DIRECT DETECTION OF MRSA IN CLINICAL SPECIMENS BY PCR

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Introduction

Rapid detection and identification of MRSA have become increasingly important for therapeutic and especially epidemiological purposes. Culture-based methods in combination with phenotypic identification are time-consuming and therefore delay decisions regarding isolation procedures for patients suspected as possible MRSA carriers. We have established a LightCycler-based assay for the direct detection of MRSA in clinical specimens using the SCCmec-orfX junction as the target. This assay is similar to a commercial MRSA-PCR (IDI-MRSA, Cepheid) [1, 2]. In order to validate our assay, we have compared it to culture as well as to another molecular strategy which is based on PCR detecting *mecA* and *femA* (allows the exclusion of MRSA carriage if either or both tests are negative).

Material and Methods

Swabs:	Oxoid Transport swabs (suspended in 1ml phys. NaCl, equal splitting for culture and PCR)
Culture:	Oxoid MRSA chromogenic agar and blood-agar; TSB NaCl 6.5% with subculture on ORSA-agar (Oxoid); cephoxitin-disk on MH-agar; PBP2-agglutination
MRSA-PCR:	SCCmec/orfX-junction; FRET-PCR (see Fig. 1)
femA-PCR:	FRET-PCR; marker for *S.aureus*
mecA-PCR:	FRET-PCR; marker for methicillin-resistance
Inhibition control:	independent FRET-PCR for all samples
Amplification	LightCycler, 20 ul capillaries, identical cycling conditions and polymerase (LC FastStart DNA Master^{PLUS} Hybeprobe, 100 ul reactions) for all parameters
Assay time	approx. 2 hours, incl. DNA extraction

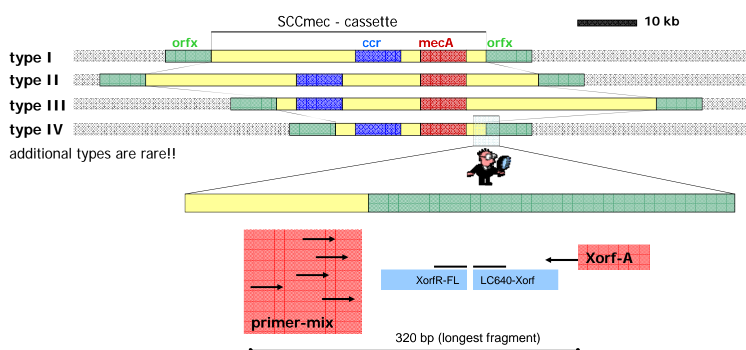

Figure 1: Design of MRSA-PCR

	<u>*femA*</u>	<u>*mecA*</u>	<u>MRSA</u>
MSSA	+	-	-
MRSA	+	+	+
MSSE	-	-	-
MRSE	-	+	-
MSSA + MRSE	+	+	-

Table 1: Expected PCR results

Results

		PCR positive			
culture	N	**MRSA**	*femA*	*mecA*	*femA+mecA*
positive	10	**10**	8	10	8
negative	161	**1***	32	82	13

* previously MRSA-positive patient after decolonization; considered true positive

Table 2: comparison of culture and PCR

	PCR for			
	MRSA	*femA*	*mecA*	*femA+mecA*
Sensitivity %	**100**	81.8	100	81.8
Specifity %	**100**	66.3	41.7	91.9
PPV %	**100**	14.3	10.3	40.6
NPV %	**100**	98.1	100	98.6

Table 3: characteristics of the various PCR assays

Conclusions

- Direct MRSA PCR showed 100% agreement with culture (if a sample from a previously MRSA-positive patient is excluded from the analysis)
- a combination of femA/mecA PCR has a high NPV but does not exclude the presence of MRSA in almost 10% of culture-negative specimens
- our direct MRSA-PCR is both rapid and reliable, significantly less expensive and more flexible (see below) than commercial systems

Further developments

Since the conclusion of this study, we have modified our MRSA-PCR as follows:

- replacement of the two FRET probes by a single TaqMan probe significantly increased the signal produced in positive specimens
- addition of a sixth forward primer now allows also the detection of a new MRSA clone associated with intravenous drug usage [3]

Acknowledgement

We thank M. Ender and B. Berger-Bächi for making available the SCCmec sequence of the ‚drug clone'

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