been utilised for typing the *Staphylococcus spp.* strains. **Sabat et al., (2006)** compared performance of six different PCR-based methods in typing the *S. aureus* strains, the pulsed

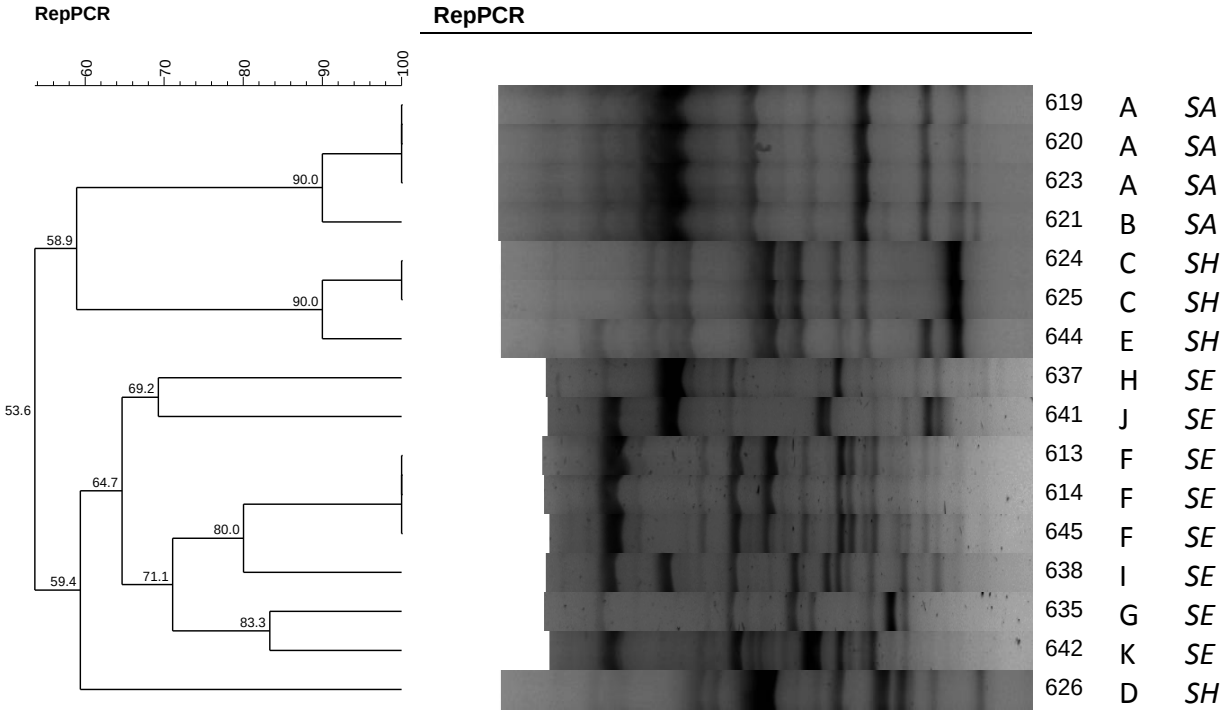


strain n. 619, 620, 621, 623 – *S. aureus*; strain n. 624, 625, 626, 644 - *S. haemolyticus*; 613, 614*, 635, 637, 638, 641, 642, 645 - *S. epidermidis*, M – ladder 100 plus (Thermo Fisher Scientific Inc., DE, USA); 614* = control strain of *S. epidermidis* (= strain 613 utilised for artificial contamination of heat-treated meat paste sample, repeatedly cultivated and analysed using the rep-PCR method)

Figure 1 Electrophoresis of rep-PCR products characteristic for *S. aureus* and *S. haemolyticus* isolates (A) and for *S. epidermidis* isolates (B).

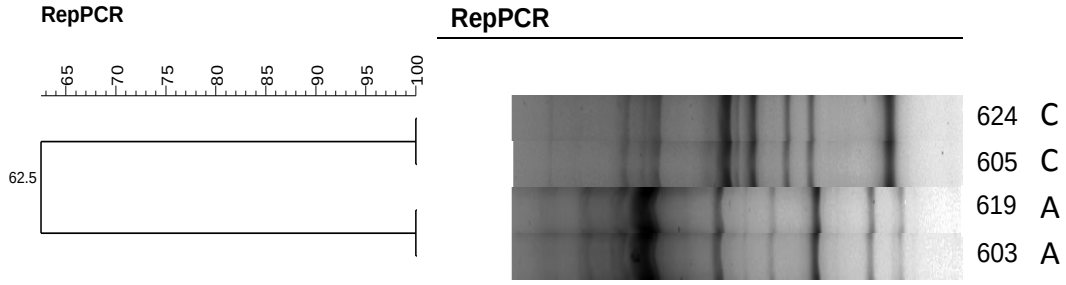
field gel electrophoresis was utilised as a reference method. The most representative results were obtained using the VNTR typing method. As we found out in our previous work, the performance of rep-PCR method in *S. aureus* typing experiment may be full comparable even superior to pulsed field gel electrophoresis or *spa* typing **(Manga et al., 2011)**. The other thing is that individual typing methods may provide results differing each other. With respect to evolution of current technology, application of whole genome sequencing methods may represent the most powerful and reproducible typing tool in the near future **(Salipante et al., 2015)**. However, these methods are still high cost and high technically demanding and usually require extensive skills in bioinformatics. In addition to this, the complex criteria and general rules for reproducible interpretation the NGS typing data have to be defined by scientific community.

As we found out, the rep-PCR typing is an alternative for low-demand and cost-effective analytic method, which may be useful in identifying the source of bacterial contamination. Besides the presented study, this may be illustrated with the work of **Zhong et al., (2009)** investigating spread of airborne *S. aureus* in and around chicken house using the rep-PCR. They found out that microbes in chicken feces can be aerosolized and spread indoor and outdoor, especially to downwind of the chicken houses.



SA = S. aureus, SH = S. haemolyticus, SE = S. epidermidis, A – K = determined strain clusters with similarity ≤ 90%

Figure 2 Clustering analysis of staphylococcal strains based on the rep-PCR data and the Applied Maths software (Applied Maths NV, Belgium); band-based UPGMA clustering (Dice correlation coefficient, Tolerance change: 1%).



cluster A type (100% similarity): 619 - *S. aureus* (coriander), 603 - *S. aureus* (raw meat paste); cluster C type (100% similarity): 624 – *S. haemolyticus* (juniper), 605 - *S. haemolyticus* (raw meat paste)

Figure 3 Confirmation the source of microbial contaminants in raw meat paste using the rep-PCR method.

CONCLUSION

We analysed meat paste production line in single Czech company to identify the source of staphylococci pathogenic strains, detected in raw meat paste. Isolates of target species *S. aureus* and *S. haemolyticus* were cultivated also from spices used in investigated manufacture.

With further rep-PCR analysis, we provided evidence that the staphylococci strains cultivated from raw meat paste were originated in spice. Moreover, molecular typing of collected isolates indicates presence of a homogeneous group of strains. This suggest on clonal spreading of the strains and their likely circulation in manufacturing or packaging link at spice manufacturer. Existence of adequate powerful diagnostic tools is one of the key prerequisite for effective monitoring of the food quality. As shown in our study, the rep-PCR method working with the (GTG)5 primer could be an effective and low cost analysis for typing microbial strains. Further, microbial contamination in spices suggests the need for regular inspection of the spices quality.

REFERENCES

Abdollahzadeh, J., Zolfaghari, S. 2014. Efficiency of rep-PCR fingerprinting as a useful technique for molecular typing of plant pathogenic fungal species: *Botryosphaeriaceae* species as a case study. *FEMS Microbiology Letters*, vol. 361, no. 2, p. 144-157. http://dx.doi.org/10.1111/1574-6968.12624 PMid:25307801

Barros, E. M., Ceotto, H., Bastos, M. C., Dos Santos, K. R., Giambiagi-Demarval, M. 2012. *Staphylococcus haemolyticus* as an important hospital pathogen and carrier of methicillin resistance genes. *Journal of Clinical Microbiology*, vol. 50, no. 1, p. 166-168. http://dx.doi.org/10.1128/JCM.05563-11 PMid:21976766

Begović, J., Jovčić, B., Papić-Obradović, M., Veljović, K., Lukić, J., Kojić, M., Topisirović, L. 2013. Genotypic diversity and virulent factors of *Staphylococcus epidermidis* isolated from human breast milk. *Microbiological Research*, vol. 168, n. 2, p. 77-83. http://dx.doi.org/10.1016/j.micres.2012.09.004 PMid:23098640

Berglund, C., Söderquist, B. 2008. The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette chromosome mec between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Clinical Microbiology and Infection*, vol. 14, no. 11, p. 1048-1056. http://dx.doi.org/10.1111/j.1469-0691.2008.02090.x PMid:19040477

Bloemendaal, A. L., Brouwer, E. C., Fluit, A. C. 2010. Methicillin resistance transfer from Staphylocccus epidermidis to methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy. *PLoS One*, vol. 29, no. 5, p. e11841. http://dx.doi.org/10.1371/journal.pone.0011841 PMid:20686601

Clinical and Laboratory Standards Institute (CLSI) 2012, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*, M100-S22, vol. 32, no. 3, CLSI, Wayne, PA, USA, [cit. 2015-03-06] Available at: http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf

Collins, D. A., Elliott, B., Riley, T. V. 2015. Molecular methods for detecting and typing of *Clostridium difficile*. *Pathology*, vol. 47, no 3, p. 211-218. http://dx.doi.org/10.1097/PAT.0000000000000238 PMid:25719853

ČSN EN ISO 6888-1: 2004. *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)* - Part 1: Technique using Baird-Parker agar medium ČNI Praha, 2004.

Gevers, D., Huys, G., Swings, J. 2001. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiology Letters*, vol. 27, no. 1, p. 31-36. http://dx.doi.org/10.1111/j.1574-6968.2001.tb10921.x PMid:11728712

Gherardi, G., Creti, R., Pompilio, A., Di Bonaventura, G. 2015. An overview of various typing methods for clinical epidemiology of the emerging pathogen *Stenotrophomonas maltophilia*. *Diagnostic Microbiology and Infectious Disease*, vol. 81. no. 3, p. 219-226. http://dx.doi.org/10.1016/j.diagmicrobio.2014.11.005 PMid:25592000

Grisold, A. J., Zarfel, G., Hoenigl, M., Krziwanek, K., Feierl, G., Masoud, L., Leitner, E., Wagner-Eibel, U., Badura, A., Marth, E., 2010. Occurrence and genotyping using automated repetitive-sequence-based PCR of methicillin-resistant *Staphylococcus aureus* ST398 in Southeast Austria. *Diagnostic Microbiology and Infectious Disease*, vol. 66, no. 2, p. 217-221. http://dx.doi.org/10.1016/j.diagmicrobio.2009.09.006 PMid:19828273

Huber, H., Ziegler, D., Pflüger, V., Vogel, G., Zweifel, C., Stephan, R. 2011. Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. *BMC Veterinary Research*, vol. 27, no. 6. http://dx.doi.org/10.1186/1746-6148-7-6 PMid:21272304

Juhas, M. 2015. Horizontal gene transfer in human pathogens. *Critical Reviews in Microbiology*, vol. 41, no. 1, p. 101-108. http://dx.doi.org/10.3109/1040841X.2013.804031 PMid:23862575

Koreňová, J., Lopašovská, J., Kuchta, T., 2009. Biofilm forming bacterial contaminants in small and medium-sized ewes'milk and meat processing enterprises in Slovakia. *Journal of Food and Nutrition Research*, vol. 48, no. 3, p. 115–120. [cit. 2015-03-09] Available at: http://www.vup.sk/index.php?mainID=2&navID=36&version=2&volume=48&article=914

Manga, I., Vyletělová, M. 2011. Occurence of antibiotic resistance at *S. haemolyticus*, *S. epidermidis* and *S. chromogenes* in the environment of milk dairy farms. *Cattle Research*, vol. 53, no. 4, p. 40-48. ISSN 0139-7265

Manga, I., Vyletělová, M., Karpíšková, R., Šťástková, Z. 2011. Molecular characterization of MRSA strains isolated from livestock milk and meat in the Czech Republic, In: *Book of abstracts from the SfAM Summer Conference*, Dublin, Ireland, 4–7.7. 2011, P85, p. 55-56.

Manga, I., Vyletělová, M. 2012: Rep-PCR-based typing as a tool for tracking of MRSA infection origin. *Acta Universitatis Agriculturae et Silviculturae Mendeleianae Brunensis*, vol 60, no. 6, p. 251-256. http://dx.doi.org/10.11118/actaun201260060251

Martineau, F., Picard, F. J., Roy, P. H., Ouellette, M., Bergeron, M. G. 1998. Species-specific and ubiquitous-DNA-

based assays for rapid identification of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, vol. 36, no. 3, p. 618-623. Available at: http://jcm.asm.org/content/36/3/618.long PMid:9508283

Moreira, P. L., Lourenção, T. B., Pinto, J. P., Rall, V. L. 2009. Microbiological quality of spices marketed in the city of Botucatu, São Paulo, Brazil. *Journal of Food Protection*, vol. 72, no. 2, p. 421-424. PMid:19350991

Nordin, S., Wahab, Z., Hussin, S., Rahman, M. 2011. Molecular characterization of nosocomial methicillin resistant staphylococci aureus by Rep-PCR. *The Internet Journal of Infectious Diseases*, vol. 9, no. 2, p. 1-10. [cit. 2015-03-04] Available at: https://ispub.com/IJID/9/2/4041

Poulsen, A. B., Skov, R., Pallesen, L. V., 2003. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. *J. Antimicrob. Chemother*, vol 51, no. 2, p. 419-421. http://dx.doi.org/10.1093/jac/dkg084 PMid:12562714

Reinoso, E. B., El-Sayed, A., Lämmler, C., Bogni, C., Zschöck, M. 2008. Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. *Microbiological Research*, vol. 163, no. 3, p. 314-322. http://dx.doi.org/10.1016/j.micres.2006.05.013 PMid:16930967

Sabat, A., Malachowa, N., Miedzobrodzki, J., Hryniewicz, W. 2006. Comparison of PCR-based methods for typing *Staphylococcus aureus* isolates *Journal of Clinical Microbiology*, vol. 44, no. 10, p. 3804-3807. http://dx.doi.org/10.1128/JCM.00395-06 PMid:17021116

Salipante, S. J., SenGupta, D. J., Cummings, L. A., Land, T. A., Hoogestraat, D. R., Cookson, B. T. 2015. Application of whole genome sequencing for bacterial strain typing in molecular epidemiology. *Journal of Clinical Microbiology*, vol. 53, no. 4, p. 1072-1079. http://dx.doi.org/10.1128/JCM.03385-14 PMid:25631811

Shamsuddeen, U. 2009. Microbiological quality of spice used in the production of kilishi a traditionally dried and grilled meat product. *Bayero Journal of Pure and Applied Sciences*, vol. 2, no. 2, p. 66-69. http://dx.doi.org/10.4314/bajopas.v2i2.63767

Sospedra, I., Soriano, J. M., Mañes. J. 2010. Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. *Plant Foods for Human Nutrition*, vol. 65, no. 4, p. 364-368. http://dx.doi.org/10.1007/s11130-010-0186-0 PMid:20848208

Švec, P., Nováková, D., Žáčková, L., Kukletová, M., Sedláček, I., 2008. Evaluation of (GTG)5-PCR for rapid identification of *Streptococcus* mutans. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, vol. 94, no. 4, p. 573-579. http://dx.doi.org/10.1007/s10482-008-9275-6 PMid:18709544

Švec, P., Pantůček, R., Petras, P., Sedláček, I., Nováková, D., 2010. Identification of *Staphylococcus* spp. using (GTG)(5)-PCR fingerprinting. *Systematic and Applied Microbiology*, vol. 33. no. 8, p. 451-456. http://dx.doi.org/10.1016/j.syapm.2010.09.004 PMid:21095086

Tulu, S., Demissie, S., Dabassa, A. 2014. Microbial Safety of some Selected Spices Sold in Jimma Town, South Western of Ethiopia. *Research Journal of Microbiology*, vol. 9, no. 1, p. 43-50. http://dx.doi.org/10.3923/jm.2014.43.50

Versalovic, J., De Bruijn, F. J., Lupski, J. R. 1998: Repetitive Sequence-based PCR (rep-PCR) DNA Fingerprinting of Bacterial Genomes. *Bacterial Genomes*, p. 437-454. Springer Science & Business Media, ISBN 978-1-4613-7925-6 http://dx.doi.org/10.1007/978-1-4615-6369-3_34

Vyletělová M., Vlková, H., Manga, I. 2011. Occurrence and characteristics of methicillin resistant *Staphylococcus aureus* and methicillin resistant coagulase-negative *Staphylococci* in raw milk manufacturing. *Czech Journal of Food Sciences*, vol. 29, p. 11-16. [cit. 2015-03-09] Available at: http://www.agriculturejournals.cz/publicFiles/54725.pdf

te Witt, R., Kanhai, V., van Leeuwen, W. B. 2009. Comparison of the DiversiLab™ system, pulsed-field gel electrophoresis and multi-locus sequence typing for the characterization of epidemic reference MRSA strains. *Journal of Microbiological Methods*, vol. 77, no. 1, p. 130–133. http://dx.doi.org/10.1016/j.mimet.2009.01.009 PMid:19318062

van der Zee, A., Verbakel, H., van Zon, J. C., Frenay, I., van Belkum, A., Peeters, M., Buiting, A., Bergmans, A. 1999. Molecular genotyping of *Staphylococcus aureus* strains: comparison of repetitive element sequence-based PCR with various typing methods and isolation of a novel epidemicity marker. *Journal of Clinical Microbiology*, vol. 37, no. 2, p. 342-349. PMid:9889215

Zhong, Z., Chai, T., Duan, H., Miao, Z., Li, X., Yao, M., Yuan, W., Wang, W., Li, Q., Zucker, B.A., Schlenker, G. 2009. REP-PCR tracking of the origin and spread of airborne *Staphylococcus aureus* in and around chicken house. *Indoor Air*, vol. 19, no. 6, p. 511-516. http://dx.doi.org/10.1111/j.1600-0668.2009.00618.x PMid:19840144

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