



## Improved DNA extraction and purification with magnetic nanoparticles for the detection of methicillin-resistant *Staphylococcus aureus*

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### ABSTRACT

Molecular methods offer fast, safe and cost-efficient detection of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). These tests depend on a rapid extraction of bacterial DNA. The aim of this study was to compare an optimized DNA extraction and purification protocol for MRSA using magnetic nanoparticles with the original method. The purity of the extracted DNA was assessed by photometric measurements and the amount of DNA was determined by real-time PCR. Three MRSA reference strains (*S. aureus* ATCC<sup>®</sup> 70699, *S. aureus* ATCC<sup>®</sup> 43300; *S. aureus* ATCC<sup>®</sup> 33592) and eleven MRSA field strains, which include SCCmec elements of types I to XI, were used in this study. The optimized protocol can save approximately 20 min time compared to the original method and the DNA yield was higher with the new protocol. Therefore, this new protocol allows a faster DNA extraction from MRSA cultures.

### 1. Introduction

*Staphylococcus aureus* can be a harmless colonizer of the skin and the mucosal surfaces of humans and animals, but represents – in addition to extended-spectrum  $\beta$ -lactamase- and carbapenemase-producing Enterobacteriaceae among others – also a major pathogen in human and veterinary medicine. Several infections can be associated with *S. aureus*, such as skin and soft-tissue infections, endocarditis, pneumonia, osteomyelitis and mastitis (Köck et al., 2014). These infections are commonly treated with  $\beta$ -lactams (Köck et al., 2014), but this option is not available for methicillin-resistant *S. aureus* (MRSA), since MRSA are considered as resistant against all  $\beta$ -lactams, except for some specific substances only licensed for human medicine (CLSI, 2018). Therefore, a rapid detection of MRSA is very important for an adequate treatment of severely diseased patients. In comparison to cultural methods requiring several days, molecular methods allow a faster detection of MRSA (Hogg et al., 2008; Stürenburg, 2009; Gao et al., 2018; Seidel et al., 2017). In this context, the DNA extraction is an important step for initiating downstream applications such as PCR assays (Hogg et al., 2008; Wang et al., 2015). Various DNA extraction methods, including either commercial kits or standard operation procedures, such as boiling,

phenol-chloroform extraction and detergent-based methods, are available (Hassanzadeh et al., 2016). These methods can come along with a low purity, time-consuming handling, use of toxic solvents and high risk of contamination of the extracted DNA (Hassanzadeh et al., 2016). Magnetic nanoparticles (MNPs) have the advantage of shorter processing times, without the use of hazardous chemicals. Their application allows automation, is easy to handle and has low costs, and provides high DNA yields of sufficient purity (Wang et al., 2015).

The aim of this study was to modify an existing MNP-based DNA extraction protocol from Hodgson (2014) established for the extraction of bacterial DNA from food and water and assess its efficiency in terms of reduced time of analysis and yield of MRSA DNA.

### 2. Material and methods

#### 2.1. Bacterial strains

Three MRSA reference strains (ATCC<sup>®</sup> 70699; ATCC<sup>®</sup> 43300; ATCC<sup>®</sup> 33592) and eleven MRSA field strains comprising the major SCCmec types I to XI were used as targets. The SCCmec types were confirmed by sequence analysis (GATC Biotech AG, Konstanz, Germany). All strains

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**Table 1**  
Comparison of the two methods.

Steps	New method (this study)	Original method Hodgson (2014)
1	<b>deleted</b>	100 µL <sup>*</sup> of bacterial culture are transferred into a sterile 1.5 mL cup and centrifuged at 10.000 rpm for 10 minutes.
2	100 µL bacterial culture are pipetted directly into 400 µl buffer.	The supernatant is discharged and 400 µL of buffer are pipetted onto the bacterial pellet.
3	<b>deleted</b>	10 µL proteinase K is added and mixed well.
4	The mixture is incubated for <b>5 min at 100 °C</b> on a thermal mixing block at 700 rpm.	The mixture is incubated for 20 minutes at 65 °C on a thermal mixing block at 700 rpm.
5	<b>deleted</b>	Centrifugation at 10.000 rpm for 5 minutes.
6	The mixture is transferred into 400 µL of binding buffer and <b>60 µL</b> of MNPs.	The supernatant is transferred into 400 µL binding buffer and 25 µL MNPs.
7	Incubation for <b>10 minutes</b> at room temperature	Incubation for 5 minutes at room temperature
8	MNPs are magnetically immobilised and the supernatant is removed.	MNPs are magnetically immobilised and the supernatant is removed.
9	MNPs are washed with 400 µL 75% ethanol. MNPs are magnetically immobilized and the washing buffer is removed. The ethanol evaporates at room temperature. <b>(Optional - only for heavily soiled samples)</b>	MNPs are washed with 400 µL 75% ethanol. MNPs are magnetically immobilized and the washing buffer is removed. The ethanol evaporates at room temperature.
10	The MNPs are re-suspended in 100 µL deionised water and incubated for 5 minutes at 65 °C.	The MNPs are re-suspended in 100 µL deionised water and incubated for 5 minutes at 65 °C.

\* in contrast to the method described from Hodgson (2014), 100 µL were used instead of the proposed 1 mL.

**Table 2**  
Efficacy test of the modified DNA extraction method and the original method.

MRSA strains	New method (this study)							Original method (Hodgson, 2014)						
	Test 1		Test 2		Test 3		Total	Test 1		Test 2		Test 3		Total
	GM	CV (%)	GM	CV (%)	GM	CV (%)		CV (%)	GM	CV (%)	GM	CV (%)	GM	
<i>S. aureus</i> ATCC <sup>®</sup> 33592	29.10	6.19	25.38	6.24	79.44	1.21	<b>4.55</b>	6.64	3.91	4.49	8.40	12.39	4.43	<b>5.58</b>
<i>S. aureus</i> ATCC <sup>®</sup> 43300	28.17	0.12	35.12	3.32	79.75	3.68	<b>2.37</b>	4.11	12.15	16.04	6.98	7.78	24.12	<b>14.41</b>
<i>S. aureus</i> ATCC <sup>®</sup> 70699	33.52	4.25	28.85	3.59	57.48	6.77	<b>4.87</b>	5.16	17.96	4.53	13.51	5.34	14.56	<b>15.34</b>
MRSA SCCmec type I	24.30	1.13	26.13	0.75	40.32	3.36	<b>1.75</b>	7.77	4.13	4.71	5.16	10.85	3.58	<b>4.29</b>
MRSA SCCmec type II	24.53	1.12	18.17	50.51	26.40	7.86	<b>19.83</b>	6.81	10.55	7.45	4.05	5.22	2.26	<b>5.62</b>
MRSA SCCmec type III	28.48	11.37	25.40	7.85	22.60	1.91	<b>7.04</b>	5.30	3.11	3.00	12.00	13.45	8.11	<b>7.74</b>
MRSA SCCmec type IV	26.61	6.41	9.23	13.71	40.59	5.65	<b>8.59</b>	5.27	21.23	5.61	1.96	16.27	5.33	<b>9.51</b>
MRSA SCCmec type V	26.95	1.43	28.71	2.66	38.40	0.23	<b>1.44</b>	3.50	7.01	5.44	5.83	13.13	3.83	<b>5.55</b>
MRSA SCCmec type VI	26.33	1.66	29.90	9.39	36.79	3.77	<b>4.94</b>	5.22	20.12	6.69	13.84	13.63	10.18	<b>14.71</b>
MRSA SCCmec type VII	25.58	1.53	41.57	2.49	21.58	4.55	<b>2.86</b>	5.97	1.85	5.55	14.30	12.86	8.28	<b>8.14</b>
MRSA SCCmec type VIII	34.76	2.29	31.51	0.62	29.62	2.56	<b>1.82</b>	8.05	8.13	8.93	32.38	8.91	9.42	<b>16.65</b>
MRSA SCCmec type IX	26.57	4.28	22.70	4.59	27.31	0.51	<b>3.13</b>	7.97	6.60	4.33	8.71	2.47	22.47	<b>12.59</b>
MRSA SCCmec type X	25.34	1.68	32.47	6.52	59.87	3.21	<b>3.80</b>	5.08	11.60	5.74	11.82	16.29	2.30	<b>8.57</b>
MRSA SCCmec type XI	40.63	12.55	67.46	34.08	51.81	3.49	<b>16.70</b>	4.03	14.35	6.40	20.44	11.65	3.28	<b>12.69</b>

DNA yield (ng/µL) of both methods was determined for the three MRSA reference strains and the eleven MRSA field strains in three independent tests. The DNA concentration of each test was determined three times by NanoDrop<sup>®</sup> measurement (technical replicates). GM = geometric mean; CV = Coefficient of variation.

were routinely maintained on Columbia blood agar (Oxoid, Wesel, Germany). The bacterial suspensions were prepared by inoculating a single colony in 40 mL Giolitti-Cantoni Broth (Merck KGaA, Darmstadt, Germany) and incubation for 48 h at 36 °C.

## 2.2. Comparison of the modified DNA extraction method and the original protocol

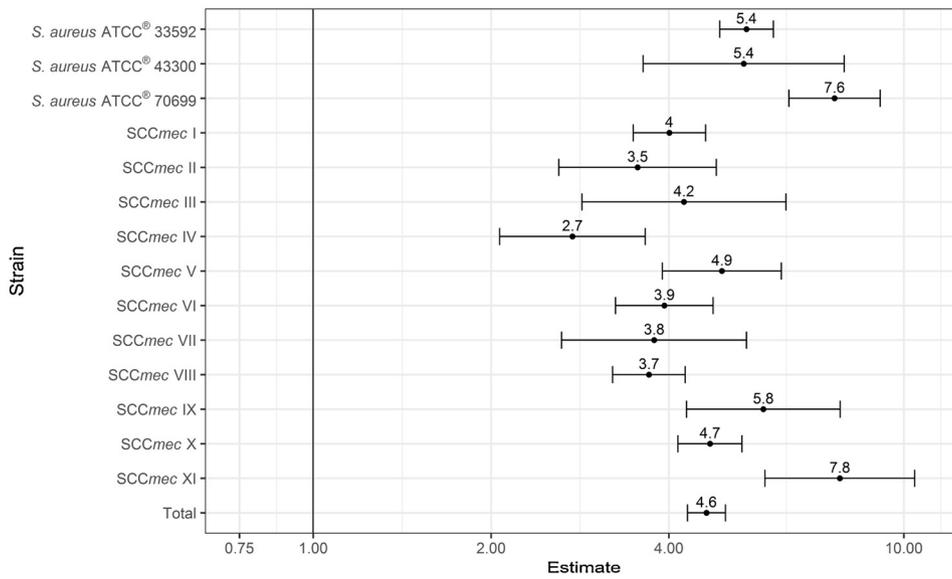
The DNA extraction method using MNPs from Hodgson (2014) was modified (Table 1). The new protocol is performed as follows: For the modified DNA extraction method 100 µL of an MRSA suspension were transferred to a sterile 1.5 mL cup with 400 µL buffer (100 mM Tris-HCl, 10 mM EDTA, 1 M NaCl, pH 8.0 (all Carl Roth, Karlsruhe, Germany)) and mixed well. The cell wall was lysed by incubating the samples for 5 min at 100 °C on a thermoshaker (700 rpm/min). The suspension was transferred to a sterile 1.5 mL cup with 400 µL of binding buffer (20% polyethyleneglycol (M<sub>r</sub> 8000) in 4 M NaCl (Carl Roth, Karlsruhe, Germany)) and 60 µL MNPs (Q-Bioanalytic large-scale amorphous silica-coated magnetite, QBLSSM, suspension density of 20 mg/mL, Q-Bioanalytic GmbH, Bremerhaven, Germany) and incubated at room temperature for 10 min to bind the DNA to the MNPs. The MNPs are magnetically immobilized in a magnetic stand and the supernatant is removed. Then, the MNPs are re-suspended in 100 µL deionised water

and incubated for 5 min at 65 °C. Finally, the MNPs are magnetically immobilized again and the DNA-containing supernatant is transferred to a new cup.

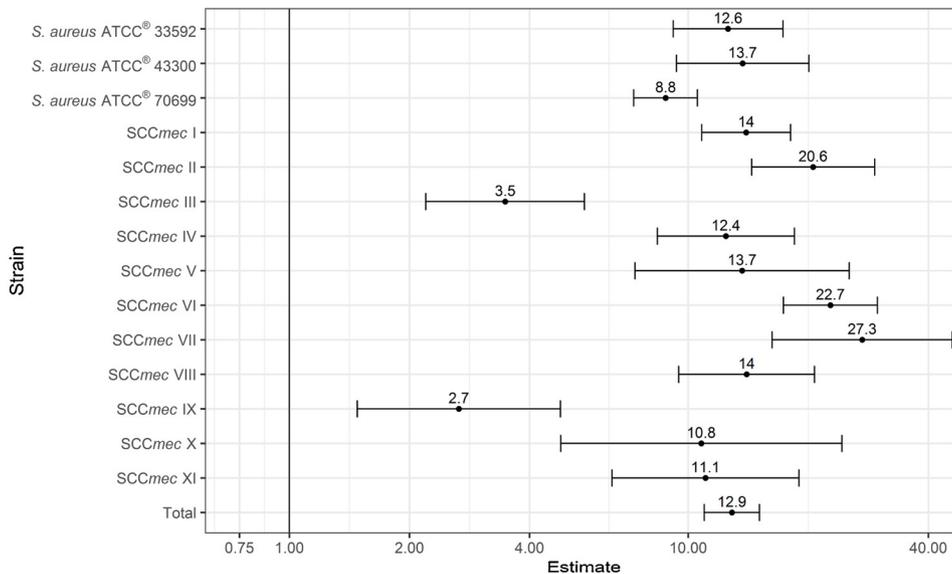
The new protocol reduced the extraction/washing steps and consequently also the time for the DNA extraction. Comparative tests using the three reference strains and the eleven field strains were performed to compare the two methods using the same bacterial suspension. Each strain was used three times at independent occasions for each of the two DNA extraction protocols (Table 1). For comparative reasons, 100 µL of the bacterial suspension were used as start point for the DNA extraction to facilitate the handling due to the use of 1.5 ml cups, even though 1 mL of the pre-enrichment is used for the detection of *Listeria* in the original method (Hodgson, 2014).

The yield and purity of the extracted DNA was assessed by photometric measurements (NanoDrop<sup>®</sup> 1000, Thermo Fisher, Waltham, Massachusetts, USA), which were performed in triplicates. In addition, 2 µL DNA were used as template for a commercial real-time TaqMan PCR kit according to the manufacturer's instructions (Quick Blue Real-Time PCR, DNA detection kit - MRSA, Q-Bioanalytic GmbH, Bremerhaven, Germany). The real-time PCR was performed by using a LC 480 Roche Cycloer equipped with a 384-well heating block (Hoffmann-La Roche, Basel, Switzerland).

**a) NanoDrop® measurements**



**b) PCR results**



**Fig. 1.** Forest plots of the DNA yields obtained by the different methods. (a) The estimate is defined as the multiple increase in extracted DNA of the new method compared to the old method (DNA amount new/DNA amount old). (b) The factors for the real time PCR were calculated from the  $C_T$  values. The numbers show the factor of the different amounts of DNA extracted; the bars indicate the 95% confidence interval.

**2.3. Data analysis and statistical analysis**

Geometric mean (GM) values of the three NanoDrop® measurements of the DNA concentrations were log transformed to achieve normal distribution. We used the coefficient of variation as a measure of dispersion for the lognormal distributed data; it is calculated as ratio of the standard deviation to the mean. The threshold cycle ( $C_T$ ) value of the real-time PCR was used to estimate the amount of amplicons from the target strain and to compare the values of different experiments. Multiple linear regression determined the statistical significance. The DNA concentration and the  $C_T$  values of the real-time qPCR were compared with respect to the method of DNA extraction, adjusting for each suspension of each strain. Additionally, we ran a stratified analysis

by strain. The differences are reported as multiples of extraction increases with the respective 95% confidence interval. Confidence intervals of differences, which do not contain one, can be considered significant. Moreover, the purity of the DNA was evaluated using the A260/A280 and A230/280 ratios, determined by NanoDrop® measurements (<http://www.nhm.ac.uk/content/dam/nhmwww/our-science/dpts-facilities-staff/Coreresearchlabs/nanodrop.pdf>). All statistical analyses were performed using R Version 3.50 (R Foundation Vienna).

**3. Results and discussion**

DNA extraction plays an important role for the use of subsequent

applications, such as PCR or sequence analysis (Wang et al., 2015). In this study, we modified an existing method originally designed for the extraction of DNA from food matrices (Hodgson, 2014). The boiling step during DNA extraction seemed to have a positive effect on the nucleic acid yield, which has already been shown previously (Hassanzadeh et al., 2016). However, the boiling of the bacteria is often associated with a low purity of the extracted DNA (Hassanzadeh et al., 2016), which can be compensated by the use of MNPs (Hodgson, 2014; Wang et al., 2015). The optimized method and the original protocol (Table 1) were tested in parallel using 100 µL of the same bacterial cultures from three MRSA reference strains and eleven strains with the SCCmec types I to XI. The two most recently identified SCCmec elements, SCCmec type XII (Wu et al., 2015) and SCCmec type XIII (Baig et al., 2018), were not included in this study as *Staphylococcus aureus* strains carrying these SCCmec elements had not been available to us at the time of performance of the experiments. The DNA extraction was performed for each strain three times using pure cultures.

The GM values of the triplicate NanoDrop® measurements obtained for the DNA amounts for each strain in the different tests ranged from 9.23 to 79.75 ng/µl for the new method compared to 2.47 to 16.29 ng/µl for the original method (Table 2). The broad range obtained for the DNA amounts might be due to different inoculum densities used. These results confirmed a higher DNA yield with the new method compared to the original protocol (Fig. 1a, Table S1a). The reason for the higher DNA yield might be due to the reduction of the washing steps, since variable amounts of DNA may be lost during each washing step. However, such washing steps might be necessary for the DNA isolation from food matrices or other samples containing potential PCR inhibitors. In addition, the variation in the amounts of extracted DNA was lower using the new method as indicated by a coefficient of variation (CV) of 5.98% (95% CI [3.16%, 8.79%]) when using the new method compared to 10.1% (95% CI [7.91%, 12.29%]) when using the original method. Moreover, the data can show that the new method resulted in higher DNA yields regardless of the amount of bacteria in the culture. However, if higher DNA yields were obtained with the old method, the added benefit of the new method was lower. Three repetitions with different suspensions and three measurements each were performed per strain, resulting in nine values obtained with the old and nine values obtained with the new method. This can provide a rough overview regarding the stability and reproducibility of the method on strain level. Since these tests have been performed for 14 strains leading to 252 measurements in total, the sample size is sufficient for estimating an overall benefit of the new method.

The A260/A280 ratios from the NanoDrop® measurements can be used to determine the purity of the DNA (Hassanzadeh et al., 2016). Values of about 1.8 demonstrate a pure DNA free from protein contamination (Dauphin et al., 2010; <http://www.nhm.ac.uk/content/dam/nhmwww/our-science/dpts-facilities-staff/Coreresearchlabs/nanodrop.pdf>). A260/A280 mean values of about 1.4 were determined for both methods (new method 1.38, original method 1.41) pointing towards protein contamination in the DNA extracts. The A230/280 ratios were 0.694 for the old versus 0.740 for the new method. Nevertheless, the target sequences could still be detected in subsequent real-time PCR assays. However, Dauphin et al. (2010) found that the purity of the DNA seemed to have a greater influence than the DNA concentration on the real-time PCR detection limit of *Yersinia pestis*, which might also be assumed for MRSA.

Real-time PCR results demonstrated lower C<sub>T</sub> values for the optimized method compared to the original method (Fig. 1b, Table S1b). The C<sub>T</sub> value represents the PCR cycle in which the threshold is crossed by the fluorescent signal. The numerical value of the C<sub>T</sub> is inversely related to the amount of amplicon, and therefore, the lower the C<sub>T</sub> value, the greater the amount of amplicons and template DNA in the sample (Schmittgen and Livak, 2008). This indicates that a greater amount of DNA was extracted using the optimized method, which was confirmed by statistical analysis.

#### 4. Conclusion

This study showed that the optimized protocol is superior to the method by Hodgson (2014) in terms of time and DNA yield. It can successfully be used to quickly and efficiently extract and purify DNA from various MRSA strains to be used for subsequent analysis like RT-PCR

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#### Conflict of interests

JH, KS and BO are present and CE, EE, JWE are former employees of Q-Bioanalytic GmbH, Bremerhaven, Germany. Other authors: none to declare.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetmic.2019.01.009>.

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