Detection of MRSA encoding *mecA*$_{LGA251}$: Problems and solutions?

Angela Kearns[1], Elizabeth Fagan[1], Robert Hill[1], Giles Edwards[2], Christopher Teale[3], Mark Holmes[4], Bruno Pichon[1], Christine Walton[1]

[1]Microbiology Services, HPA Colindale, London, NW9 5EQ, UK [2]Scottish MRSA Reference Laboratory, Stobhill Hospital, Glasgow, G21 3UW, UK
[3]Animal Health and Veterinary Laboratories Agency, Shropshire, SY1 4HD, UK [4]Dept Veterinary Medicine, University of Cambridge, UK

angela.kearns@hpa.org.uk

Introduction and Purpose

The recent identification of MRSA encoding a newly described *mecA* homologue (*mecA*$_{LGA251}$) highlighted the fact that accurate detection and identification of these organisms could pose a challenge for clinical diagnostic laboratories using standard protocols.

The United Kingdom National External Quality Assessment Service (UK NEQAS) distributes clinically relevant and educational specimens for external quality assessment (EQA). The MRSA screening scheme enables participants to assess the quality of the culture and molecular screening techniques employed for the detection MRSA.

To improve awareness and increase case ascertainment, we sought to (i) include a representative isolate in the UK NEQAS MRSA screening scheme and (ii) assess the performance of phenotypic methods for their detection.

Methods

i) In July 2011, a *mecA*$_{LGA251}$-positive MRSA (oxacillin and cefoxitin MIC = 16mg/L) was distributed to 311 UK NEQAS participants, from 19 countries (Table 1), to screen for MRSA using their standard culture and/or molecular methods.

Table 1. Countries participating in MRSA screening scheme (July 2011)

Participating countries			
Austria	France	Netherlands	Sweden
Belgium	Hong Kong	Norway	Switzerland
Croatia	Ireland	Saudi Arabia	United Arab Emirates
Denmark	Italy	Slovenia	United Kingdom
Finland	Kuwait	South Africa	

(ii) A total of 55 *mecA*$_{LGA251}$-positive MRSA were studied (Table 2). They represented 4 different lineages and were recovered from human sources in England and Scotland (n=30 and 12 respectively), and bovine mastitis cases in England (n=13). Isolates were cultured onto a non-selective medium (nutrient agar) and chromID MRSA selective agar (bioMérieux, which contains cefoxitin) prior to screening for PBP2a using two commercially available kits (Figure 1):

- MASTALEX™ MRSA (Mast) – an agglutination assay using latex particles sensitised with anti-PBP2a monoclonal antibodies
- Clearview® Exact (Alere) – an immunochromatographic membrane assay using anti-PBP2a monoclonal antibodies

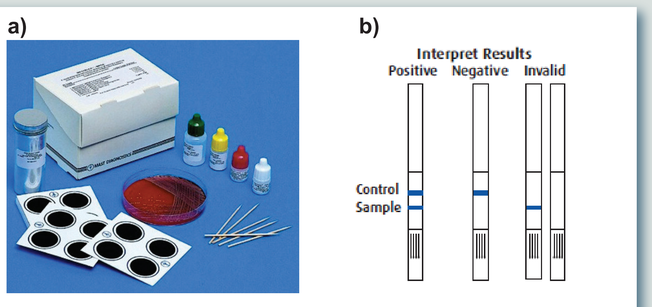

Figure 1. Two simple, rapid methods for the detection of PBP2a; a) MASTALEX™ MRSA and b) Clearview® Exact

Results

i) Analysis of UK NEQAS EQA results

301 of 311 UK NEQAS participants returned results for the *mecA*$_{LGA251}$-positive MRSA.

Molecular methods

Of 68 participants that reported on the detection of MRSA using molecular methods (Figure 2):

- 16 (24%) reported positive results
  - 3 were true positives from in-house PCRs that could detect *mecA*$_{LGA251}$
  - those using commercial molecular-based kits are presumed to have based this conclusion on test results from conventional culture run in parallel (*mecA*$_{LGA251}$ and SCC*mec* type XI are sufficiently divergent not to be detected by currently available commercial molecular-based techniques)
- 49 (72%) reported a negative result
  - 11 of these stated that based on reduced susceptibilities to oxacillin or cefoxitin they would refer the isolate for further investigation querying borderline oxacillin resistant *S. aureus* (BORSA), or new *mecA*-homologue/*mecA*$_{LGA251}$
- 3 (4%) reported an invalid result

Culture-based methods

Of the 299 participants that reported on the detection of MRSA using culture methods:

- 225 (75%) detected MRSA (Figure 3)
- 74 (25%) reported MRSA not detected (Figure 3)

The high failure rate by conventional culture with *mecA*$_{LGA251}$ isolates may be due to their low level resistance to oxacillin and cefoxitin (typical MICs 4-16 mg/L)

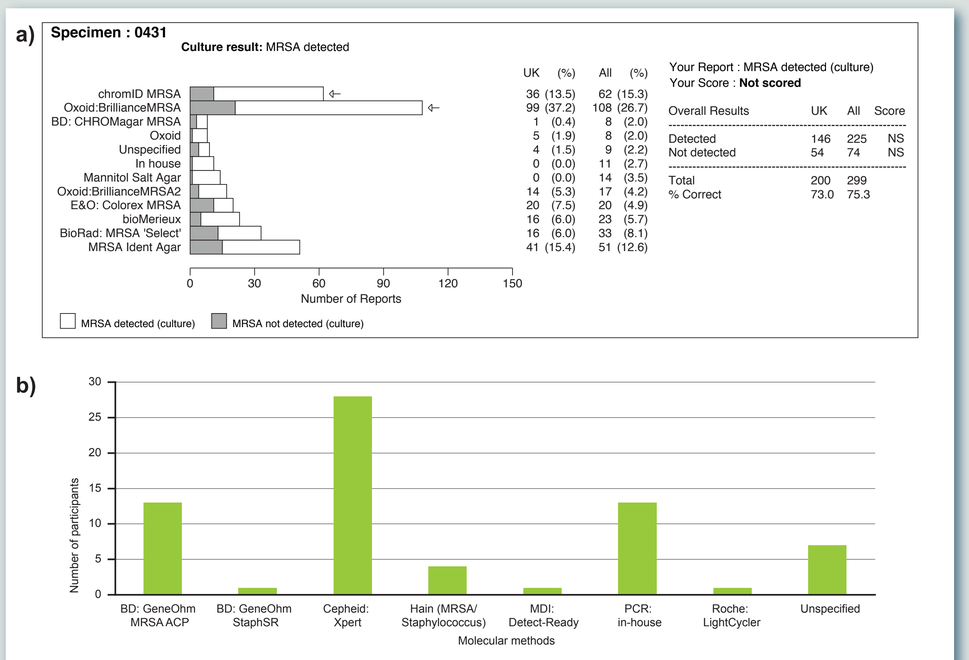

Figure 2: Molecular results of UK NEQAS participants for the *mecA*$_{LGA251}$-positive MRSA, distributed in July 2011 as specimen 0431: (a) results displayed by method in the histogram [some participants used more than one kit/method]; (b) breakdown of molecular methods used

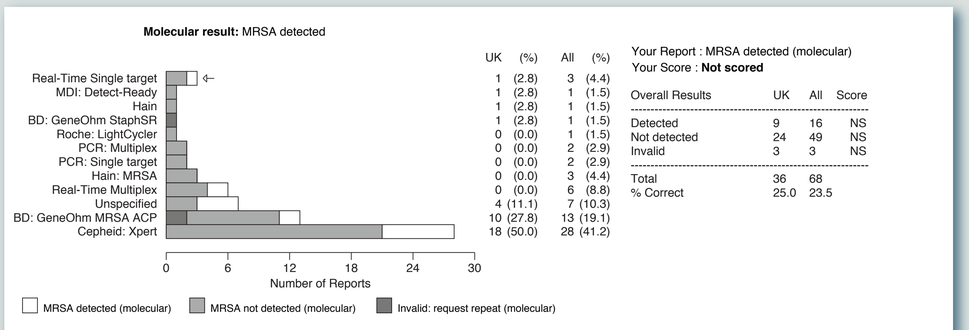

Figure 3: Culture results of UK NEQAS participants for *mecA*$_{LGA251}$-positive MRSA, distributed in July 2011 as specimen 0431: results are displayed by method in the histogram

ii) Analysis of results of phenotypic methods

MASTALEX™MRSA

- None of the *mecA*$_{LGA251}$–positive MRSA gave a positive result whether cultured on non-selective or selective media

Clearview® Exact PBP2a

- When cultured on nutrient agar, all test isolates gave a **negative** result with the Clearview® Exact assay
- When cultured on chromID™ MRSA agar (bioMérieux), all test isolates gave a **positive** result with the Clearview® Exact assay

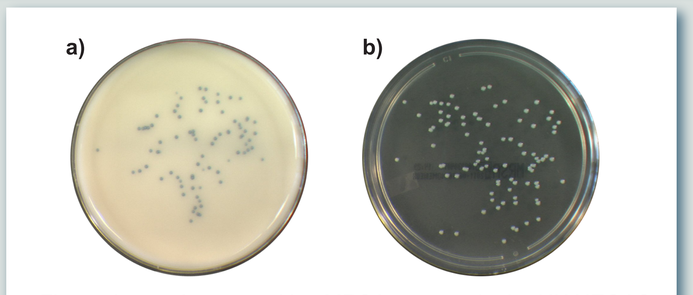

Figure 4: *mecA*$_{LGA251}$-positive MRSA sent out in the UK NEQAS MRSA screening scheme grown on (a) Brilliance MRSA agar (Oxoid) and (b) Chrom ID™ MRSA agar (bioMérieux)

Table 2. Phenotypic and genotypic characteristics of study isolates

No. isolates	MLST-CC	*Spa* type(s)	PCR for *mecA*$_{LGA251}$ [a]	Oxa MIC (mg/L)	FOX MIC (mg/L)	ChromID MRSA agar (bioMérieux)	Clearview® Exact PBP2a (Alere)		MASTALEX™ (Mast)	
							Without FOX induction[b]	With FOX induction[c]	Without FOX induction[b]	With FOX induction[c]
Human sources[d] 28	130	t843 t1535 t3256 t6293 t7485 t7734 t7946 t7947 t9605	Pos	8-32	4-64	Growth	Neg	Pos	Neg	Neg
11	425	t742 t6292 t6300 t6386 t9376	Pos	8-32	4-64	Growth	Neg	Pos	Neg	Neg
2	1943	t978 t7945	Pos	8-32	4-64	Growth	Neg	Pos	Neg	Neg
1	ST2179	t8833	Pos	8-32	4-64	Growth	Neg	Pos	Neg	Neg
Animal sources[e] 11	130	t843 t6220 t6293	Pos	2-32	4-32	Growth	Neg	Pos	Neg	Neg
2	425	t2678 t6292	Pos	12-16	4-32	Growth	Neg	Pos	Neg	Neg
Correctly identified						**100%**	**0%**	**100%**	**0%**	**0%**

[a] Pichon et al, submitted for publication; [b] Isolates grown on non-selective medium (nutrient agar); [c] Isolates grown on selective medium containing cefoxitin (ChromID MRSA agar, bioMérieux); [d] Various sites including skin, blood, nose, throat, sputum; [e] Bovine mastitis

Conclusions

- Results from laboratories participating in the EQA distribution have highlighted problems in identifying *mecA*$_{LGA251}$-positive MRSA with the commercial molecular kits used
- Where phenotypic and/or genotypic data for oxacillin/cefoxitin resistance in *S. aureus* are discrepant, the possibility of *mecA*$_{LGA251}$-positive MRSA should be considered
- MRSA harbouring either *mecA* or *mecA*$_{LGA251}$ can be detected successfully using Clearview® Exact following cefoxitin induction, but differentiation between these two gene homologues requires the use of specific PCR-based methods
- This combined study alerts routine diagnostic microbiology laboratories to the challenges of accurately detecting emerging MRSA strains

Acknowledgements: We would like to thank participants of the UK NEQAS MRSA screening scheme for their support and the team at UK NEQAS for their dedication and contribution to the service. Special thanks go to staff in the Staphylococcus Reference and Antibiotic Resistance Evaluation Units, HPA Colindale.

M372. Produced & printed by HPA Colindale Medical Illustration. www.hpa.co.uk © Health Protection Agency 2012

AUSTRIA, BELGIUM, BOSNIA AND HERZEGOVINA, BULGARIA, CROATIA, CYPRUS, CZECH REPUBLIC, DENMARK, ESTONIA, FINLAND, FRANCE, GERMANY, GREECE, HUNGARY, ICELAND, IRELAND, ITALY, LATVIA, LITHUANIA, LUXEMBURG, MALTA, NETHERLANDS, NORWAY, POLAND, PORTUGAL, ROMANIA, SLOVENIA, SPAIN, SWEDEN, TURKEY, UNITED KINGDOM