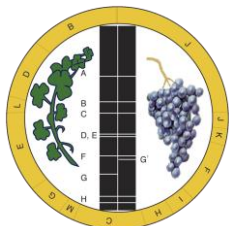


SJH CENTRE FOR LABORATORY MEDICINE & MOLECULAR PATHOLOGY			
Edition No.:	10	National MRSA Reference Laboratory	Doc No: LP-MRSA-0067
Author: Gráinne Brennan		Date: 20/07/2026	Date of Issue: 20/07/2026
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National MRSA Reference Laboratory

User's Manual

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1.	Location	3
2.	Contact Details	3
3.	Contact Names	3
4.	Contact Details for Advice	3
5.	Opening Hours	3
6.	Users of the Laboratory Service	3
7.	Data Protection	3
8.	Specimens	3
9.	Services Available	4
10.	Whole-genome sequencing	5
11.	Turnaround Times	6
12.	Completing NMRSARL Laboratory Investigation Forms	6
13.	Isolate Identification	7
14.	Dispatch of MRSA isolates to NMRSARL	7
15.	European Antimicrobial Resistance Surveillance Network (EARS-Net)	8
16.	Result Reporting	8
17.	Monitoring of MRSA Population in Ireland	8
18.	User Satisfaction	9
19.	External Agencies	9

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1. 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.

2. Contact Details

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

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

3. Contact Names

Role	Name	Telephone	Email
Consultant Microbiologist	Dr. Tee Keat Teoh	01 416 2912	TKeatTeoh@stjames.ie
Chief Medical Scientist	Dr. Gráinne Brennan	01 410 3662	gbrennan@stjames.ie

4. Contact Details for Advice

Patient treatment/management	Dr Tee Keat Teoh	01 416 2912
Laboratory aspects of MRSA	Dr Gráinne Brennan	01 410 3662
Infection control	Infection Control Team SJH	01 416 2961

5. Opening Hours

Monday to Friday 8.30 am to 4.30 pm

6. Users of the Laboratory Service

The NMRSARL service is available to all hospital microbiology laboratories in Ireland. Users of the service, at their own expense, are responsible for the packaging and transportation of samples to the NMRSARL in accordance with the ADR (European Agreement Concerning the International Carriage of Dangerous Goods by Road, 2003) regulations (see section 13 below). Details of specimen identification, request forms, turnaround times, results reporting and services available are detailed below. The NMRSARL does not currently charge hospital microbiology laboratories for the routine services provided by the laboratory.

7. Data Protection

NMRSARL will ensure that its obligations as a Data Processor under the Data Protection Act and other relevant EU Directives are fulfilled.

8. Specimens

NMRSARL investigates MRSA isolates submitted by medical practitioners and hospital laboratories in Ireland. The laboratory does not investigate patient specimens for MRSA. Isolates should be submitted as fresh subcultures on nutrient agar slopes from 18-h subcultures grown on

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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 8 isolates 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.

9. Services Available

While primarily the NMRSARL investigates MRSA additional services for investigation of MSSA, Coagulase negative staphylococci and Enterococci are available and are listed below.

Strains	Investigations
MRSA/MSSA	- Confirmation of <i>S. aureus</i> identity and meticillin resistance - Susceptibility testing against a panel of antimicrobial agents by disk diffusion in accordance with EUCAST interpretive criteria - Investigation of glycopeptide resistance - Broth MIC Determination against the following panel of antibiotics - Linezolid - Daptomycin - Ceftaroline - Vancomycin - Dalbavancin - Tigecycline - Teicoplanin - Detection of the toxin encoding genes including <i>pvl</i>, <i>etA</i>, <i>etB</i> and <i>etD</i> - Detection of <i>cfr</i>, <i>optrA</i> and <i>poxA</i> genes encoding linezolid resistance and the G2376T mutation - Characterization of selected isolates by staphylococcal protein A (<i>spa</i>) typing - Whole genome sequencing for isolate relatedness, typing and the presence/absence of resistance and virulence genes*
Coagulase negative staphylococci	- Susceptibility testing* - Confirmation of glycopeptide resistance* - Confirmation of linezolid resistance - Detection of <i>cfr</i>, <i>optrA</i> and <i>poxA</i> genes encoding linezolid resistance and the G2376T mutation
Enterococci	- Confirmation of linezolid resistance - Detection of <i>cfr</i>, <i>optrA</i> and <i>poxA</i> genes encoding linezolid resistance and the G2376T mutation - Whole genome sequencing for outbreak investigation* - Broth MIC Determination against the following panel of antibiotics

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Strains	Investigations
	○ Ampicillin ○ Dalbavancin ○ Daptomycin ○ Delafloxacin ○ Levofloxacin ○ Linezolid ○ Tedizolid ○ Teicoplanin ○ Vancomycin

**Tests are outside the scope of INAB registered laboratory Reg No. 327MT ISO15189 accreditation and will be highlighted as such on all reports issued*

Current PCR assays include detection of *mecA* and *mecC* (both of which encode meticillin resistance) and *pvl* (encoding the Panton-Valentine leucocidin) and are performed weekly on a Thursday. Investigation of linezolid resistance determinants in Staphylococci and Enterococci is performed weekly on a Wednesday. *spa* typing is performed weekly on a Friday while whole genome sequencing is performed as required. PCR for the detection of linezolid resistance is performed as frequently as requested.

Services provided to users are reviewed regularly and any changes to services that may arise as a result and which impact on the services provided to users will be communicated to users in an updated user manual.

10. Whole-genome sequencing

Whole-genome sequencing (WGS) is an essential tool for public health surveillance, characterisation of antimicrobial drug resistance and the molecular epidemiology of infectious diseases. It enables incidence monitoring of pathogens at genotype level and allows precise geographical delineation of the spread of organisms. Coupled with epidemiological and environmental investigations, it delivers ultimate resolution for tracing sources of epidemic infections.

The analysis of whole genome sequencing (WGS) data to support outbreak investigations of Staphylococci and Enterococci is available on request only. The genetic relatedness of isolates linked to a cluster is assessed by core genome multi locus sequence typing (cg-MLST) based analyses of WGS data.

Outbreak reports include:

- Lineage (Multi Locus Sequence Type – MLST)
- cgMLST
- Relatedness to other isolates included in the outbreak investigation

For the characterisation of single isolates, MSSA or MRSA may also be referred for WGS analysis for reasons including:

- Surveillance – for example, MRSA or MSSA from cases of bacteraemia;

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- Typing, antimicrobial resistance gene and/or toxin gene profiling;
- Identification of suspected Hospital, Community or Livestock-Associated MRSA.

Depending on the nature of the reason for referral, the following will be derived and reported from WGS data using SeqSphere™ analysis software:

- The lineage (MLST)
- The detection of resistance genes including:
 - *mecA* and its homologue *mecC*, which confer resistance to oxacillin
 - *mupA* and *mupB* which confer high-level and low-level resistance to mupirocin
- Toxin gene profiling providing insights into strain virulence including:
 - 9 enterotoxin genes (*sea-see* and *seg-sei*)
 - 3 exfoliative toxin genes *eta*, *etb* and *etd*
 - Toxic shock syndrome toxin gene 1 (*tst*)
 - Panton-Valentine Leukocidin toxin gene

Key factors affecting the performance of the test include:

- mixed culture
- single isolate with no indication of what it should be compared with
- lack of epidemiological information

Whole genome sequencing is undertaken on a 1-2-week basis and as such the TAT for isolates submitted for WGS analysis may be 3-4 weeks. Please contact the laboratory in advance of submitting isolates to determine an approximate TAT and to discuss the epidemiological basis for submission.

11. 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
Confirmation of meticillin resistance (phenotypic investigation)	4 working days
Reduced susceptibility to glycopeptides and broth microdilution determination	5 working days
Detection of <i>mecA/mecC</i> gene	8 working days
Detection of PVL toxin	8 working days
Detection of linezolid resistance genes (<i>cfr</i> , <i>optrA</i> , <i>poxtA</i> and G2576T mutation)	8 working days
<i>spa</i> typing	10 working days
Whole genome sequencing	20 working days

12. Completing NMRSARL Laboratory Investigation Forms

Forms are available to download from the NMRSARL section of the SJH website.

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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 the person sending the isolates.
- **Test Required:** 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 'other' box to request the investigation. If isolates are to be included with a previous NMRSARL incident, include the required incident number.
- **Patient / Isolate Information:** Provide 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 8 isolates from the same outbreak investigation can be accommodated on one form.
- **Clinical Details:** Please complete this section if there is any clinical information relevant to the required investigation or if the isolate exhibits unusual microbiological features.

13. 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).

Isolate slopes will be rejected if:

- Isolates are sent on agar plates or large agar slopes;
- Specimen slope is broken;
- Specimen form and/or slope are unlabelled, mismatched or incomplete;
- If for any other reason the specimen is not in a safe condition for processing and/or cannot be clearly identified.

When an isolate is rejected due to any of the above reasons the sender of the isolate will be contacted by phone or email, informed and requested to send a repeat culture.

14. 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 or any other suitable alternative method. NMRSARL collects isolates daily from the Microbiology Department, SJH. When sending isolates for epidemiological typing please observe the guidelines detailed in Appendix 2.

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15. 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 a completed NMRSARL request form indicating EARS-Net, a WHONET printout or other agreed documentation.

16. Result Reporting

The preferred format of returning results is through encrypted email. All institutions are requested to provide details of an email address (or several addresses) that will accept ALL NMRSARL reports. St. James's Hospital uses Transport Layer Security (TLS) technology as part of its email management system. Because symmetric cryptography is used to encrypt the data transmitted, the email connections using this technology are fully secure. The hospital has implemented forced encryption with certain commonly used email domains including all @hse.ie email addresses and the addresses for other hospitals throughout the country. All emails are automatically encrypted in both directions, so there is no need for any further action on the part of users to ensure that Data Protection requirements are met.

Where a user has not provided a nominated email to receive reports, reports will be returned to the user by post to the sender of the isolate or, to the departmental Consultant Microbiologist once the report is issued.

EARS-Net isolates from each hospital are reported bi-annually on one cumulative report. The reports are sent to the Consultant Microbiologist and the Chief Medical Scientist of laboratories from which isolates were received.

Users will be notified in writing prior to the introduction of any changes in laboratory procedures that will result in significantly different interpretation of results.

17. Monitoring of 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. In addition a summary of the epidemiological types of all isolates submitted during the previous six-month period is available on the NMRSARL website. These results provide useful background information on the MRSA population in each hospital against which potential outbreaks can be assessed.

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18. User Satisfaction

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

19. 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.

Research and Development

The NMRSARL is involved in on-going research and development collaborations with the Microbiology Research Unit, Dublin Dental School and Hospital, Trinity College Dublin 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
- Population structure of *E. faecium* circulating in Irish hospitals
- Practical aspects of use of a whole genome sequencing for routine use