Specimen Submission and Testing Manual

Bacterial Diseases Division
Silver Spring, Maryland 20910

This manual is designed to provide detailed instructions for submission of samples that will be analyzed in the MRSN. Please send a message to:
usarmy.detrick.medcom-wrair.mbx.WRAIR-MRSN@mail.mil
to obtain additional information.

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Introduction

As the sole antimicrobial resistance (AMR) surveillance network and repository in the DoD and as a named component in the White House National Action Plan for Combating Antibiotic Resistant Bacteria, the MRSN is charged with carrying out an increasing number of internal DoD-based evaluations and studies to address operationally and militarily-relevant questions involving Multidrug-Resistant isolates to include:

- *Staphylococcus aureus:* testing oxacillin resistant, or positive from molecular testing for mecA and PBP2a.
- *Enterococcus* spp.: testing resistant to vancomycin
- *Klebsiella spp.:* testing intermediate or resistant to ceftazidime or ceftriaxone OR representative agents within 3 antimicrobial classes including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.
- *Escherichia coli*: testing intermediate or resistant to ceftazidime or ceftriaxone OR representative agents within 3 antimicrobial classes including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.
- *Acinetobacter spp*: resistant to one or more antimicrobial classes*, including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.
- *Pseudomonas aeruginosa*: resistant to one or more antimicrobial classes*, including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.
- *Enterobacter spp.:* testing intermediate or resistant to ceftazidime or ceftriaxone OR representative agents within 3 antimicrobial classes including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.
- *Clostridium difficile*: isolated colonies only; no stool specimens.

Because of the public health risk, any carbapenem resistant Gram-negative organism (e.g. *Serratia*, *Proteus* etc.) should also be provided. Additional species for collection may be identified in the future, as public health concerns develop over time.

*If a facility does not include an antimicrobial agent in their testing panel, the absence of that agent should not be a reason to exclude the isolate as a MDRO.

The MRSN does not collect, accept, or archive primary human specimens (i.e. blood, tissue, urine, stool, respiratory secretions, etc.).

General Information

Specimen collection is not intended for primary research or publication outside of the rubric of public health evaluations. Use of the database for research will require appropriate institutional review board (IRB) approval. Selected uses of the database for quality assurance, quality improvement, or process improvement may not require an IRB approval, in accordance with Federal, DoD, and Service-specific regulations. Isolates held in the MRSN collection will not be distributed without acceptance of an appropriate material transfer agreement (MTA), interagency agreement (IA) or equivalent.

The MRSN has ethical and legal obligations to ensure that private information pertaining to individuals or submitting facilities stored in MRSN databases is protected in all public health practice (PHP) and research activities that MRSN supports and conducts.

All isolates are cryo-preserved on-site at the MRSN as well as off-site in a well-catalogued repository, available for later study by MRSN personnel, original isolate submitters, or other personnel (e.g., protocol-approved). Isolates obtained via public health/DoD mandate are eligible for transfer to outside entities provided appropriate agreements are provided.

Submission Guidelines

Routine Bacterial Isolate Submission

Isolates that need to be sent to the MRSN may be identified in one of two ways:

1. Your laboratory has cultured MDRO bacteria to include a Multidrug-Resistant *Staphylococcus aureus, Klebsiella spp., Escherichia coli, Acinetobacter spp., Enterobacter spp., Pseudomonas aeruginosa* and *Clostridium difficile* isolates. ***See Introduction for specific criteria.**
2. You have an approved research project/protocol that describes support by the MRSN.

Isolate Sub-culturing and Shipping

If your laboratory has an isolate that has been identified as an organism of interest, as outlined above, the following steps need to be taken:

- Ensure the isolate of interest is pure, sub-culturing as necessary.
- Subculture an isolated colony from a pure culture onto a Tryptic Soy Agar (TSA) slant at 37°C for 24 hours. The MRSN can provide these slants if needed, by request. For tracking purposes, place a unique identifier on the tube, being careful not to obscure the TSA slant surface.
- After incubation, refrigerate your slants at 4°C for no longer than 4 weeks if you plan on shipping out samples in batches. Batched isolates must be submitted to the MRSN at least monthly.
- With regards to *C. difficile*, inoculate isolated colonies into **3** cryo-vials and freeze before submission. As mentioned above **the MRSN does not collect, accept, or archive primary human specimens (i.e. blood, tissue, urine, stool, respiratory secretions, etc.).**
- Prior to shipping, the Master Data Entry Template Excel file must be completed and emailed encrypted to: usarmy.detrick.medcom-wrair.mbx.WRAIR-MRSN@mail.mil. **The MRSN will cover all shipping and handling costs.**

- Send specimens to:

  US Army Garrison-Forest Glen
  Walter Reed Army Institute of Research
  Attn: MRSN Processing Laboratory Room 2W106
  C/o Director, MRSN Laboratory Operations
  2460 Linden Lane, Bldg. #503
  Silver Spring, MD 20910

Any questions about shipping should be directed to usarmy.detrick.medcom-wrair.mbx.WRAIR-MRSN@mail.mil.

Testing Algorithm and Instrumentation

Participating laboratories submit all targeted MDROs, along with clinical and demographic information on the encrypted Master Data Entry Excel file to the central MRSN laboratory at WRAIR. The repository determines genetic-relatedness, performs extended phenotypic and phylogenetic analyses, preserves the specimens indefinitely, and relays public health relevant information to hospitals, medical leaders and policymakers.

More specifically, isolates undergo:

Clinical Microbiology Testing

Isolates undergo confirmatory identification and antibiotic susceptibility testing on commercial automated and manual platforms to include:

- BD Phoenix with Bruker MALDI-TOF
- Biomerieux Vitek 2XL with MS
- Microscan with Bruker MALDI-TOF
- Trek Sensititre ARIS® 2X (optional test)
- Biomerieux E-Test (optional test)

Molecular Microbiology Testing

The Molecular Research and Diagnostics group performs phenotypic and genomic analysis on isolates submitted to the MRSN utilizing the following platforms and assays:

- CarbaNP testing for carbapenemase production
- Illumina MiSeq
- Illumina NextSeq
- Pacific Bio RSII Long-Read sequencer
- Rapid (<1 hour after culture) specific gene detection using real-time PCR on a Biorad CFX96 RT-PCR instrument with the following primers and probes:
    - MRSA (mecA, mup, qac A/B, pvl)
    - Carbapenem-resistant GNR (bla_{KPC}, bla_{IMP}, bla_{NDM}, $bla_{OXA\ 48\text{-}like}$, bla_{VIM} – All variants)
    - 16s methylases (armA, npm, rmtA-H)
    - MDR Acinetobacter ($bla_{OXA\ 23,\ 24,\ 58}$)

Bioinformatics Analysis

The MRSN Bioinformatics group utilizes an automated, in-house designed software pipeline for the alignment, assembly, analysis, and interpretation of DNA sequencing data. The end products of each analysis include:

- Whole genome species identification/confirmation
- Antibiotic resistance gene content
- *In silico* traditional Multi-locus sequence typing (MLST) determination
- Panseq and/or core genome (cg) MLST derived molecular phylogeny
- *Long-read sequencing using the PacBio RSII or Minion Nanopore
- *Plasmid typing
- *Whole genome annotation via RAST/Prokka
- *High-resolution whole genome SNP-based molecular phylogeny

***:** Illumina (short-read) WGS will be performed on all isolates from which species identification, a list of antibiotic resistance genes, traditional MLST, and Panseq and/or cgMLST-derived phylogeny will be supplied. Any additional data acquisition or bioinformatics analysis (denoted with * above) performed by the MRSN beyond the above described will have to be requested by researchers using the form in Appendix A. The form will be sent to the MRSN with a description of the project and collaboration requests.

The MRSN will confirm project feasibility and expected timeline for completion.

ARMoR-Database (ARMoR-D) Entry

Upon completion of laboratory analysis, the MRSN will store clinical and demographic data for each isolate, identification and susceptibility results, multiplex PCR results, and sequence data (AMR and virulence genes) in the ARMoR-Database. Upon entry into the database, all personal identifying information (PII) will be separated, encrypted, and securely stored in accordance with the Army Information Assurance regulation. A de-identified MRSN number will be assigned to the isolate, which will serve as the accession number for those granted access to the database. The ARMoR-D will provide the results of its phenotypic and genotypic analyses, of the isolates, in aggregate reports and publications for the greater public health benefit of antimicrobial resistance surveillance, including suspected outbreak investigations.

Requesting Bacterial Isolates and/or Reports

Public Health Practice or Research Support

- Obtaining and analyzing data are essential to the practice of public health. For many public health activities, data are systematically collected and analyzed. Public health practice may

involve the collection and analysis of identifiable data and involve persons who do not volunteer to participate.

- The Human Subjects Protection Branch / WRAIR IRB serves as the determination officials to adjudicate the status (public health practice vs. research) of requests for human data from the MRSN database. The MRSN Director or designee is the final adjudicator regarding the feasibility of all requests for MRSN analysis or isolates.

- If a dataset or isolate(s) is/are to be provided, the transfer will be covered by a Memorandum of Understanding (MOU), Material Transfer Agreement (MTA) or Data Use Agreement (DUA), as applicable.

- Under certain circumstances patient-level information may be required to de-conflict cases, to support specific reporting requirements, or to provide granularity for aggregate reports.

Research Support

- All communications with the MRSN about the study, delivery of datasets, and arrangements for specimen pickup/shipment will be conducted directly through the military Principal Investigator.

- All protocols will be submitted to the WRAIR, HSPB for review, unless a prior determination has been made by the submitting collaborators IRB. Any problems with the protocol will be sent back to the PI for clarification/correction. The HSPB director may request additional protocol review by a WRAIR Scientific Review Committee (SRC) or the WRAIR IRB.

Bacterial Isolate Request Requirements

- Requests for bacterial isolates from the MRSN must be made in writing via letter or email to the MRSN Director/Deputy/designee, with encryption where appropriate.

- MRSN requirements ensure availability of the bacterial specimens for operational requests and for other researchers. Requests for bacterial isolates may require an Agreement, and must indicate:
    - ❖ Specific objectives pertaining to the use of the specimens;
    - ❖ Specific assays to be performed;
    - ❖ Specific requirements of the MRSN (e.g., bacterial species, quantity of requested isolates, etc.).
- Bacterial isolates from the MRSN shall be used only for purposes specifically requested and authorized prior to use. Bacterial material that remains after authorized use shall not be retained by users or used for purposes not specifically authorized.

Protection of Identifiers and Personal Health Information

- The MRSN does not release individually identifiable information or protected health information except in the following circumstances:
    - ❖ An IRB/HUC-approved protocol (e.g., for chart reviews, case studies). In such cases, the informed consent (or IRB-approved waiver) of each study subject may be required.
    - ❖ Requests made by command authorities involved in conducting outbreak investigations, audits, or other operational investigations.
    - ❖ At the discretion of the Director, MRSN for public health investigation.

- Most other investigations can be supported if all data and bacterial isolates are irreversibly de-identified.
    - ❖ Data: MRSN maintains a stand-alone database with multiple levels of safety and control limiting access to any PHI.
        - i. Human data requests that contain de-identified/coded information (i.e. sex, age) will still require a human subjects research determination.
    - ❖ Bacterial Isolates: MRSN assigns barcode numbers to each isolate within its repository without regard to location of origin or other related specimen. Barcode IDs are printed on labels that are affixed to aliquot tubes.

- Data provided to the PI will contain only MRSN barcode IDs with requested demographic and other descriptive data by MRSN ID (if approved by the IRB).

- Requests for data, including raw datasets, may require a Data Use Agreement with the WRAIR/MRSN.

Recording the Requests

- Accepted requests are catalogued, recorded and executed using the WRAIR Office of Research and Technology Applications (ORTA), Business Management Office (BMO).
    - ❖ Other communications and documents (e.g. e-mails, tasking memoranda, and templates) are included in the ORTA files along with the request forms, data/analysis plan, and/or protocol or statement of work to record the original intent and justification.

- If the request is denied, a letter from the Director, MRSN is sent for notification. Denied requests are documented in a separate worksheet including an explanation for the denial and are archived with supportive documentation.

- Cancelled requests remain archived in the ORTA with pertinent annotations in the project log.

- Requests for data exchange (e.g., between government databases) are documented in the ORTA.

Information Delivery

- Results of requests are usually sent via e-mail (preferably to ".mil or .gov" addresses, with encryption where appropriate) in the form of read-only spreadsheets or PDF documents. Identifiable or sensitive data may be encrypted or sent via SAFENET (https://safe.amrdec.army.mil/safe/Welcome.aspx). High volume data may be encrypted on fixed electronic media (e.g., CD/DVD) and sent by FedEx with tracking.

Submission of Publications/Reports

- Any abstract, manuscript, or presentation resulting from use of the MRSN data and/or specimens shall be sent to the MRSN Director/Designee prior to publication for review of methodological accuracy. Non-compliance with this requirement may result in rejection of future requests.

- Manuscripts by WRAIR/MRSN staff will be processed and cleared in accordance with WRAIR publication clearance guidance and the WRAIR Strategic Communications Office.

- When indicated, manuscripts submitted to peer-reviewed journals will include a statement of formal ethical review in accordance with 32 CFR §219. The statement should also indicate whether or not the study was deemed exempt under 32 CFR §219 and/or if the study was classified as non-research.

Acronyms and Definitions

- *Bacterial Isolate:* Any bacterial organism in culture or DNA/RNA derived from a bacterial organism.

- *Individually Identifiable:* The identity of the subject is known or may readily be ascertained by the investigator or associated with the information.

- *Private Information*: Information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may readily be ascertained by the investigator associated with the information) in order for obtaining the information to constitute research involving human subjects.

- *Protected Health Information*: Individually identifiable health information that is transmitted by electronic media; maintained in electronic media; or transmitted or maintained in any other form or medium. It excludes individually identifiable health information in education records, records described at 20 U.S.C. 1232g, and employment records.

- *Research*: A systematic investigation designed with the intent to generate, produce, or contribute new and generalizable knowledge.

- *Surveillance*: The ongoing, systematic collection, analysis, and interpretation of outcome-specific data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease or injury.

- *ARMoR*: Antimicrobial Resistance Monitoring and Research

References

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2. Title 45 Code of Federal Regulations §164, Privacy of Individually Identifiable Health Information, Oct 1, 2009.

3. DoD Directive 5400.11, "DoD Privacy Program," May 8, 2007.

4. DoD 5400, 11-R, "Department of Defense Privacy Program," May 14, 2007.

5. DoD Instruction 6025.18, Privacy of Individually Identifiable Health Information in DoD Health Care Programs. Dec 2, 2009.

6. DoD 6025.18-R, "Health Information Privacy Regulation," Jan 24, 2003.

7. Public Law 104-101, "Health Insurance Portability and Accountability Act of 1996," Aug 21, 1996.

8. DoD Directive 3216.02, Protection of Human Subjects and Adherence to Ethical Standards in DoD-Supported Research, Apr 24, 2007.

9. Title 32 Code of Federal Regulations §219, Protection of Human Subjects (OSD Common Rule), Sep 2005.

10. Title 45 Code of Federal Regulations §46, Protection of Human Subjects (The Common Rule), 15 Jan 2009.

11. Title 10 U.S. Code, Chapter 55 §1074f, Medical tracking system for members deployed overseas, 17 Oct 2006.

12. Public Law 105-85 sec. 765, Improved Medical Tracking system for Members Deployed Overseas in Contingency or Combat Operations, 18 Nov 1997.

13. USAMRMC, Guidelines for Investigators, 1 Oct 2007.

14. AFHSC IPM 2009-1, Clearance; Publication; and Presentation Routing Guidance, 28 Jul 2009.

15. Title 45 Code of Federal Regulations §160.103, General Administration Requirements, Definitions 16 Feb 2006.

16. CDC Policy, "Distinguishing Public Health Research and Public Health Nonresearch," 29 July 2010.

17. USAMRMC, Human Subjects Research Review Board Policies and Procedures, 14 Mar 2005.

18. Public Law 89-554: 80 Statute 383, “Freedom of Information Act,” 6 Sep 1966.

19. Memorandum, Deputy Assistant Secretary of Defense (Force Health Protection and Readiness), Revised Service Guidelines for Reportable Medical Events, 30 Jun 2009.

20. Title 21 Code of Federal Regulations §56, Institutional Review Boards, 1 Apr 2008.

21. Federal Register Volume 63, No.216, pg 60364-60367, Protection of Human Subjects: Categories of Research that May be Reviewed by the IRB through an Expedited Review Procedure, 9 Nov 1998.

22. Office of Human Subjects Research, National Institutes of Health, Information sheet #14, “Guidance on the research use of stored samples or data.” 12 Jun 2006.

23. Army Regulation 40-38, Clinical Investigation Program, 1 Sep 1989.

24. USACHPPM MEMO 70-25-1, Human Research Protection Program: Organizational Policies and Procedures, 3 Sep 2009.

25. Public Law 89-554: 80 Statute 383, “Freedom of Information Act,” 6 Sep 1966.

26. Memorandum, Deputy Assistant Secretary of Defense (Force Health Protection and Readiness), Revised Service Guidelines for Reportable Medical Events, 30 Jun 2009.

27. Army Regulation 25-2, Information Assurance, 24 OCT 2007.

28. Army Regulation 380-5, Department of the Army Information Security Program, 29 Sep 2000.

Appendix A: MDRO Data Acquisition and/or Analysis Request

Contact: **Lab:**

Email/Phone:

MRSN Contact: Director, MRSN

GEIS Project Number and Title:

On-going Workplan: Y/N **1-Year Funding:** Y/N

Project Overview: (brief description of project objectives)

Isolate(s) Description: (e.g. MRSA, MDR *Enterobacteriaceae)* **# of Isolates:**

MRSN Isolate Master Sheet Sent: Yes No

Additional Notes:

Requested Data Acquisition: (brief description of requested data acquisition, e.g. PacBio sequencing)

Requested Data Analysis: (brief description of requested data analysis, e.g.. plasmid characterization, isolate relatedness)

Final Data Usage: Meeting Presentation { } Publication { } Funding Agency Report { }

Details:

Requested Timeline: