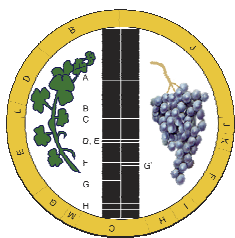


ST. JAMES'S HOSPITAL LABMED DIRECTORATE

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Author: Gráinne Brennan			Date:04/04/2011	Date of Issue: 04/04/2011
Authorised By: Brian O'Connell			Date:04/04/2011	

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(Only to be used if there are no modifications at review)



National MRSA Reference Laboratory

Users Manual

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Author: Gráinne Brennan		Date: 04/04/2011	Date of issue: 04/04/2011
Authorised By: Brian O'Connell		Date: 04/04/2011	

Table of Contents	Page
Contents.....	2
General Information.....	3
Location	3
Postal Address.....	3
Contact Details.....	3
Contact Names	3
Contact Details for Advice.....	3
Opening Hours	3
User Satisfaction	3
External Agencies	4
Specimens	5
Services Available in NMRSARL.....	5
Turnaround Times.....	6
Completing NMRSARL Laboratory Investigation Forms	6
Isolate Identification	7
European Antimicrobial Resistance Surveillance Network (EARS-Net).....	7
Monitoring the MRSA Population in Ireland	7
Dispatch of MRSA isolates to NMRSARL	7
Appendix 1. Sample of National MRSA Reference Laboratory Investigation Request Form.....	8
Appendix 2. Sample of EARS-Net <i>S. aureus</i> Record Form.....	9
Appendix 3. Guidelines for sending MRSA isolates for epidemiological typing	10

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General Information

Location

The National Meticillin-resistant *Staphylococcus aureus* (MRSA) Reference Laboratory (NMRSARL) is located in St. James's Hospital (SJH) at the Rialto end of the SJH campus in a building separate from the Central Pathology Laboratory Building.

Postal Address

National MRSA Reference Laboratory
 St. James's Hospital
 James's St.
 Dublin 8
 Ireland.

Contact Details

Tel: +353 1 410 3662
 Fax: +353 1 410 3666
 Webpage: www.nmrsarl.ie
 Email: mrsarl@stjames.ie
 DX Address: 6000310
 DX Exchange: James Street 6 IE

Contact Names

Medical Director:	Dr Brian O'Connell	Tel: 01 416 2912
Chief Medical Scientist:	Ms. Gráinne Brennan	Tel: 01 410 3662.

Contact Details for Advice

Patient treatment/management	Dr Brian O'Connell	01 416 2912
Infection control	Infection Control Team SJH	01 416 2961
Laboratory aspects of MRSA	Ms. Gráinne Brennan	01 410 3662

Opening Hours

Monday to Friday 9.00 am to 5.00 pm

User Satisfaction

NMRSARL operates an on-going process of service evaluation and quality improvement to meet users' needs. This process includes meeting with its Users Group and undertaking user satisfaction surveys. All comments and complaints are welcome and are evaluated by laboratory management so that the service may be improved to ensure that NMRSARL provide the best possible level of service to its users.

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External Agencies

The NMRSARL utilises expertise available from a number of external agencies to ensure the best possible service is provided to our users and has a continuing commitment to research and development activities relevant to the work of the laboratory.

Strategic Development

Professor Hilary Humphreys, Consultant Microbiologist, Beaumont Hospital and Royal College of Surgeons Ireland chairs a users group and advises the laboratory on users needs and aspects of strategic development.

Research and Development

The NMRSARL is involved in on-going research and development collaborations with Professor David Coleman, Dublin Dental School and Hospital, Trinity College Dublin Dr. Celine Herra, Dublin Institute of Technology along with European and international laboratories

Areas of interest include:

- Community associated MRSA
- MRSA in animals
- Molecular characterisation of MRSA clones
- *S. aureus* with reduced susceptibility to vancomycin
- Panton-Valentine Leukocidin toxin in *S. aureus*
- Development of real-time PCR assays for use in NMRSARL
- Practical aspects of use of a DNA array for routine use

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Specimens

NMRSARL investigates MRSA isolates submitted by medical practitioners and hospital laboratories in Ireland. It does not investigate patient specimens for MRSA. Isolates should be submitted as fresh subcultures on nutrient agar slopes from 18-h subcultures grown on blood agar. Isolates should be submitted as soon as possible after isolation or should be fresh subcultures from isolates stored at -20°C or -70°C as soon as possible after isolation. Isolates must be submitted in pure culture. All isolates for NMRSARL laboratory investigation must be accompanied by an NMRSARL laboratory investigation form (see Appendix 1). The form is designed to accommodate up to 10 isolates per form to facilitate users when sending a number of isolates for outbreak investigation. Forms are available to download from the NMRSARL website at www.nmrsarl.ie.

Services Available in NMRSARL

NMRSARL provides the following services:

1. Investigation of MRSA isolates for
 - confirmation of *S. aureus* identity and meticillin resistance
 - epidemiological typing using
 - antibiogram-resistogram (AR) typing using a panel of 23 antimicrobials in conjunction with a number of heavy metal agents
 - biotyping
 - DNA macrorestriction analysis using pulsed field gel electrophoresis (PFGE) (available in limited circumstances)
 - investigation of glycopeptide resistance
 - detection of the *pvl* gene encoding the Panton-Valentine leucocidin toxin
 - Characterization of selected isolates by staphylococcal protein A (*spa*) typing is available in NMRSARL in exceptional circumstances

2. Investigation of meticillin susceptible *S. aureus* for
 - detection of the *pvl* gene that encodes for the Panton-Valentine leucocidin Toxin
 - Outbreak investigation of strains using PFGE
Please contact the NMRSARL prior to sending MSSA isolates for outbreak investigation.

3. Advice
 - on treatment and management of patients with MRSA through its medical director
 - on infection control through the infection control team of SJH
 - on laboratory aspects of MRSA through the scientific staff of NMRSARL.

Resource constraints dictate that the present epidemiological typing service is limited to AR typing and biotyping for institutions experiencing acute problems with MRSA.

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Meticillin resistance confirmation is generally limited to phenotypic confirmation. PFGE is available in restricted circumstances only and polymerase chain reaction (PCR) assays are available for research purposes only. Current PCR assays include detection of *mecA* (which encodes meticillin resistance) and *pvl* (encoding the Panton-Valentine leucocidin).

Turnaround Times

The turn-around time (TAT) is calculated from the date of receipt of an isolate in pure culture. Due to workload levels some investigations must be 'batched' and TATs vary accordingly. Please see table below for approximate TATs.

Test	TAT
AR Typing	4 consecutive working days
Confirmation of meticillin resistance (phenotypic investigation)	4 consecutive working days
Reduced susceptibility to glycopeptides	5 consecutive working days
Detection of <i>mecA</i> gene	10 working days
Detection of PVL toxin	10 working days
Pulsed field gel electrophoresis	15 working days

Completing NMRSARL Laboratory Investigation Forms

Forms are available to download from the NMRSARL website at www.nmrsarl.ie

Please complete the form as indicated below.

- **Sender:** Indicate the sender's name; sender's staff position (e.g. Consultant Microbiologist, Senior Medical Scientist, Surveillance Scientist, etc); hospital address; contact details and date. Include a contact name if other than above.
- **Reason for Sending Isolate(s):** Please tick the appropriate box to request the required laboratory investigation (i.e. confirmation of *S. aureus* identity, confirmation of meticillin resistance, confirmation of mupirocin resistance etc. or epidemiological typing). If the laboratory investigation request is other than those listed above, use the 'Sender's Comments' box to request the investigation.
- **Sender's Results:** Please indicate your laboratory results in this section.
- **Patient / Isolate Information:** Please supply the specimen number from which the isolate was recovered, the date the specimen was taken, the patient's name (or initials), hospital number, date of birth and ward. Please complete any known antibiogram details. If sending isolates for epidemiological typing, up to 10 isolates from the same outbreak investigation can be accommodated on one form.
- **Sender's Comment:** Please complete this section if there is any clinical information relevant to the required investigation or if the isolate exhibits unusual microbiological features.

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Isolate Identification

Isolate slopes must be clearly identified with **at least two identifiers** (e.g. isolate number and patient's hospital number, name, initials or date of birth).

European Antimicrobial Resistance Surveillance Network (EARS-Net)

NMRSARL provides laboratory support for the MRSA component of EARS-Net in Ireland. All Irish hospital laboratories participating in EARS-Net send MRSA isolates from blood cultures (one per patient per quarter) to NMRSARL. NMRSARL performs minimum inhibitory concentration (MIC) determinations of oxacillin and screens isolates for reduced susceptibility to vancomycin using the E-test™ macro-method. NMRSARL also provides data on rates of resistance to other clinically useful antibiotics. All EARS-Net MRSA isolates must be accompanied by copies of the EARS-Net *S. aureus* Record form, a WHONET printout or other agreed documentation. A copy of the EARS-Net *S. aureus* Record form is included in Appendix 2. EARS-Net forms are available from the NMRSARL website at www.nmrsarl.ie or from the Health Protection Surveillance Centre's website at: <http://www.hpsc.ie/hpsc/A-Z/MicrobiologyAntimicrobialResistance/EuropeanAntimicrobialResistanceSurveillanceSystemEARSS/ProtocolsandForms/File.2295,en.pdf>.

Monitoring the MRSA Population in Ireland

NMRSARL investigates MRSA from Irish EARS-Net participant hospitals to monitor the strains of MRSA that constitute the Irish MRSA population. Isolates are typed by AR typing, biotyping and PFGE typing. These data are combined to generate AR-PFG types¹. Quarterly reports are issued to each participant laboratory detailing the overall AR-PFG type distribution among EARS-Net MRSA isolates. NMRSARL also provides separate reports for each individual laboratory of that laboratory's AR typing results. These results provide useful background information on the MRSA population in each hospital against which potential outbreaks can be assessed.

Dispatch of MRSA isolates to NMRSARL

All required safety precautions for the packing and transport of MRSA isolates must be observed. MRSA isolates may be sent to the Microbiology Department, SJH with the DX courier service. NMRSARL collects isolates daily from the Microbiology Department, SJH. When sending isolates for epidemiological typing please observe the guidelines detailed in Appendix 3.

¹ Rossney AS, Lawrence MJ, Morgan PM *et al.* *Eur J Clin Microbiol Infect Dis* 2006; **25**: 79-89.

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Appendix 1. Sample of National MRSA Reference Laboratory Investigation Request Form

National Meticillin-Resistant <i>Staphylococcus aureus</i> Reference Laboratory (NMRSARL)															
Laboratory Investigation Request Form															
Sender			Date:			Reason for sending isolates:				Indicate Sender's Results here:					
Name: Position: Address: Telephone: Fax: Email: HaysDx: Contact Name: (if other than above) Telephone:			Please tick 1) Confirmation of: S. aureus identity: <input type="checkbox"/> Meticillin resistance: <input type="checkbox"/> Mupirocin resistance: <input type="checkbox"/> Glycopeptide resistance: <input type="checkbox"/> 2) Epidemiological typing <input type="checkbox"/> Nature of incident: <input type="checkbox"/>				Sender's Result (Pos. Neg. S. M. R) Tube: <input type="checkbox"/> Slide/Kit: <input type="checkbox"/> Tnase/Dnase: <input type="checkbox"/> Met5: <input type="checkbox"/> Met10: <input type="checkbox"/> Ox1: <input type="checkbox"/> Ox5: <input type="checkbox"/> Mup5: <input type="checkbox"/> Mup200: <input type="checkbox"/> Agar screen: <input type="checkbox"/> Van5: <input type="checkbox"/> Tei30: <input type="checkbox"/>								
Patient/Isolate information (please use date format dd/mm/yyyy)							Sender's Susceptibility Results					Ref Lab Use Only			
Sender Lab No.	Specimen Date	Patient Last Name	Patient First Name	Hospital No.	DoB	Ward	Gen	Ery	Cip	Tet	Rif	Tmp	Fuc	Mup	MRSA Ref Lab No.
Sender's Comments							Send isolates to: Director, National MRSA Reference Laboratory, St James's Hospital, James's St, Dublin 8.					Ref Lab Use Only Incident No: Date Rec'd: Comment:			
Isolates must be packed and dispatched according to appropriate safety regulations. Improperly packaged isolates (including use of staples) will not be processed.															

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Appendix 3. Guidelines for sending MRSA isolates for epidemiological typing

Best value is obtained from epidemiological typing of MRSA if a number of guidelines for sending isolates are followed. These include that, for all requests:

- there is a definite hypothesis to be tested² or a clear question that typing results will answer and that results will make a difference to management of the problem
- typing is only requested if there is a definite plan to act upon results
- a consultant microbiologist and/or infection control team has confirmed there are good reasons for sending isolates.

NMRSARL asks users to follow these guidelines when requesting epidemiological typing of MRSA. If in doubt, please contact NMRSARL prior to sending isolates.

As a general guide, NMRSARL requests that:

- In the event of a clear-cut common source outbreak
Users should send the minimum number of isolates needed to confirm the source and extent of the outbreak. A smaller number of isolates epidemiologically unrelated to the outbreak should also be sent for comparative purposes. (This may be unnecessary for institutions sending EARS-Net MRSA as these isolates are routinely typed and provide useful background information).
- In the event of protracted or complex outbreaks involving several wards or units
Users should send the minimum number of isolates needed to test the hypotheses of time and place and store other temporally-related isolates (frozen if possible)¹.
- As an aid to choosing appropriate isolates, use surrogate markers (such as antimicrobial resistance, and/or urease) and include isolates with significantly different phenotypes
- Do not send more than one isolate per patient unless the patient appears to carry more than one strain of MRSA (as indicated by, for example, differences in the routine susceptibility testing pattern)
- Environmental isolates should be sent only after prior discussion with NMRSARL
- MRSA should be sent as soon as possible after isolation unless isolates are stored frozen. Where isolates have been stored frozen, fresh subcultures should be sent.
- Where possible, isolates from each putative outbreak incident should be sent in a single batch.
- Management of endemic MRSA
When users plan to investigate endemic MRSA, they are requested to contact NMRSARL before sending isolates so that best use can be made of scarce resources.

² Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. *Journal of Hospital Infection* (1998) **39**: 253-290.