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Laboratory Testing Strategy

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1. Introduction

As part of investigating national or EU-level outbreaks of antimicrobial resistance (AMR) threats in priority pathogens, including investigation of emerging resistance, the EURL shall provide phenotypic and functional reference testing on selected isolates. Hence, we aim to provide a framework for consistent and high-quality AMR monitoring, ensuring that members of EURGen-Net have access to the resources and expertise necessary to address this critical public health challenge.

This testing strategy outlines a harmonized approach to ensure timely and accurate characterisation of national or EU-level outbreaks of AMR threats, through the provision of phenotypic and functional testing on selected isolates, using European standards for testing and interpretation. Priority pathogens are defined as carbapenem- and/or colistin-resistant *Enterobacterales* (CCRE), carbapenem-resistant *Acinetobacter baumannii* (CRAb) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPa), by the definitions of EURGen-Net. Testing of species other than these will be provided when agreed upon beforehand between ECDC and the EURL.

The EURL provides **preparedness** for antimicrobial susceptibility testing (AST) of up to a maximum of 1000 isolates per year. All testing will be performed according to EUCAST guidelines, normally with disk diffusion as the initial test, followed by broth microdilution (BMD) and/or functional testing when considered necessary.

2. Roles and responsibilities

ECDC:

- Initiating reference testing by contacting the EURL and assigning an identifier/number to the suspected outbreak or emerging resistance investigation
- Deciding on the number of isolates to test and forwarding this request to NRLs in affected countries as well as to the EURL
- Together with the EURL decide on antibiotics to be tested in the outbreak or emerging resistance investigation in question
- Receiving, analysing and summarising results from the EURL, and participating in discussion of results with the EURL and NRLs

EURL-PH-AMR:

- Providing instructions for (1) sampling and (2) transportation to the EURL (always via the NRL)
- In collaboration with ECDC deciding on relevant antibiotics for testing.
- Performing phenotypic AST on selected isolates and/or functional testing for antimicrobial resistance mechanisms according to EUCAST guidelines, as agreed with ECDC
- Delivering written reports of results to ECDC and the NRL. On request from ECDC, EURL or the NRL, results, analysis and conclusions will be discussed in online meetings
- Short-term storage of isolates, and on request transportation of these to the ECDC contracted provider of long-term storage
- Forwarding strains for genotypic characterisation and collection of reference material, either within the EURL or to the ECDC contracted provider of next-generation sequencing (NGS), as agreed between the EURL and ECDC

NRLs:

- In national and cross-country outbreaks or emerging resistance investigations and in agreement with ECDC, collect relevant isolates from national laboratories
- In agreement with ECDC and the EURL submit isolates to the EURL as described in the instructions provided by the EURL
- Respond to questions and requests from the EURL and ECDC related to phenotypic AST performed as part of outbreak or emerging resistance investigation

3. Sampling strategy

EURL reference testing of isolates is always initiated by ECDC. ECDC contacts the EURL per email (eurl@kronoberg.se, inbox monitored during office hours) or phone (+46767259607 or +46709844685, available 24/7) for agreement on what species to test, number of isolates, antibiotics to test and desired turn-around time. ECDC also assigns a unique identifier to the outbreak or emerging resistance investigation in question, which can be referred to in coming discussions and reporting of results.

After this agreement, ECDC informs the NRL(s) in question and refers them to the EURL website for sample submission guidelines.

4. Sample submission guidelines

These will be published on the EURL website, and will include sample handling and transportation.

5. Testing algorithm

All antimicrobial susceptibility testing will be performed in accordance with the latest published EUCAST guidelines. Testing will normally be tiered: (1) disk diffusion to establish if the isolate is wild type or non-wild type. For wild type isolates, further testing is not warranted. (2) For non-wild type isolates, additional phenotypic, functional and, when deemed appropriate, genotypic testing is organised. Broth microdilution can be performed with a wide array of MIC broth panels, sometimes including novel agents, such as new beta-lactam/beta-lactamase inhibitor combinations and, when appropriate, agents that are still under development.

For antimicrobial agents where agar dilution is the reference method, this can be organised.

When breakpoints are lacking, either because the species has not been assigned breakpoints for the agent (agent lacking in tables, or agent/species represented by “IE” in tables), the analysis will instead include the categorisation wild type or non-wild type to help decide whether or not resistance mechanisms are present or not.

The EURL has access to all relevant phenotypic methods for functional testing and confirmation of resistance mechanisms recommended by EUCAST (The EUCAST guideline on detection of resistance mechanisms v 2.0 or an updated version). Commercially available rapid tests can be introduced if needed when found to meet performance requirements and approved by the EURL. Referral to SSI for sequencing to characterise the genetic background and mechanisms of resistance will be performed for isolates of interest.

Antibiotic panels

The EURL preparedness for reference testing of priority pathogens includes the following antibiotics:

***Enterobacterales*:**

Piperacillin-tazobactam, cefiderocol (caveat: reference method still not available), cefotaxime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ertapenem, imipenem, imipenem-relebactam, meropenem, meropenem-vaborbactam, aztreonam, aztreonam-avibactam, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, tigecycline, colistin, trimethoprim-sulfamethoxazole, fosfomycin (*E. coli* only)

***Pseudomonas aeruginosa*:**

Piperacillin-tazobactam, cefiderocol (caveat: reference method still not available), ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, imipenem, imipenem-relebactam, meropenem, meropenem-vaborbactam, aztreonam, ciprofloxacin, levofloxacin, amikacin, tobramycin, colistin

***Acinetobacter baumannii*:**

Cefiderocol (caveat: reference method still not available), imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, tigecycline, colistin, trimethoprim-sulfamethoxazole

Testing of other species and antibiotics can be performed after agreement with ECDC, but may not be available at similar annual volumes as for standard panels.

6. Data sharing and reporting

Results of reference testing will be shared with ECDC and the NRL as written reports (pdf and/or excel files). These will include qualitative results (S, I and R, wild type vs. non-wild type) and when relevant, quantitative results in the form of MIC values and/or inhibition zone diameters.

Upon request, the EURL shall arrange online meetings to discuss the findings. ECDC may invite representatives of relevant NRLs to these meetings. In this forum, additional testing can also be decided on.

Ownership. This may refer either to isolates transported to the EURL or to the results of the analysis or both. This will be agreed between ECDC and the NRL prior to each effort to help investigate the origin and severity of a presumed outbreak or emerging resistance investigation. MTAs (Material Transfer Agreements) will be signed if required by any of the parties or after further consultation with HaDEA.

7. Turnaround time

Turnaround time for testing and reporting of results is dependent of several factors, including the number of isolates submitted simultaneously, the purity of isolates and the need for additional testing after initial AST has been performed. For up to 10 isolates, concomitantly submitted, the timeline is usually as follows (counted as workdays):

- 1-2 days for culture, purity control and verification of species ID
- 1 day for disk diffusion testing
- 1 additional day for BMD and/or functional testing

The total turnaround time for reporting AST results, including those from BMD, is hence a maximum of 4 workdays. Service is not provided during weekends and formal holidays unless specifically agreed.

8. Quality control and proficiency testing

- All reference testing will be performed under ISO 15189 accreditation.
- Quality controls (QC) will be performed in accordance with EUCAST recommendations.
- Proficiency will be assessed through the participation in external quality assessment (EQA) schemes.

9. Evaluation and review

The testing strategy is evaluated by the EURL and ECDC annually. Adjustments to meet the introduction of new agents, new species and new algorithms for testing will be agreed between ECDC and the EURL.

10. Laboratory testing quality indicators

1. Turnaround time (≤4 workdays, under standard settings)

2. AST EQA results (UK NEQAS, Labquality) ≥ 95% correct

These will be reviewed yearly together with ECDC, and furthermore analysed in written reports at month 12 and 48, as stated in the grant agreement.