

EFSA-EURL Confirmatory testing 2019 data

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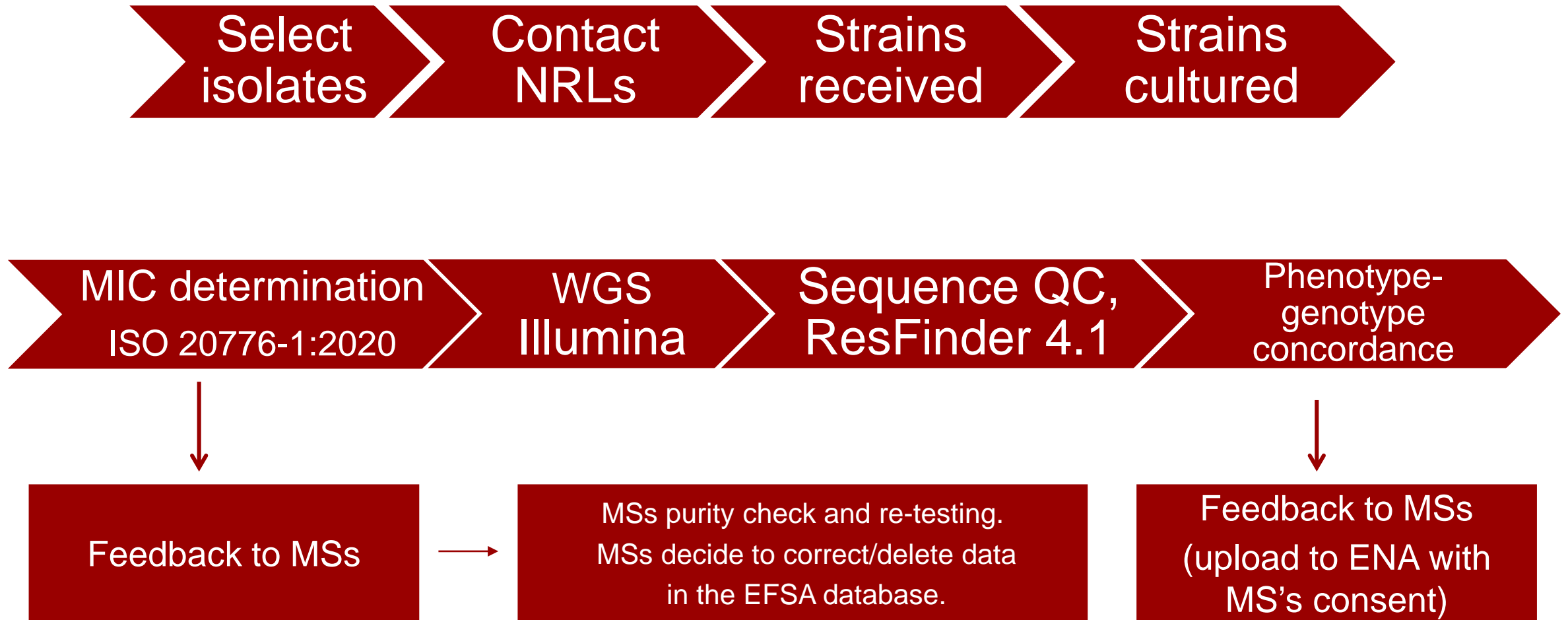
Term of Reference, EURL-AR

The EURL-AR will provide confirmatory testing on bacterial isolates of particular relevance upon request by the NRLs, the European Commission and EFSA:

- To support the activities related to the implementation of Decision 2013/652/EU
- To confirm the AMR phenotypes observed by MSs
- To determine the AMR genotypes and to evaluate phenotype-genotype concordance

Relevant Material Transfer Agreements (MTAs) between the EURL and MSs ensure the legal aspects of strain ownership.

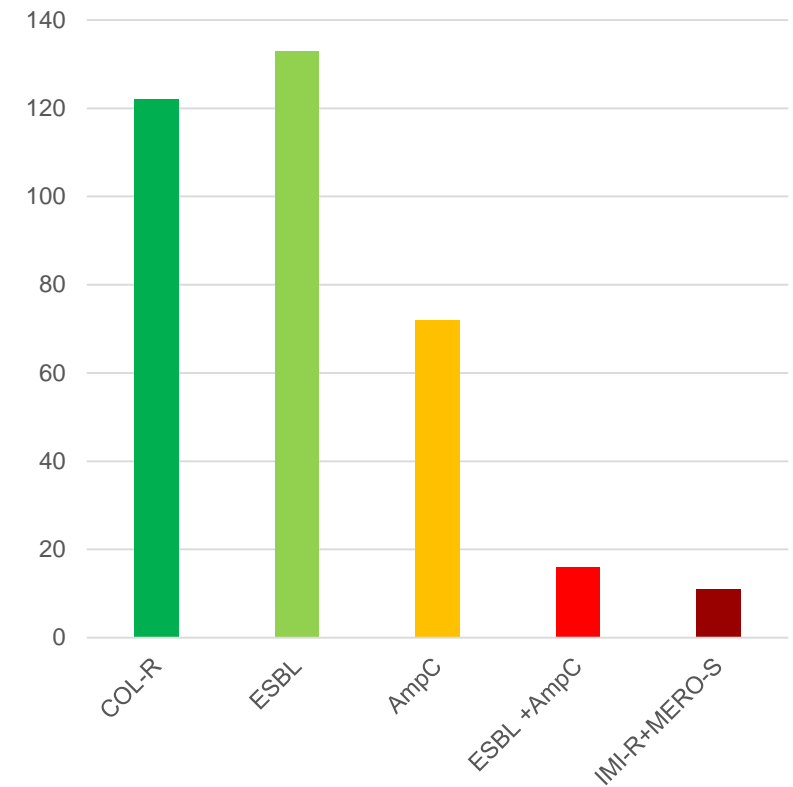
Workflow for the confirmatory testing activities



Selection criteria by EFSA

- Selection criteria for the 2019-isolates related to colistin resistance and to the presumptive ESBL/AmpC profiles and carbapenem resistance:

- COL-R profile: 122 isolates
- ESBL profile: 133 isolates
- AmpC profile: 72 isolates (FOX MIC=16; FOX MIC>16)
- ESBL+AmpC profile: 16 isolates
- IMI-R+MERO-S profile: 11 isolates (all cephalosporin-resistant)



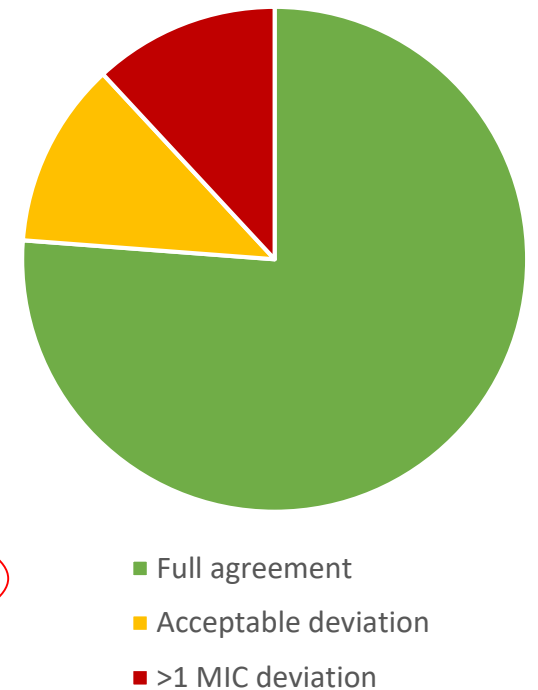
Isolates

- **349 isolates selected (30 countries)**
 - 345 *E. coli*, 4 *Salmonella* (range 1-60 per country)
- **339 isolates received at the EURL (30 countries)**
 - 3 isolates contaminated with *Proteus* → not included
 - 336 isolates MIC-tested → 8,064 bug/drug tests (i.e. 8,064 MIC-values)
- **10 isolates not received**
 - Data changed by MS for 8 isolates (3 COL-R and 5 IMI-R+MERO-S)

Comparison of phenotypes at MS and EURL (1)

- 256 isolates (76.2 %) in fully agreement:
 - Identical R/S phenotypes according to the applied ECOFFs
 - MIC-values within the acceptable deviation of the method (± 1 dilution step difference).
- R/S discrepancies observed for 40 isolates (11.9 %)
- differences in MIC-values within the acceptable deviation of the method
→ No critical issues identified.
- R/S discrepancies observed for another 40 isolates (11.9 %)
- differences in MIC-values above the acceptable deviation of the method (13 countries)

Comparison of phenotypes



→ Actions by MS were requested like purity check and re-testing

→ MS reported their result and final decisions back to the EURL

Comparison of phenotypes at MS and EURL (2)

- **Outcome of the 40 requested actions after re-testing by the MSs:**
 - Data for 29 isolates were corrected in the EFSA database (3 isolates deleted). Half of these corrections were for colistin (from R to S profile).
 - Data for 11 isolates were proved to be valid. All results indicated that the observed discrepancies were caused by testing of non-identical material at MS and EURL-AR.

Comparison of phenotypes at MS and EURL (3)

- Phenotypic profiles in regard to the selection criteria:
 - COL-R profiles: 87.7 % of 122 profiles were confirmed as COL-R.
 - ESBL profiles: 99.2 % of 133 profiles were confirmed as ESBL.
1 isolate was susceptible to all cephalosporins in the panel.
 - AmpC profiles: 100 % of 72 profiles were confirmed as AmpC.
 - ESBL + AmpC: 100 % of 16 profiles were confirmed as ESBL.
50 % were confirmed as AmpC (FOX MIC-value 8 vs. 16 mg/L).
 - IMI-R + MERO-S: 0 % of the 11 profiles were confirmed as IMI-R.

Phenotype-genotype comparison

- All 336 isolates MIC-tested at the EURL-AR were sequenced at the EURL-AR (*1 isolate pending*)
- The comparison was performed by use of the EURL MIC-data.
- Only a selection of data is presented in the following.
- A total list of the detected gene/mutation pool will be presented in the Newsletter 2021.

Phenotype-genotype concordance

- 1,550 phenotypic resistant bug/drug combinations (cephalosporins NOT included):
 - **98.3 % were in concordance with the genes/mutations detected by WGS**
 - **1.7 % (27 combinations) were not in concordance with genes/mutations detected** (i.e. no genetic background was detected to explain the observed phenotypic resistance)
- 38 apparently functional genes or mutations were detected for isolates that were phenotypic susceptible.
- These non-concordance cases were observed for different classes of antimicrobials.
- 100 % concordance was observed for: AMP, TET, GEN, IMI, MERO, ETP.

Genotypic profiles - azithromycin and colistin

- **Azithromycin**

Phenotypic resistant (41 isolates):

mphA (24 isolates),

mefB (4 isolates),

*mefC-mphG** (5 isolates),

msrE (1 isolate),

unknown (7 isolates, MIC >32 mg/L)

Phenotypic susceptible isolates with

*mphA** (23 isolates) and

mefB (3 isolates)

**Publication pending.*

- **Colistin**

Phenotypic resistant (104 isolates):

mcr-1.1 (98 isolates),

mcr-3.2 (1 isolate),

mcr-4.2 (1 isolate),

unknown (4 isolates, MIC 8-16 mg/L)

Phenotypic susceptible isolates with

mcr-4.6 (1 *E. coli*) and

mcr-9 (2 *Salmonella*)

Genotypic profiles – cephalosporins and carbapenems

All cephalosporin-resistant isolates were confirmed by MIC-testing (except for 1 isolate)

- **AmpC profiles:** All correctly categorized by the MSs and in concordance with genes/mutations detected: 63% AmpC promoter (g.42C>T) and 37% CMY-2
- **ESBL profiles:** All correctly categorized by the MSs and in concordance with genes detected: 88.5% CTX-M genes, 6% SHV-12, 4% TEM-207, 1% OXA-1, 0.5% TEM-52B/C
- **ESBL+AmpC profiles:** 8 of the 16 reported profiles were not categorized as AmpCs by the EURL after MIC-testing (EURL FOX MIC 8 mg/L vs. MS FOX MIC 16 mg/L).
Only CTX-M genes were detected and one of the isolates also had an AmpC promoter mutation.
- **No carbapenem-resistance genes were detected.**

Final words...

- Thanks to all NRLs and to EFSA for a great collaboration!
- Thanks to our lab technicians at DTU for all the work done in the lab!
- The EFSA/EURL confirmatory testing for 2020-data are running right now and we expect to send the MIC-results to the NRLs in the beginning of December.

THANK YOU FOR YOUR ATTENTION