Examining resistance gene expression using reverse transcriptase qPCR

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Objective

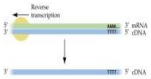
The expression of the mecA gene in cefoxitin induced clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates was measured using reverse transcriptase qPCR (RT-qPCR). The aim was to examine if there was a correlation between the inducibility and staphylococcal cassette chromosome *mec* (SCC*mec*) type or minimum inhibitory concentration (MIC) of cefoxitin.

Methods

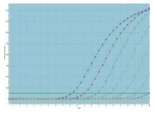
Growth for 40 min at 35°C with or without 3 mg/L cefoxitin.

Determining the cefoxitin minimum inhibitory concentration (MIC) using Etest.

RNA protection and RNA extraction from 1 mL cell culture.



cDNA synthesis using random hexamers.



qPCR measuring the relative expression of mecA compared to 16S rRNA.

Conclusion

Most RNA was obtained when applying both enzymatic and mechanical cell rupture.

The best RNA quality was obtained when an additional gDNA Eliminator Column step was carried out.

It was possible to apply RT-qPCR for the detection of changes in the *mecA* expression upon cefoxitin induction. Significant changes (P<0.05) were observed for seven of the examined MRSA isolates. There was no correlation between the SCC*mec* type and the induced baselines, but it seemed that isolates with a low cefoxitin MIC also had a low expression level of *mecA*.

Reference

Shang *et al*, Antimicrob Agents Chemother 2010, p. 956-59.

Optimization of RNA extraction

Strain #	RNA yield [ng/µL] (no bead beating)	RNA yield [ng/µL] (with bead beating)
46 (uninduced)	12	115
46 (induced)	21	110
190 (uninduced)	17	53
190 (induced)	23	107

Table 1: Mechanical lysis (bead beating) markedly increases the yield of total RNA.

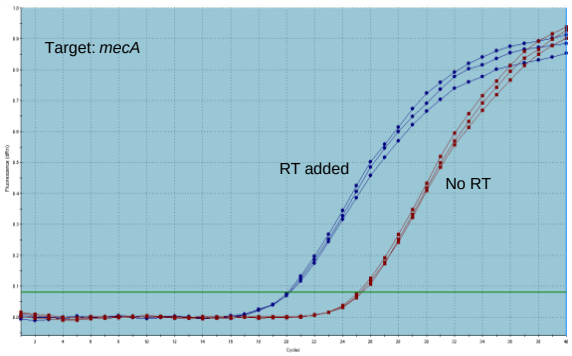


Figure 1: gDNA co-elutes with the RNA.

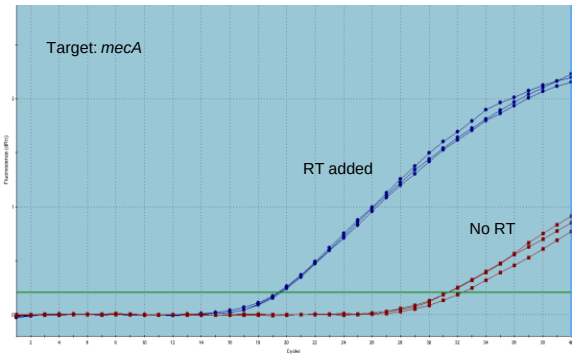


Figure 2: Markedly reduction of co-eluting gDNA upon on-column DNAse treatment and an additional gDNA Eliminator Column step.

Changes in *mecA* expression

Strain #	SCC*mec* type	Cefoxitin MIC (µg/mL)	Induced baseline	P-value	MFC
155 (MSSA)	-	3	0	1.00	0
156 (MSSA)	-	4	0	1.00	0
9	I	256	60 ± 19	0.21	1 ± 0.5
1468	I	256	87 ± 45	0.25	1 ± 0
920	I	256	153 ± 64	0.06	36 ± 22
39	II	128	135 ± 26	0.01	24 ± 13
11	II	256	34 ± 10	0.03	11 ± 1
2	III	256	33 ± 33	0.30	7 ± 6
13	IIIA	256	49 ± 17	0.06	6 ± 3
927	IV	24	51 ± 21	0.05	5 ± 1
886	IV	48	9 ± 3	0.11	1 ± 0
560	IV	48	83 ± 28	0.03	9 ± 4
557	IV	64	74 ± 35	0.09	5 ± 2
320	IV	256	40 ± 17	0.02	3 ± 1
559	IV	256	80 ± 11	0.01	6 ± 2
306	V	12	5 ± 3	0.11	7 ± 3
460	V	12	13 ± 2	0.004	16 ± 2
321	V	96	36 ± 15	0.03	12 ± 3

Table 2: Seven strains showed significantly (P<0.05) inducible *mecA* expression upon cefoxitin induction. Whether the *mecA* expression was constitutive or inducible did not correlate with the level of phenotypic resistance (cefoxitin MIC) or the SCC*mec* type. However it seemed that isolates with a low cefoxitin MIC also had a low *mecA* expression as well.