

Current problems in the detection and management of methicillin-resistant *Staphylococcus aureus* (MRSA)

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Classical microbiology has focussed on the cultivation of infectious agents: replication of such agents on (semi)synthetic culture media or within host cells grown *in vitro* provided proof of infection. Currently, the detection of microbial antigens or antibodies directed towards these antigens generates similar proof. The most recent development capitalises on the detection of a specific type of antigen: nucleic acids. DNA technology has developed into a major industry over the past two decades and many examples of commercially available nucleic acid test kits for the detection of infections have been brought forward. DNA hybridisation and amplification methods are central to these successful developments. A variety of MRSA-specific molecular tests has been developed and will be discussed.

When not detected in time, outbreaks of MRSA infection can lead to nosocomial endemicity and, although not always solidly supported by cost-benefit analyses, it is assumed that this leads to additional morbidity, maybe even mortality, but surely enhanced costs of hospitalisation. In The Netherlands and various other European (primarily Nordic) countries extensive efforts are put into the prevention of MRSA importation. In The Netherlands patients at risk are isolated until proven “free of MRSA”. In addition, in case of MRSA-positive patients an active “search and destroy” policy, supported by generally accepted national guidelines, is strictly implemented. On the basis of microbiological screening cultures departments may be closed and newly discovered carriers will be isolated as well. In addition to the active surveillance systems, Dutch microbiologists advocate prudent usage of antibiotics. To date MRSA is not (yet) a major problem in Dutch hospitals and the situation seems to be well controlled. Current hot topics involve the cost-effectiveness of the combined approach. Is the effort that is put in worthwhile? Can we really stop the flow of MRSA importation from the surrounding countries (with ICU patients in Belgium being colonised by MRSA in sometimes more than 50% regionally)? Does community-acquired MRSA colonisation or infection jeopardise our approach? Would molecular diagnostics be of additional value? How important is national surveillance for MRSA and should all MRSA be genotyped for defining (hyper)epidemic clones? How should such a system be quality controlled? These and other issues will be discussed in detail and the value of the Dutch “search and destroy” policy will be assessed.

In case of outbreaks, a multitude of molecular and biophysical methods suited for sub-species identification of MRSA strains are available to the infection control team. Raman spectrometry can, for instance, be used for high-speed identification of amplified DNA molecules, but such and other approaches can even be used in a nucleic acid-independent manner. Raman and UV spectrometry can be used to identify bacteria grown in microcolonies and in very short timespans (seconds). In conclusion, modern technology has revolutionised diagnostic approaches in clinical microbiology. This does not only improve detection and identification, but this also has a clear impact on our understanding of the fundamentals of infectious diseases.

Selected References for Further Reading

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**QUALITY CONTROL OF
DIRECT MOLECULAR DIAGNOSTICS OF
METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS***

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Abstract

Ten samples containing various amounts of MRSA, MSSA, MRSE and combinations thereof were distributed to 51 laboratories for molecular diagnostics. Samples containing 10^2 - 10^3 MRSA cells were frequently reported negative. MRSE were scored negative by all commercial tests, but only by 2 out of 3 in-house tests.

Methicillin-resistant *Staphylococcus aureus* (MRSA) requires timely detection in order to prevent infection and nosocomial transmission (1, 4). Classical microbiologists routinely use culture-based enrichment assays as the basis for detection and subsequent identification. Inclusion of elevated salt concentration and specific antibiotics in the growth medium allows for highly specific detection (8). However, culture requires prolonged incubation periods and, in general, confirmatory assays are required upon positive culture. Molecular assays may offer benefits over more traditional culture-based assays such as reduced time to identification and better specificity and sensitivity. The commercial diagnostics industry has introduced a range of molecular-based assays in recent years. However, there is little information available on their performance and some studies suggest that they may suffer from reduced sensitivity as a result of sample inhibition (3). Specificity may also be an issue as the presence of methicillin-resistant coagulase negative staphylococci including *Staphylococcus epidermidis* (MRCoNS and MRSE) may result in false positives. Given the number of commercially available test systems, the clinical impact of MRSA, and the need for timely diagnostics, a multi-centre External Quality Assessment (EQA) study on the efficiency and efficacy of molecular testing for MRSA was initiated by Quality Control for Molecular Diagnostics (QCMD, see also www.qcmd.org).

The QCMD MRSA EQA panel was distributed in October 2006 to 51 participating laboratories from 11 countries; along with detailed instructions on how to process the panel samples. Participants were given six weeks to report their results back to the QCMD Neutral Office using an online data collection system

The QCMD 2006 MRSA panel consisted of five samples containing various amounts of MRSA, three samples containing various amounts of

staphylococci other than *S. aureus* and one sample containing *Escherichia coli*, see Table 1). The content of the samples was quantified on the basis of culture and molecular testing. The IDI MRSA test was employed [Becton Dickinson] in combination with the Sigma Plant DNA isolation kit according to the manufacturers' instructions.

Out of the 51 participants, 46 responded (90%). Non-respondents indicated technical problems (n=2) or 'test under development' (n=2) as the reason for not returning results. Overall, 58 datasets were returned, 55 of which stated qualitative data only; three labs reported both qualitative and quantitative data. All participants received the expected results following the close of the programme. Subsequently, the QCMD Neutral Office analysed the data, which was released to participants in the form of a detailed EQA Final Report.

Most of the real-time data were generated on the Roche LightCycler System (n=14), Roche LightCycler 2.0 (n=6), Corbett Research Rotor-Gene 3000 and the Applied Biosystems systems (ABI 7500 Real Time PCR System (n=6), ABI PRISM 7000 Sequence Detection System (n=5) and ABI PRISM 7900 Sequence Detection System (n=1). Two Bio-Rad machines were included and single examples of the Cepheid Smart Cycler II System, Roche LightCycler 480 and the Stratagene MxP3000 Real Time System. For the in-house conventional PCRs the Eppendorf MasterCycler, MWG AG Biotech Primus 96 and the Perkin-Elmer 9600 were used. The diversity of the equipment covers the commercially available spectrum quite well.

QCMD used a simple scoring system for qualitative EQA data, which was: 2 points for a correct result and 0 points for all other results (including 'not determined' and 'equivocal'). The scores obtained are summarised in Table 2. The panel sample with the highest number of MRSA cells (MRSA06-08) was reported correctly in 97% of the datasets. This indicates that usage of molecular tests downstream of positive cultures is reliable: all tests can be used for culture confirmation. However, the samples containing lower amounts of MRSA (MRSA06-09 and MRSA06-06) were below the limit of detection of most participants' assays. Only in case of 12% and 52% of the datasets returned correct results for these samples. MRSE sample MRSA06-07 was correctly reported negative by all commercial PCR tests but incorrectly

reported as positive in 33% (1/3) of datasets generated with conventional in-house assays and 21% (9/43) of datasets generated using real time in-house PCR. The MSSA containing sample MRSA06-10 was scored incorrectly by both commercial and in house tests (conventional PCR 17%, real time PCR 9%), indicating moderate specificity. These levels of false positives underscore the need of improved specificity for these MRSA tests. Even the *E. coli* specimen could not be adequately tested (indeterminate in 7% (4/58)). Table 3 summarises the performance scores for the various PCR platforms. The performance of the commercial conventional PCRs and the in-house real time PCRs can be considered satisfactory. However, only three participants attained the maximum score of 20.

Quality control of molecular diagnostic tools is important in order to maintain high-quality clinical care in medical institutions. Multi-centre studies on the sensitivity and specificity of several bacterial and viral DNA tests have been performed in the past and the outcome of such studies has provided important information on performance (2, 5, 6, 7). Here we report on an EQA study for one of the most important nosocomial bacterial pathogens, MRSA. In conclusion, molecular identification of MRSA using samples with high CFU counts is reliable and can be implemented in the laboratory setting with confidence. Essentially, all testing formats perform equally well. However, for direct molecular diagnostics we have to conclude that the current array of tests does not meet the clinical quality criteria. Sensitivity of many tests is (too) low and the specificity needs to be improved. The reasons for poor assay sensitivity may be grounded in the common practice of pre-enriching samples by culture before confirmation by molecular assays. Pre-enrichment may lead to concentrations of MRSA in excess of 1×10^{10} CFU / ml, which is greater than the concentrations of MRSA likely to be encountered in an unmodified patient sample (and represented in this EQA panel). Some assays may have been designed specifically for this higher target concentration range leading to poor levels of sensitivity. The pre-enrichment approach also reduces one of the key improvements offered by molecular assays, which is more rapid diagnosis. The sensitivity of molecular assays for the detection of MRSA will only improve if the molecular diagnostics community moves away from pre-enrichment and tests directly from the clinical sample. Performance

would also be improved by provision of proper quality control materials and international standards for MRSA testing.

Although the stage has been set for direct clinical diagnosis of MRSA, our data here indicate that the current testing systems are insufficient. And clinical practice may even be worse: we here used clean samples and in case of samples such as urine, blood and sputa inhibitory compounds within these samples may even deteriorate the test' performance. These data clearly support the need for improvements in the molecular diagnosis of MRSA.

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Table 1 Panel composition and independent testing.

Sample	Sample Contents	Target Sample Conc (CFU/ml)	<u>In process testing result</u>		Sample Status
			qualitative	Ct value	
MRSA06-01	MRSA	10 ³	+	20.0	pos
MRSA06-02	MSSA+MRSE	10 ³ +10 ⁵	-	>45	neg
MRSA06-03	E. coli	10 ⁹	-	>45	neg
MRSA06-04	MRSA	10 ⁶	+	18.0	pos
MRSA06-05	MSSA+MRSE	10 ³ +10 ⁴	-	>45	neg
MRSA06-06	MRSA	10 ³	+	20.0	pos
MRSA06-07	MRSE	10 ⁹	-	>45	neg
MRSA06-08	MRSA	10 ⁹	+	15.0	pos
MRSA06-09	MRSA	10 ²	+	25.0	pos
MRSA06-10	MSSA	10 ⁹	-	>45	neg

All samples were provided in Mueller-Hinton broth. The MRSA strain was *S. aureus* N315, the MSSA was ATCC 29213 and the MRSE was 260. *E. coli* strain ATCC 35218 was used. For in process testing the IDI MRSA test (Becton Dickinson) was employed with the Sigma Plant DNA isolation kit as the processing unit for DNA extraction from the samples. The Ct value identifies the cut-off value for a positive score: when Ct>45 a sample can be considered negative. In case of Ct<45 a sample is positive, with the highest tittered samples scoring the lowest Ct value.

LANDSCAPE TABLES 2 AND 3

Table 2 Technology types and numbers of correct qualitative results per panel member.

Sample	Sample Contents	total datasets n=58		PCR							
				CONVENTIONAL				REAL TIME			
				Commercial (n=12)		In house (n=3)		In house (n=43)			
n	(%)	n	(%)	n	(%)	n	(%)	n	(%)		
MRSA06-01	MRSA 10 ³	30	52	6	50	1	33	23	54		
MRSA06-02	MSSA+MRSE 10 ³ +10 ⁶	55	95	12	100	3	100	40	93		
MRSA06-03	E. coli 10 ⁹	54	93	9	75	3	100	42	98		
MRSA06-04	MRSA 10 ⁶	48	83	12	100	2	67	34	79		
MRSA06-05	MSSA+MRSE 10 ³ +10 ⁴	56	97	12	100	3	100	41	95		
MRSA06-06	MRSA 10 ³	30	52	4	33	1	33	25	58		
MRSA06-07	MRSE 10 ⁹	48	83	12	100	2	67	34	79		
MRSA06-08	MRSA 10 ⁹	56	97	12	100	3	100	41	95		
MRSA06-09	MRSA 10 ²	7	12	2	17	0	0	5	12		
MRSA06-10	MSSA 10 ⁹	51	88	10	83	2	67	39	91		

The commercial tests were the Hain GenoQuick MRSA (n=2), Hain GenoType MRSA Direct (n=1) Hain GenoID MRSA (n=1), hyplex Staphyloresist (n=1), and Becton Dickinson IDI-MRSA (n=7).

Table 3 Qualitative performance scores per technology type.

PCR TECHNOLOGY	Total Datasets	MEAN±SD	MEDIAN	quartile		SCORE										
				25%	75%	6	8	10	12	14	16	18	20			
Conventional commercial	12	15.17±1.99	14	14	16.5	0	0	0	1	6	2	3	0			
Conventional in house	3	13.33±2.31	12	12	14	0	0	0	2	0	1	0	0			
Real time, in house	43	15.07±2.84	16	14	18	0	1	2	10	17	13	12	3			

For a correct result two points were scored, 0 points for any other result. A maximum of 20 points could be obtained. SD: standard deviation; The 25 % quartile may be taken to be the median of the lower half of the scores provided for each technology group, the 75% quartile represents the median of the upper half of the scores.

