Molecular epidemiology of *Staphylococcus aureus* from milk of crossbreed dairy cows in North-West Ethiopia

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Abstract

Staphylococcus aureus (*S. aureus*) is an important cause of subclinical mastitis (SCM), which is a problem across the world, but especially in developing countries. The Ethiopian situation is different than in the Western world, as in Ethiopia many smallholders milk by hand. The aim of this research was to type *S. aureus* strains to learn more about the molecular epidemiology of *S. aureus* in North-West Ethiopia and discover if strains are clustered within herds or randomly distributed over herds. Also, the place of strains in the bigger family of *S. aureus* was discovered. A total of 169 strains which were probably *S. aureus* were included in this study and were screened for different genes. Of these strains, 81.7% was found to be *S. aureus.* In *S. aureus* strains, the proportion of LukMF' and of MRSA was 2.2% and 0.0%, respectively. The predominant strain in Gondar was t042, while in Bahir Dar t042 and t15786 were the predominant strains. Furthermore, a minimum spanning tree (MST) showed that all *spa* types were closely related to each other. In the majority of the herds clustering of *S. aureus* strains was found which proves the presence of within herd transmission. When Ethiopian strains were placed in the bigger family of *S. aureus* strains, half of *spa* types were related to strains derived from cows that contained clonal complex 97 (CC97). The other strains were related to strains derived from small ruminants which contained CC130, CC133 and CC522.

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List of abbreviations

Abbreviation	Meaning
CC	clonal complex
CLSI	clinical and laboratory institute
CMT	California mastitis test
HF	Holstein Friesian
MRSA	methicillin-resistant *Staphylococcus aureus*
MST	minimum spanning tree
MQ	milliQ
NCBI	National Center for Biotechnology Information
PFGE	pulsed-field gel electrophoresis
spa	protein A gene
S. aureus	*Staphylococcus aureus*
SCM	Subclinical mastitis

Introduction

Subclinical mastitis (SCM) is a worldwide problem among dairy cows, especially in developing countries (Abrahmsén et al. 2014). One of the most important causes of SCM is *Staphylococcus aureus* (*S. aureus*), which is probably the best studied mastitis pathogen (Barkema et al. 2006, Zadoks et al. 2011). From an economic point of view, research shows SCM has a higher negative impact than clinical mastitis (Abrahmsén et al. 2014). In fact, the pathogen in dairy cattle which causes the greatest economic losses is *S. aureus* (Scali et al. 2013). SCM induces a restriction for the further economic growth in developing countries, in particular because of production losses and decrease in livestock (Abrahmsén et al. 2014). Worldwide, much research to different *S. aureus* genotypes in mastitis milk is done (Anderson et al. 2012, Lundberg et al. 2014).

However, in Ethiopia, not much research according to mastitis has been done so far. The situation in Ethiopia differs from that in the Western world, as milking of cows happens manually. Besides, herd size in Ethiopia is often very small (Mekonnen et al. In preparation). Recent research in North-West Ethiopia showed a prevalence of SCM of 33% at quarter level and 62% at cow level based on the California Mastitis Test (CMT) (Mekonnen et al. In preparation). (Souza et al. 2012) have proven that milking machines, in particular the teat cup liners, contribute to the spread of infections.

However, Ethiopian farmers milk cows with their hands by squeezing or stripping without washing their hands in-between the milking of cows (Mekonnen et al. In preparation). In the same way as the teat cup liners, hands of farmers may also contribute to transmission of infections. The clustering of strains within herds suggests transmission of *S. aureus*. However, only the degree of transmission was investigated in this research and not the different transmission routes.

This study will focus on the molecular epidemiology of *S. aureus* in North-West Ethiopia, since Mekonnen found a high prevalence of SCM in this region compared to other studies (Haftu et al. 2012, Mungube et al. 2005). The diversity of *S. aureus* strains within and between herds gives an indication of the importance of within and between herd transmission (Zadoks et al. 2011). Because of this, mapping the different types of *S. aureus* strains in dairy herds in North-West Ethiopia, the importance of within- vs. between-herd transmission may be quantified. Tarekgne et al. (2016) have found *spa* types derived from bulk milk in Ethiopia, but did not look at cow or quarter level.

(Zadoks et al. 2011) have shown that the heterogeneity of virulence gene profiles differs between countries and even between regions. LukMF' is an important virulence factor and is found in genes of *S. aureus* strains which were derived from bovine mastitis (Barrio et al. 2006). As strains of *S. aureus* differ in their capacity to produce virulence factors, characterising strains by presence or absence of LukMF' may yield insight in their capacity to cause mastitis (Vrieling et al. 2015).

The principle aim of this research is to characterise Ethiopian *S. aureus* strains and discover if the *spa* types of *S. aureus* are clustered within herds or are randomly distributed over herds. Also, the number of samples which contain methicillin-resistant *Staphylococcus aureus* (MRSA) will be tested. This study will search for differences between types in Gondar and Bahir Dar. Also, the existence of an association between LukMF' with region (Gondar vs. Bahir Dar) or with the clinical severity of the mastitis will be studied. This information can be useful to develop new strategies to fight infections with *S. aureus* (Souza et al. 2012).

Materials and methods

Bacterial strains. Strains used in this research were obtained from previous research by Mekonnen et al. (in preparation), in which bacterial strains were derived from 400 crossbreed (Holstein Friesian (HF) with indigenous Zebu) dairy cows. Cows were located in Gondar and Bahir Dar, North-West Ethiopia. Finally, 1,523 samples were available for bacteriological culturing. This research focussed only on *S. aureus* strains (n = 169).

DNA-extraction. To grow the bacteria, 2 strains per blood-agar plate were inoculated. Plates were incubated at 37˚C overnight. The next day, a separate colony was taken to isolate DNA from. This colony was suspended in 1 mL of milliQ (MQ), vortexed and centrifuged on 13.000 rpm for 1 minute. Subsequently, 1 mL MQ was removed and 200 µL MQ was added to the cell pellet. This was vortexed again and heated with a heat block for 10 minutes at 100˚C afterwards. This suspension was centrifuged for 1 minute at 13.000 rpm. From the supernatant, 20 µL was taken and was put in a new Eppendorf tube with 180 µL MQ. This resulted in 200 µL 1:10 diluted DNA. These tubes were stored in the refrigerator while undiluted DNA was stored in the freezer at -25 ˚C.

Detection of the *femA, nuc, lukF, LukM* and *mec*A genes by PCR. A duplex PCR with primer sets for *femA* and *lukF* (Table 1) was performed. Therefore, PCR-master mix was made per unit consisting of 5 µL 5x GoTAQ green buffer, 1.5 µL $MgCl_2$ solution (25mM), 1.25 µL PCR Nucleotidemix (4 mM), 2.25 µL MQ, 1.25 µL *femA* forward primer, 1.25 µL *femA* backward primer, 1.25 µL *lukF* forward primer and 1.25 µL *lukF* backward primer. After this, 0.125 µL DNA-polymerase was added to the suspension. When the PCR-master mix was finished, it was added to the wells together with 10 µL of the 1:10 diluted DNA. Subsequently, tubes were centrifuged. Because of ongoing contamination using the duplex PCR, the *nuc* gene was also used to confirm that DNA was derived from *S. aureus*. The same PCR-mastermix was used, with the primer set for the *nuc* gene (Table 1). The volume of the second primer set was replaced by MQ. When strains were found positive for *lukF*, a PCR with the primer set for *lukM* (Table 1) was done. Finally, for *S. aureus* positive strains, a PCR was performed by using the primer set for the *mecA* gene (Table 1), the gold standard test for MRSA detection (Melo et al. 2014). Products were placed in the T100™ Thermal Cycler of Biorad to amplify the DNA by means of corresponding protocols (Table 1).

Gel-electrophoresis. A 1.5% agarose gel with 5 µL Midori Green Nucleic Acid Staining Solution was used to load 10 µL of the PCR-product and run at 100 V for 40 min. A GeneRuler™ 50/100-bp ladder (Thermo Scientific, Waltham, MA) was used. DNA products were shown with a molecular Imager® Gel Doc™ XR + Imaging system which was connected to a computer with the program Image Lab 5.1 (Bio-Rad).

***Spa* typing.** *Spa* typing was done by sequencing of the X region of the protein A gene (*spa*) (Shopsin et al. 1999). This was done for 65 randomly selected *femA* positive samples. First, a PCR was performed with the primer set for the *spa* gene (Table 1). Afterwards, DNA of samples which showed a result was cleaned up using 2 µL ExoSAP-IT each well with 5 µL DNA-product. This was put in the PCR machine at 37˚C for 15 min and 80˚C for 15 min. The PCR product was submitted for Sanger sequencing. Results were processed using BioNumerics version 7 (Sint-Martens-Latem, Applied Maths) and the sequence of *spa* types was found by using the Ridom SpaServer (http://www.spaserver.ridom.de). Furthermore, a minimum spanning tree (MST) was created by using the *spa* typing plugin of BioNumerics. The following mutations were considered; duplications, substitutions and indels. A cost matrix was made by the program and default settings were used. Afterwards, *spa* types were clustered according to the grey areas in the MST which

Table 1 - Sequences of primer sets with their corresponding PCR-protocol and product size

Target gene	Nucleotide sequence (5'-3')	PCR-protocol	Product size	Reference
femA	F: TGCCTTTACAGATAGCATGCCA R: AGTAAGTAAGCAAGCTGCAATGACC	1	142 bp	(Francois et al. 2003)
nuc	F: TCACAAACAGATAACGGCGTAAATAG R: CACTTGCTTCAGGACCATATTTCTC	2	235 bp	(Cressier et al. 2011)
mecA	F: GGCTATCGTGTCACAATCGTT R: TCACCTTGTCCGTAACCTGA	3	689 bp	(Melo et al. 2014)
lukF	F: ACTCAGGCTATACCCAACCCA R: CGAGCTACTCTGTCTGCCAC	1	425 bp	Developed in house
lukM	F: TGAGTGGGTATGGCATGAAAGA R: TGGACATTTTGTGTTACACCCC	2	572 bp	Developed in house
spa	F: AGACGATCCTTCGGTGAGC R: GCTTTTGCAATGTCATTTACTG	4	Variable	(Harmsen et al. 2003)

[1] 95°C for 2 min, 35x (95°C for 30 s, 59.5°C for 30 s, 72°C for 35 s), 72°C for 5 min and 12°C for ∞
[2] 95°C for 2 min, 33x (95°C for 30 s, 58.2°C for 30 s, 72°C for 30 s), 72°C for 5 min and 12°C for ∞
[3] 95°C for 1.5 min, 30x (95°C for 45 s, 55°C for 30 s, 72°C for 45 s), 72°C for 5 min and 12°C for ∞
[4] 95°C for 2 min, 30x (95°C for 45 s, 60°C for 45 s, 72°C for 1.30 min), 72°C for 5 min and 12°C for ∞

Table 2 - *spa* types with their repeat succession and prevalence

spa type	Repeat Succession	Prevalence (%)
t042	26-23-13-34-34-33-34	49.2 (32/65)
t15786	26-23-12-34-34-34-33-34	15.4 (10/65)
t355	07-56-12-17-16-16-33-31-57-12	4.6 (3/65)
t488	07-12-21-17-13-13-34-33-34	3.1 (2/65)
t1376	07-12-21-17-13-13-34-34-33-13	3.1 (2/65)
t14061	04-31-17	3.1 (2/65)
t223	26-23-13-23-05-17-25-17-25-16-28	1.5 (1/65)
t273	07-23-21-17-13-34-16-34-33-13	1.5 (1/65)
t306	26-23-17-34-17-20-17-12-17-17-16	1.5 (1/65)
t409	60-61-34-22-34-17	1.5 (1/65)
t3202	07-12-21-17-13-13-34-13-33-34	1.5 (1/65)
t3341	26-12-21-17-13-34-34-34-33-34	1.5 (1/65)
t4206	26-23-34-34-33-34	1.5 (1/65)
t4701	26-12-21-17-34-34-34-33-34	1.5 (1/65)
t9300	26-23-21-34	1.5 (1/65)
t10018	04-31-17-25-17-25-17	1.5 (1/65)
Unknown1	07-500-22-31-17	1.5 (1/65)
Unknown2	26-23-13-17-25-17-28	1.5 (1/65)
Unknown3	14-22-75-16-24-24-24-24-24	1.5 (1/65)
Unknown4	07-23-02-12-23-02-02-02-34	1.5 (1/65)

showed a close genetic relatedness between them (Figure 2). *Spa* types that were not invaded in a cluster according to the MST were placed in one group which was considered as remaining *spa* types.

Statistical analyses. Statistical analyses were performed for 116 samples. To test the different hypotheses the Fisher's exact test was used while a $p < 0.05$ was considered as significant. *Spa* cluster (4 categories according to the MST: 1, 2, 3, 4) was tested for an association with region (2 categories: Bahir Dar (0) vs. Gondar (1)), herd size (2 categories: ≤ 6 cows (0) and > 6 cows (1)), CMT-score (2 categories: N, T (0) and 1, 2, 3, C (1)), and Holstein blood level (3 categories: less than 25% (0), between 25-50% (1) and 50% or higher (2). Current mastitis status (2 categories: no current mastitis status present (0) and current mastitis status present (1)) was tested for an association with CMT-level. Also, clinically mastitic cow present (2 categories: no clinically mastitic cow present (0) and clinically mastitic cow present (1)) was tested for an association with CMT-level. Subsequently, region was tested for an association with *lukM* (2 categories: no *lukM* present (0) and *lukM* present (1). Finally, region was tested for an association with herd size and both regions were tested separate for an association between herd size and *spa* cluster. Statistical analyses were performed using SPSS Statistics 24 (Armonk, NY: IBM Corp).

Results

Proportion of genes. The proportion of *S. aureus* was 81.7% (138/169). The proportion of strains positive for the *lukF* gene was 4.3% (6/138) and 2.2% (3/138) for

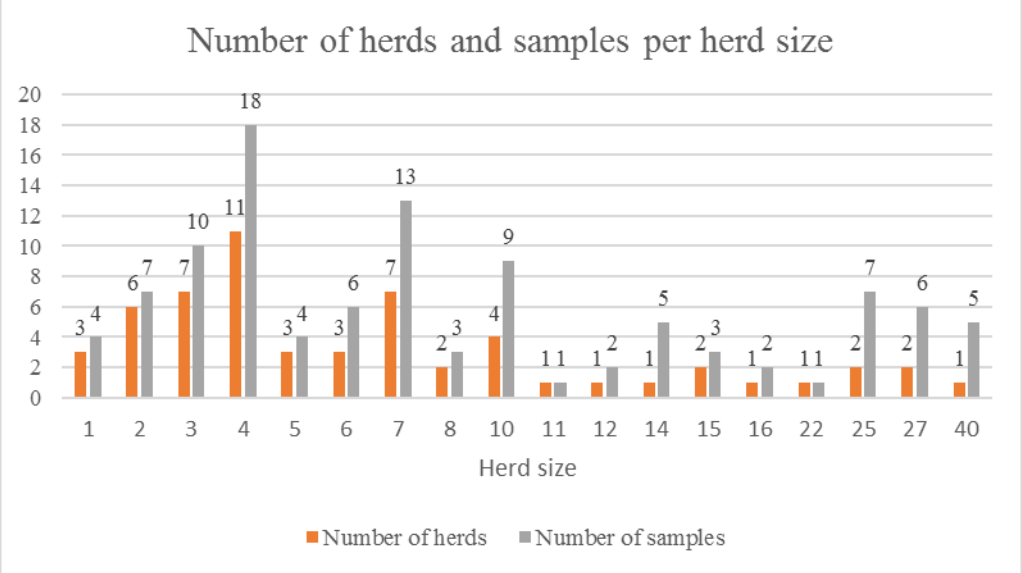

Figure 1: Distribution of the samples (n = 116) and number of herds (n = 58) among herd size. Herd size of 10 samples was unknown.

Table 3 - p-values of tested associations

Association	p-value
***Spa* cluster vs. region**	p = 0.009
S*pa* cluster vs. herd size	p = 0.015
- ***Spa* cluster vs. herd size (Bahir Dar)**	p = 0.017
- ***Spa* cluster vs. herd size (Gondar)**	p = 0.084
Region vs. herd size	p = 0.002
***Spa* cluster vs. CMT-score**	p = 0.033

the *lukM* gene. The LukMF' complex was only found in Gondar. Of the LukMF' complexes, 2 out of 3 were found in 2 different quarters of 1 cow while the third complex was found in another cow. Both cows were in different herds. The *mecA* gene was not found.

***spa* typing.** Of the 65 strains, a total of 20 different *spa* sequences were found (Table 2). Of these sequences, 4 have not been known in the Ridom SpaServer yet and can be classified as novel *spa* types. In Bahir Dar we found 39 strains and in Gondar 26. Although more *spa* types were found in Bahir Dar, in Gondar we found a greater number of different *spa* types. The predominant *spa* type for both regions is t042 (Figure 3). In Bahir Dar, t15786 is also a predominant strain (Figure 3).

Spa types were identified from 36 different herds. In general, the number of *spa* types per herd was small: in 22 herds, only 1 strain was found. In 9 herds 2 strains were found per herd. In 2 of these herds, 2 different *spa* types were detected which were not classified in the same cluster. However, in both herds, these *spa* types were closely related. In 7 herds, both strains were of the same *spa* type. We found 1 herd which contained 3 *spa* types which were all different. However, 2 of these *spa* types were in the same cluster. In 2 herds 4 strains per herd were found. In 1 of these herds all *spa* types were the same while in the other herd 2 different strains were found which are not in the same cluster. We found 1 herd with 5 *spa* types including 4 the same *spa* type and one differed. However, they were in the same cluster. In the herd with 6 *spa* types, all types were the same. The majority of the herds (78.6%) show clustering of *spa* types, but there is also clustering between herds.

Associations between *spa* cluster, region and herd size. Figure 1 shows the samples which were used for the statistical analyses. For the tested hypotheses, 5 significant associations were found (Table 3). It appears that *spa* cluster 1 (n = 43) is much more common in Bahir Dar compared to Gondar, with 31 and 12 samples respectively. Besides, *spa* cluster 2 is 6 times more present in Gondar than in Bahir Dar. For the 65 *spa* types, in Bahir Dar 10 strains were derived from smaller herds while 28 strains were found in the bigger herds. For Gondar, the opposite is true, most of the strains (17) were found in the smaller herds, while only 8 strains were found in the bigger herds. This makes a total of 27 strains in small herds and 36 strains in big herds. In both small and big herds, the predominant *spa* type was t042. The origin of 2 strains was unknown because of missing data.

Spa cluster 3 is not present in herd size > 6. In the bigger herds, *spa* cluster 1 is circa twice as common as in the smaller herds. When regions were tested separate for an association between herd size and *spa* cluster only in Bahir Dar a significant association was found. Here, the majority of the bigger herds were found in *spa* cluster 1 while in Gondar more small herds were found in *spa* cluster 1.

Furthermore, 100% of *spa* cluster 2 was found for a positive CMT-level while 75% of *spa* cluster 3 was found for a negative CMT-level. Finally, no association was found for LukMF' and the clinical severity of mastitis.

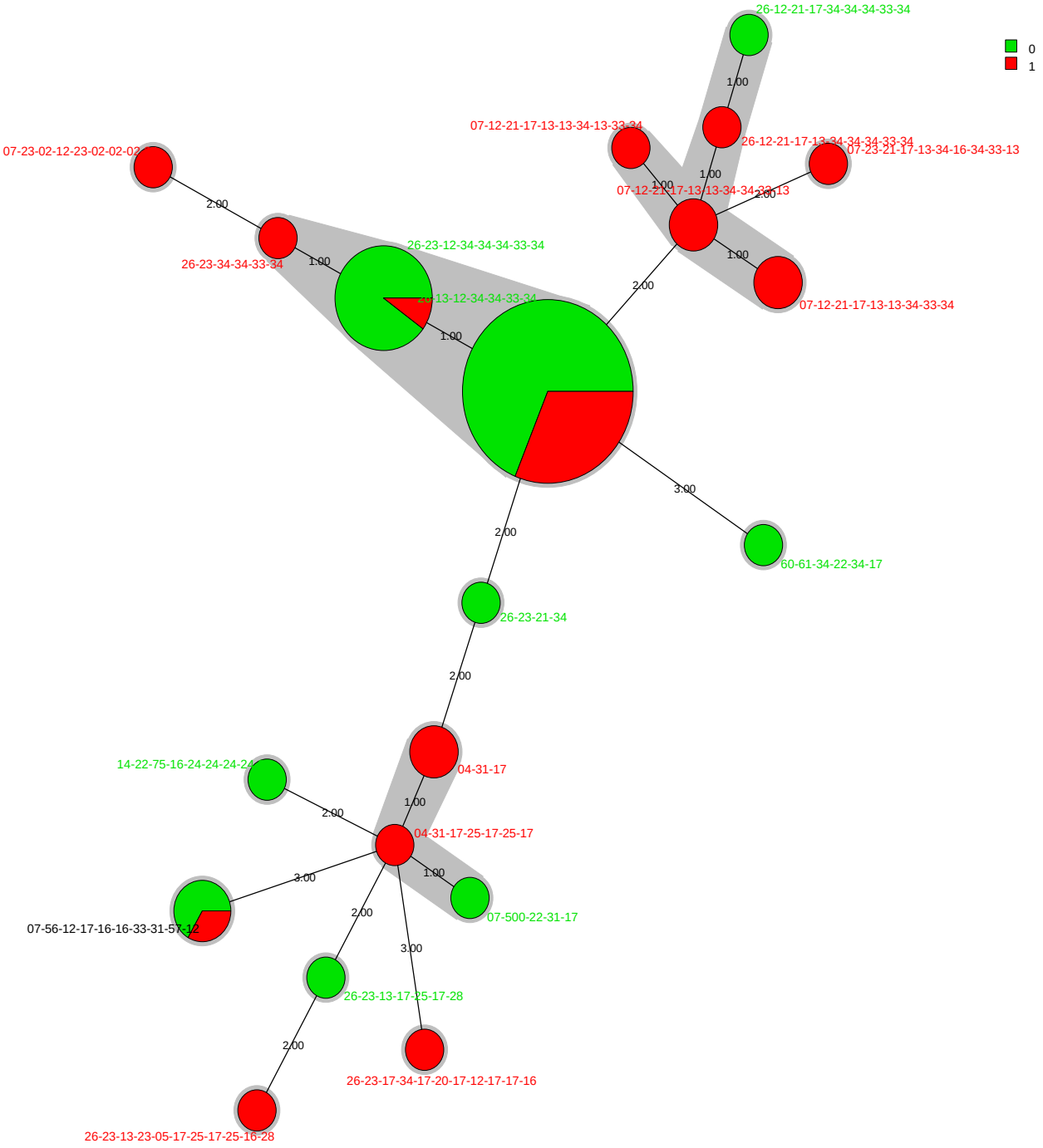

Figure 2: Minimum spanning tree of 65 strains according to their *spa* repeat succession.

The size of the circle is proportional to the number of strains reported. The closer circles are to each other, the closer they are genetically related. The colours of the circles represent the region where the *spa* type was found, Bahir Dar (0) and Gondar (1). Grey areas represent the clustering of *spa* types based on a maximum difference between 2 circles of 1.00.

Discussion

***S. aureus* proportion.** A high proportion of *S. aureus* was expected in the present study, because of the preselection of samples in Ethiopia. However, when testing the proportion of the *S. aureus* samples, it turned out to be only 81.7%. In Ethiopia, a coagulase reaction was used to confirm *S. aureus*. Specificities of 99% and 97% were found respectively for an incubation period of 3 and 24 hours while the sensitivities

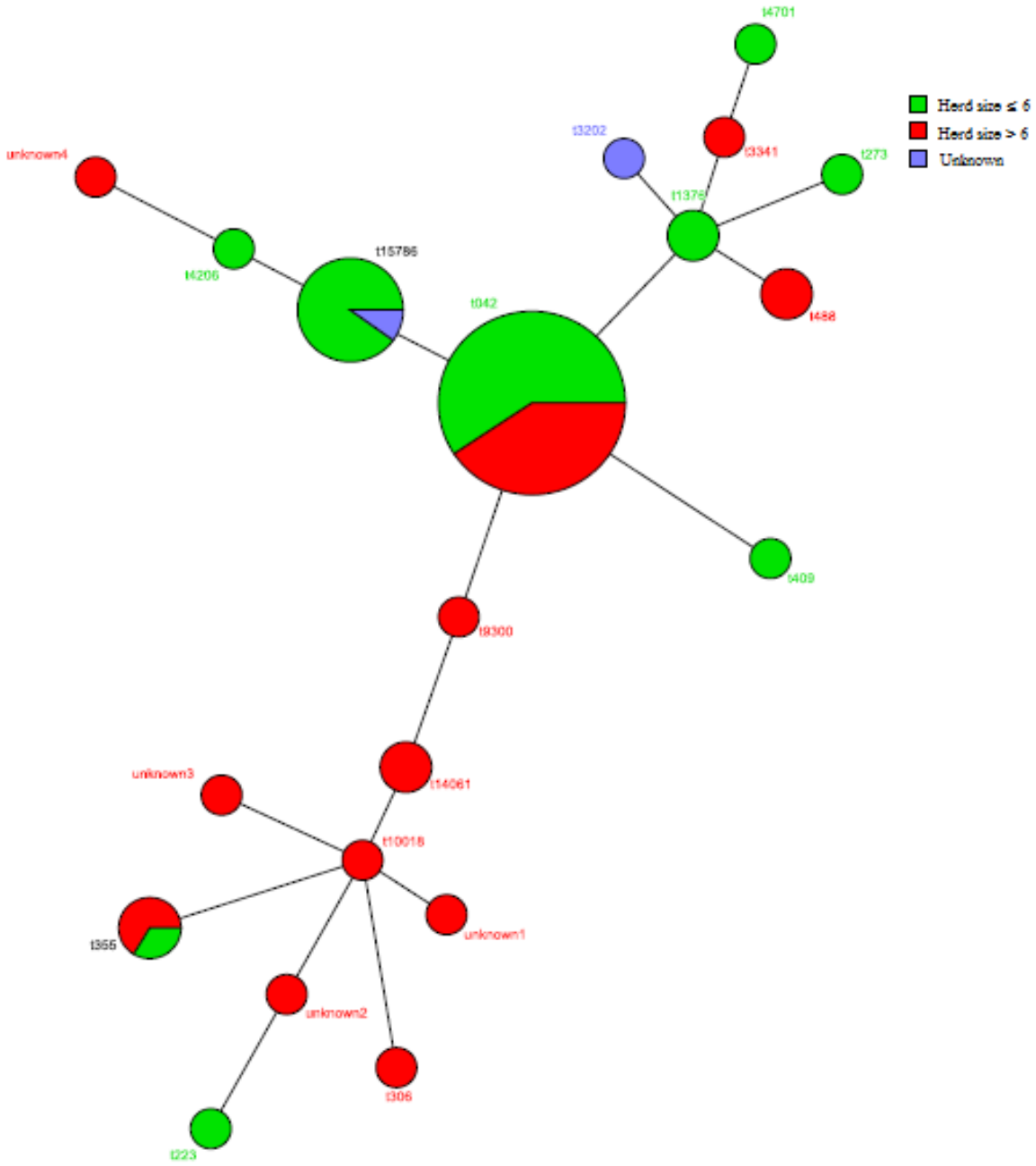

Figure 3: Minimum spanning tree which shows the relatedness of the 65 strains according to their *spa* type. Colours represent herd size ≤ 6, herd size > 6 and no information of a *spa* type.

were 97% and 99%, respectively (Graber et al. 2013). However, *S. aureus* is not the only bacterium which can test positive for the coagulase test. *S. delphini*, *S. intermedius*, *S. lutrae* and *S. schleiferi* subspecies *coagulans* can also become positive for this test (Graber et al. 2013). This may explain the lower proportion of *S. aureus* when testing with the *nuc* and *femA* genes.

LukMF'. The found prevalence of LukMF' in Ethiopia was 2.2% (3/138). The prevalence of LukMF' varies between regions (Vrieling et al. 2016). (Yamada et al. 2005) have shown prevalences of LukMF' of respectively 62.5% and 86.1% in 2 Japanese regions, while research in Germany described a LukMF' prevalence of 79.9% in milk samples (Schlotter et al. 2012). Another study performed in Germany, including Swiss isolates, showed a LukMF' prevalence of 53.1% (Monecke et al. 2007). With 2.2%, the LukMF'

prevalence in Ethiopia is considerably lower. However, the prevalence of 10-86% reported by Vrieling et al. (2016) has a large range. Since few research has been done so far concerning the prevalence of LukMF', a prevalence of 2.2% is a possible finding. Given the different circumstances in Ethiopia such as milking by hand, it is possible that human *S. aureus* strains will be transmitted to cows. However, lukMF' is not present in human *S. aureus* strains (Vrieling et al. 2015). This may explain the low prevalence of LukMF' in this research.

MRSA prevalence. In Germany, a MRSA prevalence of 4.4% was found in bulk milk (Kreausukon et al. 2012) while a prevalence of 4% was found in the United States (Haran et al. 2012). However, for both studies, herds with more than 20 and 40 cows were included, respectively. These herds are much larger than the Ethiopian herds. Other research by (Puacz et al. 2015) reported no MRSA positive cows whereby the number of cows in a herd varied from 8 to 27.

Use of antibiotics. Information about antibiotic use in Ethiopia is scarce (Groot et al. 2016). The increase in milk yield and crossbreeding have led to an increase of antibiotics in the developing countries. There is large uncontrolled use of antibiotics which is partly due to crossbreeding, because HF cows are not accustomed to the climate (Groot et al. 2016). In response to this data and in combination with MRSA prevalences reported in the literature, we expected to find positive strains for MRSA.

***MecA*.** Research by (Melo et al. 2014) shows that only bovine samples differ in the sequence of the *mecA* gene. A primer, which is known to be effective on cow milk, was selected from the article by (Melo et al. 2014). Despite the careful selection of the primer, none of the samples turned out to be positive for *mecA*.

In previous research by Mekonnen et al. (in preparation) samples were tested for beta-lactam resistance with Cefoxitin. With this test, 15 samples were found to be resistant against Cefoxitin (Mekonnen et al. (in preparation). (Mendonça et al. 2012) reported that the Cefoxitin disk diffusion test is the best option to detect of beta-lactam resistance with a specificity of 85% and a sensitivity of 87%. However, samples which do have beta-lactam resistance can have a various phenotypic expression (Melo et al. 2014). Because of this, the gold standard test for MRSA-positive samples is the presence of the *mecA* gene according to the Clinical and Laboratory Standards Institute (CLSI) (Melo et al. 2014).

MecA is carried by the staphylococcal cassette chromosome *mec* (SCC*mec*) which plays a role in the molecular epidemiology of MRSA. There are in total eleven types (I to XI) of SCC*mec* (Liu et al. 2016, Tsubakishita et al. 2010). Since there was no *mecA* gene found, the used primer set was checked in the GenBank ® founded by the National Center for Biotechnology Information (NCBI). According to the NCBI GenBank ®, the used primer set should work for types I to X, but for type XI the DNA-sequence wass unknown. Type XI SCC*mec* is a partly new SCC*mec* element, which contains a *mecA* gene that differs for 30% at the nucleotide level from the original *mecA* gene. Because of this difference, SCC*mec* type XI will not be detected with the routine PCR (Melo et al. 2014).

***Spa* typing.** *Spa* typing was used to find genetic relatedness of the *S. aureus* strains with each other and with a large collection of ruminant *S. aureus* reported in the literature and collected through a systematic literature review by (Baede 2013). Tarekgne et al. (2016) reported 25 different *spa* types in the Tigray region of Ethiopia which is located north of Gondar and Bahir Dar. For 4 of the spa types that were found, a match with the *spa* types detected in this research was observed. These types are t042, t223, t306 and t355 (Tarekgne et al. 2016). However, t042 is not predominating in the Tigray region (Tarekgne et al. 2016). Most of the strains of our research are corresponding with strains derived from the

Tigray region according to their repeat successions. However, the repeat succession of some strains totally differs between both regions.

When the found *spa* types are placed in the global picture using the literature (Baede 2013), it shows *spa* types t10018 (*n* = 1) and t14061 (*n* = 2) in the middle of the MST. This suggests that those Ethiopian strains are closely related to strains derived from other parts of the world. *Spa* type t223 has also been found before in Tunisia and was derived from sheep while most of the other Ethiopian *spa* types are more related to strains that have been linked to cows (Baede 2013). The Ethiopian *spa* types we found are only found in Africa and not in other continents. However, some African strains derived from other research are also found in Europe and North-America (Baede 2013).

Clonal complexes. When looking at clonal complexes, part of the Ethiopian strains cluster with clonal complex 97 (CC97), while the other part of strains cluster with strains commonly associated with small ruminants which are CC130, CC133 and CC522 (Porrero et al. 2012, Smith et al. 2014). CC97 is often found in bovine mastitis (Spoor et al. 2013) but has as far as we know not been reported in the zebu yet. In the 90s, crossbreeding with HF cows was introduced. It is possible that the relatedness to CC97 can be due to the crossbreeding of HF cows. However, more research is needed according to clonal complexes in the zebu. Clonal complexes CC130, CC133 and CC522 can be derived from small ruminants from the area.

Transmission of strains. Because of its contagiousness (Zadoks et al. 2011), *S. aureus* can spread fast within a herd and can lead to many new infected cows (Sommerhäuser et al. 2003). (Sommerhäuser et al. 2003) have shown that *S. aureus* infections will not decrease when trying to lower the *S. aureus* transmission during the milking process. For the decrease of *S. aureus* mastitis in a herd it is more effective to cull cows which have a chronical *S. aureus* infection.

In addition to the predominant strains, there are also *S. aureus* strains which behave as environmental pathogens (Sommerhäuser et al. 2003). When most of the mastitis cases are caused by environmental pathogens, more different *S. aureus* types are found than when a contagious *S. aureus* predominates the mastitis cases (Sommerhäuser et al. 2003). If there is no predominant *S. aureus* strain in the herd, many different rare types are found (Sommerhäuser et al. 2003).

Distribution of strains is herd-specific (Zadoks et al. 2011). If environmental strains are detected, they are found in only one cow in the herd and hardly ever spread to other cows (Larsen et al. 2000). However, the environmental strains do have a considerably less prevalence than the contagious *S. aureus* strains (Tenhagen et al. 2007). When multiple strains are found in a herd it is proven that not every intramammary infection is transmitted from cow-to-cow (Zadoks et al. 2011).

In contrast to the abovementioned studies, in our study less isolates per herd were used. Sabour et al. (2004) have also used less isolates per herd compared to the other studies. (Sabour et al. 2004) have shown that in most herds (58.6%), only one *S. aureus* strain was isolated. Because of the low number of samples, it is possible that strains with a lower prevalence were not detected (Sabour et al. 2004). However, in this research pulsed-field gel electrophoresis (PFGE) typing was used instead of *spa* typing. A comparative study by (Koreen et al. 2004) shows that *spa* typing is equally suitable as other typing methods such as PGFE. Individual *spa* types and PFGE-types have a concordance of 98% (Koreen et al. 2004) so they are comparable to each other. So, the same is true to a certain extent for this study where in 61.1% of the herds only one *S. aureus* strain was isolated.

Conclusion

All *spa* types of our study were closely related to each other. Clustering of *spa* types within and between herds was found, which suggests a within herd and between herd transmission. However, the way of transmission was beyond the scope of this study. To gain knowledge about transmission routes in Ethiopian herds, more research is required.

Acknowledgement

Credits go to dr. G. Koop and J. Hoekstra, MSc who respectively supported me in the writing process and assisted me in the laboratory. I also want to thank S.A. Mekonnen for answering questions relating to the strains obtained from his research and M.P. Spaninks for helping me with Bionumerics.

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