Characterisation of Bacterial Isolates in Cheese Production Surveillance in an Organic Farming Site

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Objectives: During the last decade, organic farming became more and more popular in Germany. Therefore, demand for active surveillance of potential microbial contamination throughout the production process in such facilities increased accordingly. In cheese production from sheep milk, for instance, it is not only necessary to screen the final product, but also to screen the milk and the udders of the animals are recommended. Furthermore, transmission of pathogens from the farmers and their families to live-stock and to raw milk are another potential source of contamination and have to be ruled out.

Methods: At an organic farm, milk and cheese samples as well as swabs from sheep and farmers and their families were collected. Subsequently, they were subjected to analysis using standard microbiological screening as well as molecular biological techniques, such as PCR, DNA sequencing and diagnostic microarrays to characterise and assign the bacterial isolates.

Results: By growing the different samples from cheese, milk, humans and sheep on Columbia blood agar plates and subsequent microbiological and molecular analysis, 13 different bacterial species were identified and confirmed by DNA sequencing, PCR and/or diagnostic microarrays. Most of them belonged to the genus *Staphylococcus*, representing the species *S. warneri, S. chromogenes, S. fleurettii, S. xylosus, S. equorum, S. epidermidis* and *S. aureus*. But also other bacteria as *Kocuria* spec., *Acinetobacter* spec., *Moraxella* spec., *Corynebacterium* spec. and *Streptococcus pyogenes* were identified. All staphylococci were analysed for 334 different sequences (~200 genes and alleles thereof) representing species, virulence and antimicrobial resistance markers using the StaphyType - Kit. By this method, a unique fingerprint of a *S. aureus* isolate was generated allowing to trace transmission of the agent. No MRSA or PVL positive isolate was detected. Most interestingly, the isolates from a human skin lesion swab and from the cheese samples were completely identical. It was an *agr*IV / CC121, capsule type 8 isolate that contained no antimicrobial resistance genes but yielded signals for enterotoxins I, M, N, O and U. PVL and *mecA* were not present. Another isolate cultured from a human nasal swab was identified by 16S rRNA gene sequencing as *S. fleurettii*. It was *mecA* positive and additionally contained the genes *mecI, mecR, ugpQ* and *xylR*. The isolate 34 from sheep was *agr*I / cc133 MSSA containing enterotoxins C and L as well as leukocidin components *lukM* and *lukF-P83*.

Discussion: The surveillance of organic farming, *e.g.*, cheese production is necessary and can be done economically and fast with the described techniques. A diversity of different bacteria was detected and humans were shown to be a potential source for food contamination. This is highly relevant if one takes the potential of antimicrobial resistance and enterotoxins / food poisoning into account. Furthermore, a *mecA* positive *S. fleurettii* was found as a potential source for a SCCmec element. This has, to our knowledge, not yet been described.

History and Analysis (Table 1):

History: From an organic farming site a sample of cheese suspected to be contaminated with bacteria, was send to the FLI. Analysis revealed *Staphylococcus aureus*. As a consequence, samples were collected from sheep, milk, farmers and related persons and subsequently analysed.

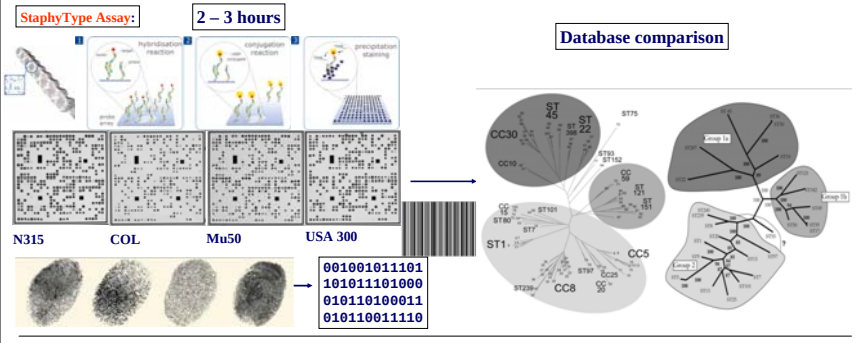
Sample analysis: Samples were taken in order to the listing in table 1. Samples 1 – 5 originated from sheep milk and wounds. The flock size was 24. A swab was collected from every dug of the udder and analysed if staphylococci were identified. Samples 6 – 24 were of human origin, samples 6 – 12 came from the farmer and her family. Samples 13 – 24 were taken from contact persons of the family. At the same time, samples 35 and 36 were collected from accumulative milk at the farm. Samples 25 – 27 were analysed from the cheese sample sent to the FLI. Two weeks later, samples 28 – 30 were taken from the children of the farmer suffering from an obvious skin infection. At the same time sheep with lesions at the mouth and skin were sampled (31 – 34). All samples were collected and analysed within one month in 2008. In an early 2009 follow-up examination no *S. aureus* were detectable in cheese, milk and farmers.

Number	Source of the Sample	Clinical Picture	Culture /Haemolysis	16 S rRNA Gene Sequencing Result	StaphyType Microarray Test	Result	Typing	Resistance and Toxin Genes
1	Sheep / Milk Sample	Mastitis	Positive / Positive	*Staphylococcus warneri*	*Staphylococcus (non aureus)*	*Staphylococcus warneri*		
2	Sheep / Milk Sample	Mastitis	Positive / Positive	*Staphylococcus warneri*	*Staphylococcus (non aureus)*	*Staphylococcus warneri*		
3	Sheep / Milk Sample	Mastitis	Positive / Positive	*Staphylococcus warneri*	*Staphylococcus (non aureus)*	*Staphylococcus warneri*		
4	Sheep / Milk Sample	Mastitis	Positive / Positive	*Staphylococcus chromogenes*	*Staphylococcus (non aureus)*	*Staphylococcus chromogenes*		
5	Sheep / Lesion Swab	Lesion	Positive / Positive	*Staphylococcus chromogenes*	*Staphylococcus (non aureus)*	*Staphylococcus chromogenes*		
6	Human / Swab	Lesion	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
7	Human / Swab	Lesion	Positive / Negative	*Moraxella nonliquefaciens*	negative	*Moraxella nonliquefaciens*		
8	Human / Swab	Lesion	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
9	Human / Swab	Lesion	Positive / Negative	*Moraxella nonliquefaciens*	negative	*Moraxella nonliquefaciens*		
10	Human / Swab	Lesion	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
11	Human / Swab	Lesion	Positive / Negative	*Streptococcus pyogenes*	negative	*Streptococcus pyogenes*		
12	Human / Swab	Lesion	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	ST 121 MSSA	*entI, entM, entN, entO, entU*
13	Human / Swab	Lesion	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
14	Human / Nasal Swab	None	Positive / Negative	*Staphylococcus fleurettii*	*Staphylococcus (non aureus)*	*Staphylococcus fleurettii*		*mecA (mecI, mecR, xylR, ugpQ)*
15	Human / Nasal Swab	None	Positive / Negative	*Corynebacterium spec.*	negative	*Corynebacterium spec.*		
16	Human / Nasal Swab	None	Positive / Negative	*Staphylococcus xylosus*	*Staphylococcus (non aureus)*	*Staphylococcus xylosus*		
17	Human / Nasal Swab	None	Positive / Negative	*Moraxella spec.*	negative	*Moraxella spec.*		
18	Human / Nasal Swab	None	Positive / Negative	*Actinobacterium spec.*	negative	*Actinobacterium spec.*		
19	Human / Nasal Swab	None	Positive / Negative	*Staphylococcus fleurettii*	*Staphylococcus (non aureus)*	*Staphylococcus fleurettii*		*mecA (mecI, mecR, xylR, ugpQ)*
20	Human / Nasal Swab	None	Positive / Negative	*Staphylococcus epidermidis*	*Staphylococcus (non aureus)*	*Staphylococcus epidermidis*		
21	Human / Throat Swab	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
22	Human / Nasal Swab	None	Positive / Negative	*Staphylococcus epidermidis*	*Staphylococcus (non aureus)*	*Staphylococcus epidermidis*		
23	Human / Nasal Swab	None	Negative					
24	Human / Swab	Lesion	Negative					
25	Cheese	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	ST 121 MSSA	*entI, entM, entN, entO, entU*
26	Cheese	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	ST 121 MSSA	*entI, entM, entN, entO, entU*
27	Cheese	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	ST 121 MSSA	*entI, entM, entN, entO, entU*
28	Human / Nasal Swab	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
29	Human / Nasal Swab	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 22 MSSA	*blaZ, entG, entM, entN, entO, entU*
30	Human / Nasal Swab	None	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	ST 121 MSSA	*blaZ, entCM14, entG, entM, entN, entO, entU*
31	Sheep / Nasal Swab	None	Positive / Negative	*Mycobacterium spec.*	negative	*Mycobacterium spec.*		
32	Sheep / Nasal Swab	None	Positive / Negative	*Kocuria spec.*	negative	*Kocuria spec.*		
33	Sheep / Nasal Swab	None	Positive / Negative	*Paenibacillus spec.*	negative	*Paenibacillus spec.*		
34	Sheep / Nasal Swab	Pus at Nose	Positive / Positive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	CC 133 MSSA	*luk-F-PV-P83/ lukM* (bovine PVL), *entC, entL, tst-1* (bovine *tst*)
35	Collected Milk Sample 1	None	Positive / Negative	*Staphylococcus equorum*	*Staphylococcus (non aureus)*	*Staphylococcus equorum*		
36	Collected Milk Sample 2	None	Positive / Negative	*Staphylococcus xylosus*	*Staphylococcus (non aureus)*	*Staphylococcus xylosus*		

Table 1:
Summary of all analysed samples. The same colours in column 1 (number) mark the same origin. The same colour in a complete row corresponds to the same strain with regard to all 334 sequences measured with the StaphyType Assay.

Results and Discussion:

From the collected samples a variety of different bacteria were detected. Interestingly, we found the identical ST121 MSSA strain in the contaminated cheese samples as well as in a skin lesion swab from the farmer. This clearly shows a possible transfer route within the cheese production at this farm. Cheese contamination with some bacteria is of great concern since, *e.g.*, staphylococci can contain many different toxin genes as *e.g. entA* and *entB* responsible for food poisoning and located on mobile genetic elements. Furthermore, cheese could be theoretically a source of infection or reservoir with more harmful strains like MRSA or PVL positive strains. We found a *mecA* positive *Staphylococcus fleurettii* coexisting in the same farm with different MSSA. This bears the theoretical risk of a transfer of the *mecA* gene resulting in a MRSA. Altogether active surveillance is advisable.



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