Supplementary data

Staphylococcus aureus Sequences from Osteomyelitic Specimens of a Pathological Bone Collection from Pre-Antibiotic Times

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Supplementary Text

S1. Primer design

To ensure the specificity of the final analysis system BLASTN 2.2.32+ search [1,2] with the newly designed primers was carried out and control experiments were conducted with the primer sets and amplification parameters as described in the paper,. To test the specificity of the PCR amplification system several different control samples were chosen:

- two commercially available human control DNAs (Abi 007 control DNA, Applied Biosystems® and 9947A control DNA, Promega), to check for any unspecific interaction of the primers with the human genome,
- two prehistoric human DNA extract (Do 901 9/99, from the Lichtenstein cave near Osterode, Lower Saxony Germany) to check for suitability of the primer system to investigate archaeological skeletal material.
- DNA extracts of two different soil samples to check for any interaction of the primers with various genomes of bacteria

The amplification success was checked via gel electrophoresis. As an example we show one of our PCR amplification of the *nuc* locus where the two Do 901 9/99 extracts were amplified alongside the pathological samples (figure A).

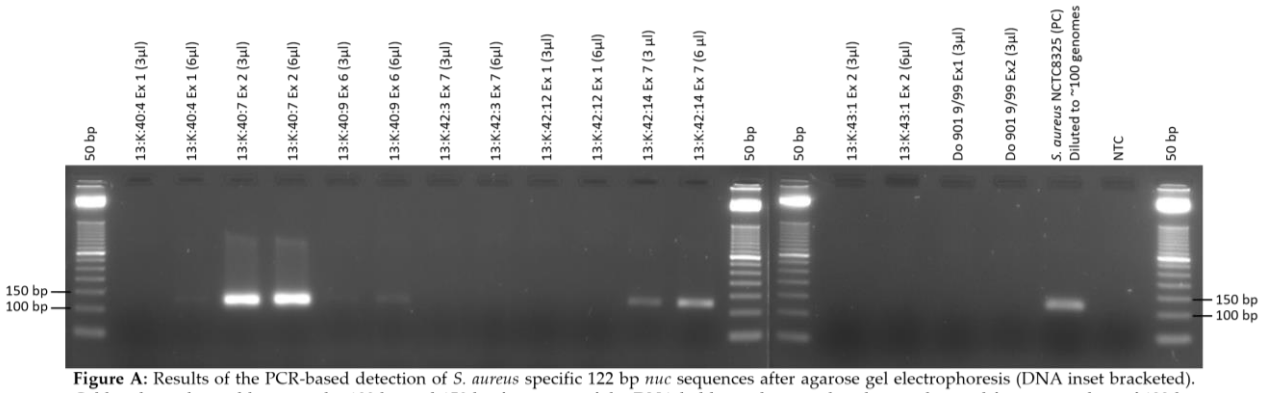

Figure A: Results of the PCR-based detection of *S. aureus* specific 122 bp *nuc* sequences after agarose gel electrophoresis (DNA inset bracketed). Gel bands are located between the 100 bp and 150 bp fragments of the DNA ladder, indicating that the sought amplification product of 122 bp was generated. 7/16 amplifications resulted in visible bands; the control samples Do 901 9/99 Ex1 and Ex2 showed no evidence of *S.aureus* DNA sequences; the negative template control (NTC) shows no evidence of pathogenic DNA presence in the PCR reaction mix. As a positive control DNA of the strain NCTC8325 (approximately 100 genomes) was used. Electrophoresis parameters: 8 µl PCR product with 2 µl Loading Dye, 4 µl size standard: 50 bp DNA ladder (Invitrogen™), 2.5% gel, 120 V, exposure time: 0.8 seconds.

Furthermore, to ensure both, sensitivity and specificity of the analysis system, several DNA extracts from the above mentioned control samples were spiked with low concentrations (10-100 genomes) of *S. aureus* DNA (strain NCTC 8325). Every spiking experiment resulted in a PCR product with expected fragment length (analysed in gel electrophoresis), ensuring both, sensitivity and specificity of the primers (data not shown).

To screen for any contaminants in extraction reagents or laboratory disposables extraction blanks were prepared and tested with the *nuc* amplification system. Additionally, swab samples from the environment of the pathological samples were extracted and tested (figure B):

- swab: swab blank control
- cupboard: storage unit of the pathological collection
- storage box 11:K:9 and 11:K:16:2: boxes where two samples unrelated to any infectious diseases were stored
- swab 11:K:9 in: swab from the inner surface of scull 11:K:9 (scull base trauma)
- swab 11:K:9 out: swab from the outer surface of scull 11:K:9 (scull base trauma)
- extraction blank 1 to 3
- 11:K:8 Ex1, 11:K:11 Ex1, and 11:K:16:2 Ex1: DNA extracts of pathological samples from the trauma collection

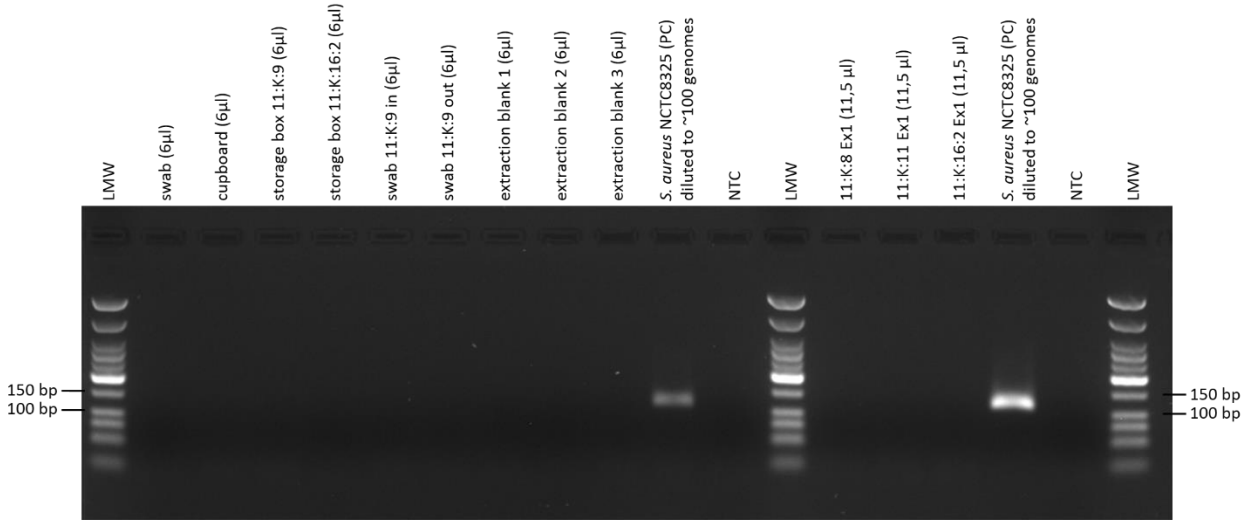

Figure B: Results of the PCR-based detection of *S. aureus* specific 122 bp *nuc* sequences in several control samples and extraction blanks after agarose gel electrophoresis (DNA inset bracketed). Two PCRs (differing in cycling parameters) are displayed (separated by LMW size standards). On the left the PCR was performed by using 40 cycling steps whereas samples on the right were amplified with 45 cycling steps. Gel bands are located between the 100 bp and 150 bp fragments of the DNA ladder, indicating that the sought amplification product of 122 bp was generated in the positive control DNAs (NCTC8325, approximately 100 genomes); the negative template control (NTC) shows no evidence of pathogenic DNA presence in the PCR reaction mix. Neither of the control samples show evidence of *S. aureus* DNA. Electrophoresis parameters: 8 µl PCR product with 2 µl Loading Dye, 4 µl size standard: Low Molecular Weight (New England Biolabs® GmbH), 2.5% gel, 100 V, exposure time: 1.0 seconds.

S2. DNA extraction from osteomyelitic bone specimens

In our department it is a common practice that every DNA extract from a specimen is numbered consecutively, regardless of which investigation it was sampled in. Three bone specimens (13:K:40:9, 13:K:42:3, and 13:K:42:14) were sampled in previous investigations, hence, extract numeration for this study starts with Ex6 and Ex7 (cf. supplementary text table S1). Furthermore, during this investigation several DNA extraction protocols were tested and some proved to be less suitable and were thus not used for PCR-based detection of specific *S. aureus* sequences (cf. supplementary text table S1, [1] and [2]).

Table S1: DNA extracts of the historic bone specimens produced during this investigation

Bone specimen (Ex=Extracts produced in this study)	Sample position	Extraction protocol
13:K:40:4		
Ex1	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex2	diaphysis	QIAvac-System (Qiagen)[1]
Ex3	metaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex4	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex5	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex6	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
13:K:40:7		
Ex1	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex2	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex3	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex4	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
13:K:40:9		
Ex6	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex7	diaphysis	QIAvac-System (Qiagen)[1]
Ex8	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex9	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex10	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
13:K:42:3		
Ex7	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex8	diaphysis	QIAvac-System (Qiagen)[1]
Ex9	metaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex10	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex11	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex12	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction

Table S1: continued

Bone specimen (Ex=Extracts produced in this study)	Sample position	Extraction protocol
13:K:42:12		
Ex1	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex2	diaphysis	QIAvac-System (Qiagen)[1]
Ex3	metaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex4	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex5	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex6	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
13:K:42:14		
Ex7	diaphysis	QIAvac-System (Qiagen)[1]
Ex8	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex9	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex10	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex11	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
13:K:43:1		
Ex1	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction, lysozyme and boiling[2]
Ex2	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex3	metaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex4	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction
Ex5	diaphysis	QIAvac-System (Qiagen) with phenol-chloroform extraction

[1]Extraction protocol was not repeated and extract was not used for PCR based detection of specific *S. aureus* sequences

[2]Extraction protocol was not repeated due to technical problems. Extract was not used for PCR-based detection of specific *S. aureus* sequences

S3. Direct sequencing parameters

Direct sequencing was performed to verify the specificity of the successfully amplified sequences (as shown by gel electrophoresis) from the historical samples. The PCR products were purified by using either the MinElute™ PCR purification kit (Qiagen) following the manufacturer's protocol or enzymatically with a protocol as follows: 2.5 U rAPid (Roche) and 1 U Exo I (New England BioLabs®Inc.) was added to 7.5 µl of the PCR product. The purification reaction was performed in a Mastercycler® Gradient (Eppendorf) for 1 hour at 37 °C, following an enzyme denaturation step for 15 minutes at 75 °C. Following the sequencing reaction a second purification step was performed by using nucleoSEQ® columns (Macherey-Nagel) following the manufacturer's protocol.

Two PCR products (12:K:40:9 Ex 6 and 13:K:42:12 Ex3) were reamplified before sequencing reaction, following the protocol as described in this paper using only 30 cycling steps and 46 °C annealing temperature.

S4. Sample materials from the processors

To detect a possible *S. aureus* colonization of the processors (ALF and JM), nasal swabs were taken by Dr. Raimond Lugert (Department of Medical Microbiology of the Medical Centre of the University Göttingen), plated on blood agar and incubated. Cultures were determined by morphological differentiation.

To ensure that the generated allele profiles of the examined historical bone specimens are authentic and not caused by external contamination by a processor or another person who handled the bone specimens, STR typing was carried out. Hence, every DNA extract was analysed with a miniSTR multiplex system that meets the requirements of degraded DNA ([3], cf. chapter S5 STR-Typing). For the DNA extract preparation, buccal swabs were taken and the cotton part was transferred to approximately 400 µl G2 buffer and 5 µl proteinase K solution in Tris/HCl (pH 7.5, 0.01 mol/l, 600 mAnson-U/ml, Merck). Incubation was performed in a thermal mixer (Comfort, Eppendorf) for 2 hours at 56 °C and 300 rpm (rotation per minute). The DNA extraction was automatically conducted via BioRobot® EZ1® (Qiagen) by accessing the trace protocol on the forensic card and using the Tissue Kit (100 µl elution volume). The DNA extracts were stored frozen at -20 °C.

S5. STR typing

STR typing was conducted with a short amplicon autosomal short tandem repeat (miniSTR) system [3]. The forward primer of each primer pair is labelled with a fluorescence dye (supplementary text table S2).

Table S2: Primer sequences of the miniSTR multiplex system [3]

STR locus		Primer sequence	Dye	Product length	Genbank accession
Amelogenin[1]	Up	5′-CCCTGGGCTCTGTAAAGAATAGTG-3′	6-FAM	X-allele: 87 bp	X: M55418
	Low	5′-AGCTGATGGTAGGAACTGTAAAAT-3′		Y-allele: 93 bp	Y: M55419
D13S317	Up	5′-CTAACGCCTATCTGTATTTACAAATA-3′	6-FAM	Allele 8-15:	AL353628
	Low	5′-AGCCCAAAAAGACAGACAGA-3′		102-130 bp	
D21S11	Up	5′-CAATTCCCCAAGTGAATTGC-3′	6-FAM	Allele 27-33.2:	AP000433
	Low	5′-GGAGGTAGATAGACTGGATAGATAGAC-3′		170-196 bp	
D18S51	Up	5′-CACTGCACTTCACTCTGAGTGAC-3′	HEX	Allele 10-20:	AP0001534
	Low	5′-GTGTGTGGAGATGTCTTACAATAACA-3′		152-192 bp	
D5S818	Up	5′-GGTATCCTTATGTAATATTTTGAAGAT-3′	NED	Allele 7-16:	AC008512
	Low	5′-ATCATAGCCACAGTTTACAACATT-3′		108-144 bp	
TH01	Up	5′-GCCTGTTCCTCCCTTATTTC-3′	NED	Allele 6-10:	D00269
	Low	5′-ATTCCGAGTGCAGGTCACAG-3′		87-103 bp	
FGA	Up	5′-AATAAAATTAGGCATATTTACAAGCTAG-3′	NED	Allele 18-26:	M64982
	Low	5′-ATTGCTGAGTGATTTGTCTGTAATTG-3′		149-181	

[1]Amelogenin dye labelling differing from **[3]**

Every PCR approach had a total volume of 25 µl, containing one part Qiagen Multiplex PCR Master Mix (Qiagen), the primer set containing every primer in a specific concentration (supplementary text table S3), DNA inset varied between 0.5 and 5 µl and RNAse-free water (Qiagen). Two-step cycling was performed in a Mastercycler gradient (Eppendorf) and cycling conditions were as follows: initial phase at 95 °C for 5 minutes, 40 cycling steps at 94 °C for 1 minute and 59 °C for 2.5 minutes, 60 °C for 45 minutes and soak for 10 minutes at 10 °C.

Table S3: Primer concentration in the miniSTR multiplex system [3]

Primer	**µM in PCR**
D13 S317 up	0.16
D13 S317 low	0.16
D21S11 up	0.24
D21S11 low	0.24
Amelogenin up	0.2
Amelogenin low	0.2
D18S51 up[1]	0.28
D18S51 low[1]	0.28
TH01 up	0.24
TH01 low	0.24
D5S818 up	0.15
D5S818 low	0.15
FGA up	0.32
FGA low	0.32

[1]Primer concentration differing from [3]

The allele determination was performed by capillary electrophoresis via 3500 Series Genetic Analyser (Applied Biosystems®), POP-7™ polymer (Applied Biosystems®) and a 50 cm capillary (Applied Biosystems®). Various quantities of the PCR products (between 0.1 and 2 µl) were used for capillary electrophoresis. The respective aliquot of the PCR products was added to 12 µl Hi-Di™ Formamid (Fluka) and 0.25 µl GeneScan™ 500 ROX™ dye (Applied Biosystems®). Data processing was applied with the GeneMapper5 (Applied Biosystems®) software.

Table S4: STR-Typing results of the bone specimen 13:K:40:4

Probe 13:K:40:4	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex1_2a	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex1_2b	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex1_2c	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex2_2a	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex2_2b	X/Y	(10)/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex2_2c	X/Y	(10)/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex3_3a	X/Y	n.a.[1]	28/-	13/(24)	8/9.3	9/12	22/24
Ex3_3b	X/Y	n.a.[1]	28/-	13/(24)	8/9.3	9/12	22/24
Ex4_14a	X/Y	10/-	-/-	13/(18)/(24)	6/8/9.3	(9)/-	22/24
Ex4_14b	X/Y	(10)/13	28/-	13/(24)	8/9.3	12/-	22/24
Ex4_14c	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex4_15a	X/Y	10/-	-/-	13/(24)	8/9.3	9/12	22/-
Ex4_15b	X/Y	(10)/13	28/-	13/(24)	8/9.3	9/-	22/24
Ex4_15c	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex5_34	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex6_34	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex1_39	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex3_39	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex5_39	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24
Ex6_39	X/Y	10/13	28/-	13/(24)	8/9.3	9/12	22/24

[1]n.a. = not analysed

- = no amplification product

Table S5: STR-Typing results of the bone specimen 13:K:40:7

Probe 13:K:40:7	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex1_14a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_14b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_14c	/-	-/-	-/-	-/-	9/-	-/-	-/-
Ex1_15a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_15b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_15c	X/-	-/-	-/-	-/-	-/-	-/-	21/-
Ex2_18a	X/Y	10/11/12	-/-	((13))/-	9/-	13/-	21/((23))
Ex2_18b	X/Y	11/12	((27))/29	13/-	9/-	10/-	20/21/23
Ex2_18c	X/Y	11/12	31.2/-	13/-	9/-	10/12	21/23
Ex3_34	X/Y	11/12	31.2/-	(9)/13	9/-	10/12	21/23
Ex4_34	X/Y	11/12	-/-	13/-	9/-	10/12	23/24
Ex2_39	X/Y	11/12	29/31.2	13/-	9/-	10/-	21/23
Ex3_39	X/Y	11/12	-/-	13/-	9/-	10/-	21/23
Ex4_39	X/Y	11/12	(29)/-	13/-	9/-	10/11/12	21/23
Ex5_6a	X/Y	11/12	-/-	13/-	9/-	-/-	21/23
Ex5_6b	X/Y	11/12	29/(30.2)/31.2	(9)/13	9/-	10/12	21/23
Ex6_6a	X/Y	12/-	-/-	13/-	9/-	-/-	21/23
Ex6_6b	X/Y	11/12	29/(30.2)/31.2	(9)/13	9/-	10/12	21/22/23

- = no amplification product

Table S6: STR-Typing results of the bone specimen 13:K:40:9

Probe 13:K:40:9	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex6_2a	Y/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex6_2b	X/Y	-/-	-/-	-/-	6/-	-/-	-/-
Ex6_2c	X/Y	-/-	-/-	-/-	6/-	-/-	-/-
Ex7_2a	Y/-	13/-	-/-	-/-	6/-	-/-	20/-
Ex7_2b	X/-	10/-	-/-	-/-	(6)/-	-/-	-/-
Ex7_2c	X/Y	-/-	-/-	-/-	6/-	-/-	((23))/-
Ex6_4	X/Y	10/12	-/-	-/-	6/10	-/-	-/-
Ex8_14a	Y/-	-/-	28/-	-/-	-/-	-/-	-/-
Ex8_14b	Y/-	-/-	-/-	-/-	(5)/-	-/-	-/-
Ex8_14c	Y/-	-/-	-/-	-/-	7/-	-/-	-/-
Ex8_15a	-/-	-/-	-/-	-/-	10/-	12/-	-/-
Ex8_15b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex8_15c	Y/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_34	X/Y	-/-	(27)/28	(9)/17	(6)/-	-/-	-/-
Ex10_34	Y/-	-/-	-/-	-/-	6/9.3	-/-	22/-
Ex6_28A	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex6_28B	X/Y	-/-	-/-	-/-	6/-	-/-	-/-
Ex6_39	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_39	Y/-	12/-	-/-	-/-	6/-	-/-	-/-
Ex10_39	Y/-	12/-	-/-	9/-	6/9.3	-/-	-/-

- = no amplification product

Table S7: STR-Typing results of the bone specimen 13:K:42:3

Probe 13:K:42:3	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex7_2a	X/Y	9/12	29/-	12/17	6/9.3	11/12	22/23
Ex7_2b	X/Y	9/12	29/-	12/17	6/9.3	11/12	22/23
Ex7_2c	X/Y	9/12	29/-	12/17	6/9.3	11/12	22/23
Ex8_2a	X/Y	9/12	29/-	12/17	6/9.3	11/-	22/23
Ex8_2b	X/Y	9/12	29/-	12/17	6/9.3	11/12	22/23
Ex8_2c	X/Y	9/12	-/-	(17)/-	6/9.3	11/(12)	22/23
Ex9_3b	-/-	n.a.[1]	-/-	-/-	6/-	-/-	-/-
Ex9_3a	-/-	n.a.[1]	-/-	-/-	(6.3)/8	-/-	-/-
Ex9_4	(X)/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_5a	X/-	-/-	-/-	-/-	8/-	-/-	-/-
Ex9_5b	X/-	9/-	-/-	-/-	6/9.3	-/-	-/-
Ex10_14a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_14b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_14c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_15a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_15b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_15c	-/-	-/-	-/-	-/-	-/-	-/-	20/-
Ex11_34	X/Y	9/12	-/-	12/17	6/9.3	11/(12)	22/23
Ex12_34	X/Y	9/12	29/-	12/17	6/9.3	11/-	22/23
Ex7_39	X/Y	9/12	-/-	12/17	6/9.3	11/12	22/23
Ex9_39	-/-	-/-	-/-	-/-	6/-	12/-	-/-
Ex11_39	X/Y	9/12	(28)/29	12/17	6/9.3	11/12	22/23
Ex12_39	X/Y	9/12	29/-	12/17	6/9.3	11/-	(22)/23

[1]n.a. = not analysed

- = no amplification product

Table S8: STR-Typing results of the bone specimen 13:K:42:12

Probe 13:K:42:12	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex1_2a	Y/-	(11)/12	32.2/-	15/-	8/9/9.3	12/-	21/-
Ex1_2b	(Y)/-	-/-	-/-	13/-	8/9	(12)/-	21/22
Ex1_2c	X/-	12/-	28.2/32.2	-/-	9/9.3	12/-	20/21
Ex2_2a	X/-	11/(12)	29/-	15/-	9.3/-	11/-	22/23
Ex2_2b	X/Y	12/14	-/-	-/-	-/-	-/-	24/-
Ex2_2c	Y/-	12/-	-/-	(13)/-	9.3/-	12/-	20/-
Ex3_3a	Y/-	n.a.[1]	-/-	((19))/-	-/-	-/-	21/-
Ex3_3b	-/-	n.a.[1]	-/-	-/-	-/-	-/-	-/-
Ex3_4	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex3_5a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex3_5b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex4_14a	-/-	-/-	-/-	-/-	-/-	(10)/-	-/-
Ex4_14b	-/-	-/-	-/-	-/-	8/-	-/-	-/-
Ex4_14c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex4_15a	(Y)/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex4_15b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex4_15c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_28A	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_28B	X/Y	-/-	32.2/-	-/-	7/9.3	-/-	-/-
Ex5_34	X/Y	(13)/14	-/-	-/-	-/-	-/-	-/-
Ex6_34	X/-	-/-	32.2/-	12/-	8/9.3	12/-	20/21
Ex1_39	X/Y	12/14	-/-	13/-	8/9.3	12/	20/-
Ex3_39	X/-	-/-	-/-	18/-	-/-	-/-	-/-
Ex5_39	X/Y	-/-	32.2	-/-	9/9.3	-/-	18/21/22
Ex6_39	X/Y	10/-	-/-	-/-	6/8/9	11/12	20/21

[1]n.a. = not analysed

- = no amplification product

Table S9: STR-Typing results of the bone specimen 13:K:42:14

Probe 13:K:42:14	**Amelogenin**	**D13S317**	**D21S11**	**D18S51**	**TH01**	**D5S818**	**FGA**
Ex7_2a	-/-	12/-	-/-	-/-	9.3/-	-/-	-/-
Ex7_2b	X/Y	12/-	-/-	-/-	9.3/-	-/-	22/-
Ex7_2c	X/Y	12/-	27/28	15/-	9/-	-/-	21/-
Ex8_2a	-/-	-/-	-/-	-/-	-/-	-/-	20/-
Ex8_2b	Y/-	11/-	-/-	-/-	8.3/9.3	12/-	-/-
Ex8_2c	X/Y	-/-	-/-	-/-	9.3/-	11/-	-/-
Ex7_4	X/-	14/-	28/-	15/16	9.3/-	-/-	22/-
Ex9_14a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_14b	-/-	-/-	-/-	-/-	9/-	-/-	-/-
Ex9_14c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_15a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_15b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex9_15c	Y/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_34	X/Y	12/-	-/-	11/-	9.3/-	15/-	-/-
Ex11_34	Y/-	11/-	28/31	18/19	9/9.3	-/-	20/22
Ex7_28A	Y/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex7_28B	X/-	-/-	(26)/-	-/-	6/8/9	-/-	-/-
Ex7_39	X/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex10_39	X/Y	-/-	-/-	-/-	7/-	11/-	21/-
Ex11_39	Y/-	11/12	-/-	12/-	8/9/9.3	11/-	-/-

- = no amplification product

Table S10: STR-Typing results of the bone specimen 13:K:43:1

Probe 13:K:43:1	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
Ex1_14a	X/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_14b	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_14c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_15a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex1_15b	-/-	-/-	-/-	-/-	(6)/-	-/-	-/-
Ex1_15c	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex2_18a	X/Y	-/-	-/-	-/-	(9)/-	-/-	21/-
Ex2_18b	X/Y	-/-	-/-	15/16	6/9	-/-	20/-
Ex2_18c	Y/-	10/11/13	-/-	-/-	6/9	11/-	24/-
Ex3_18a	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex3_18b	Y/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex3_18c	Y/-	10/13	-/-	-/-	6/-	-/-	-/-
Ex4_34	X/Y	10/-	-/-	15/-	6/9/9.3	-/-	21/22
Ex5_34	X/Y	10/13	28/30.2	-/-	6/9	-/-	24/-
Ex2_28A	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ex2_28B	-/-	10/12	-/-	-/-	6/-	-/-	-/-
Ex2_39	X/Y	10/13	28/-	15/16/18	6/9	12/-	21/-
Ex3_39	-/-	13/-	-/-	(17)/18	-/-	-/-	-/-
Ex4_39	X/Y	10/13	-/-	12/-	6/9	11/-	21/24
Ex5_39	X/Y	10/13	-/-	13/-	6/9	-/-	-/-

- = no amplification product

Table S11: Genetic fingerprint of the processors

Processor	Amelogenin	D13S317	D21S11	D18S51	TH01	D5S818	FGA
ALF	X/X	12/12	30.2/31	13/19	9/9.3	11/12	22/23
JM	X/X	8/10	29/32.2	12/16	8/9.3	12/12	22/25

S6. *spa* typing

Amplification of the X region of the protein A gene (spa) was initially done as described by Strommenger et al. [4], but showed no results visible by gel electrophoresis. Hence, cycling conditions and primer insets were adjusted to parameters used in the amplifications of the *nuc* and *fib* genes. Subsequent amplification reactions each consisted of a total volume of 25 µl with one part AmpliTaq Gold® 360 Master Mix (Applied Biosystems®), 0.4 µM of each primer (forward: spa-1113f 5′ TAA AGA CGA TCC TTC GGT GAG C 3′ and reverse: spa-1514r 5′ CAG CAG TAG TGC CGT TTG CTT 3′), and a DNA inset varying between 0.5 and 6 µl (DNA amount in the extracts varied because of the differential state of sample preservation). PCR conditions were as follows: initial phase at 94 °C for 7 minutes, 40 cycling steps consisting of 94 °C for 1 minute, 58 °C for 1 minute, and 72 °C for 1 minute, and soak for 10 minutes at 10 °C. The amplified PCR products were separated by gel electrophoresis. Run time was approximately 30 minutes at 100 V in a 2.5% gel in 1xTBE buffer.

Figure C shows the results of the amplification of the *spa* locus. Aside from the positive control (NCTC 8325, expected product length 419 bp) only sample 13:K:40:7 reveals a product (fragment size between 350 and 500 bp).

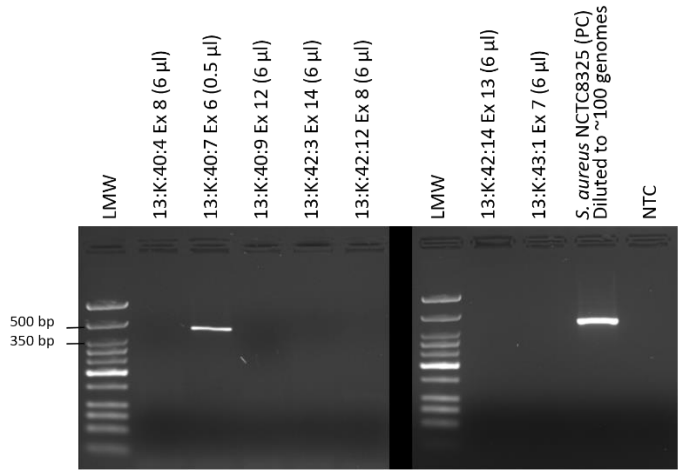

Figure C: Amplification results of the *spa* sequences after agarose gel electrophoresis (DNA inset bracketed). Gel bands are located between the 350 bp and 500 bp fragments of the DNA ladder. One PCR reaction (sample 13:K:40:7) resulted in a visible band; the negative template control (NTC) shows no evidence of pathogenic DNA presence in the PCR reaction mix. As a positive control DNA of the strain NCTC8325 (approximately 100 genomes) was used. Electrophoresis parameters: 8 µl PCR product with 2 µl Loading Dye, 4 µl size standard: Low Molecular Weight DNA Ladder (New England BioLabs®Inc.), 2.5% gel, 100 V, exposure time: 1.00 second.

S7. References

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