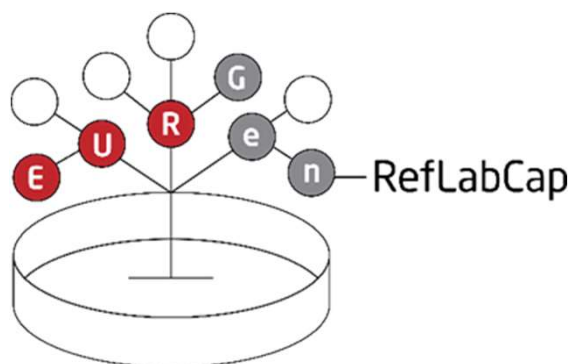




**Survey of the current molecular or genomic methods used
in the European NRLs for investigating
carbapenem- and/or colistin-resistant
Acinetobacter baumannii and *Pseudomonas aeruginosa***

**EURGen-RefLabCap
Work stream (WS) 2**



Notes

Abbreviations used in the report:

carbapenem- and/or colistin-resistant *Acinetobacter baumannii*: **C/CRAb**

carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa*: **C/CRPa**

National Reference Laboratories (including National Expert Laboratories): **NRLs**

Whole genome sequencing: **WGS**

Kosovo*: 'This designation is without prejudice to positions on status, and is in line with United Nations Security Council resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.'



Background

The aim of the **Workstream 2 questionnaire** was to assess the current laboratory functions and capacity for molecular and/or genomic methods used in the national reference laboratories for **C/CRAb** and **C/CRPa** and related **NRL Core Functions**.

27 questions covered:

1. Provision of NRL services
2. Reference diagnostics and typing, including molecular and genomic methods
3. Quality assurance and proficiency
4. Surveillance and NRL support to outbreak response
5. Data management in relation to monitoring, alert and response
6. Needs for further capacity building

The information is used to assess the current capacity for C/CRAb and C/CRPa in European NRLs and countries, and to plan and target capacity building activities within EURGen-RefLabCap in 2023-2024

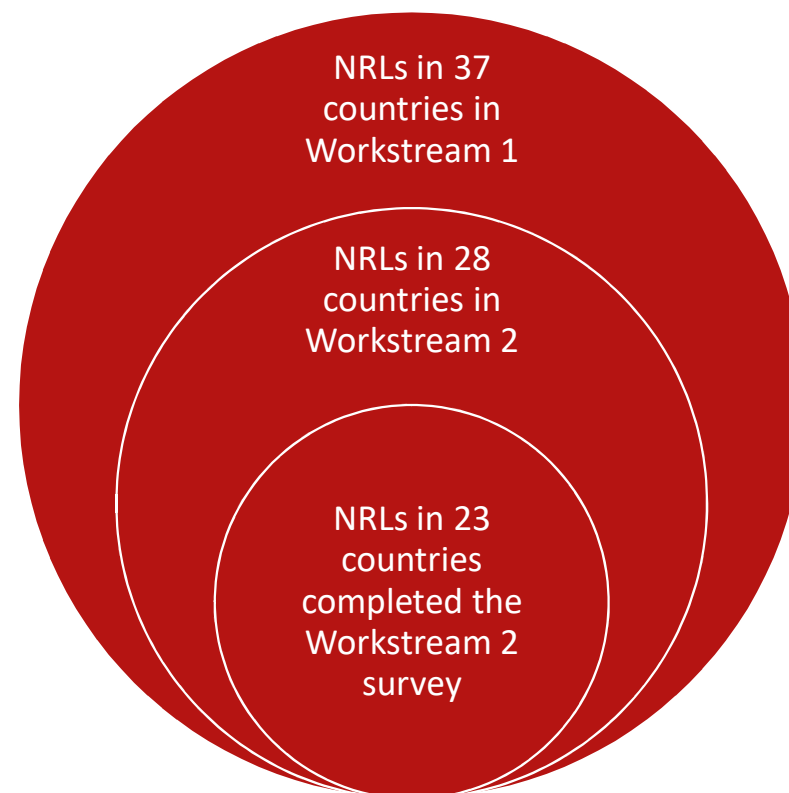
Disclaimer:

- Some replies to the survey need further clarification with the respondents
- Country tables are not shown in this webinar but will be available in the report on the survey

Response to the WS2 survey

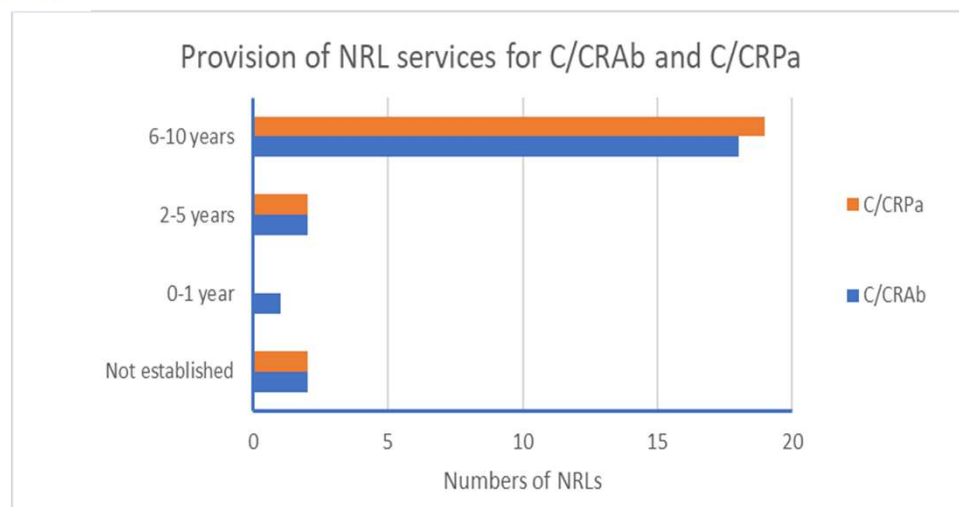
- **NRLs in 23 out of 28 countries with nomination for WS2** completed Workstream 2 survey (Hungary, Ireland, North Macedonia, Portugal and Turkey did not reply to the survey)
- **NRLs in 9 countries** have not yet been nominated for WS2 (including 5 Priority Countries, 4 non-Priority Countries)
- **Denominators** for the individual questions are related to the context of each question:

e.g. all NRLs, NRLs that provide reference services for WS2 pathogens (one/both) and NRLs that have implemented WGS.



Please note that in some countries different NRLs provide reference services for WS1 and WS2 pathogens, respectively

1.1 Provision of NRL services



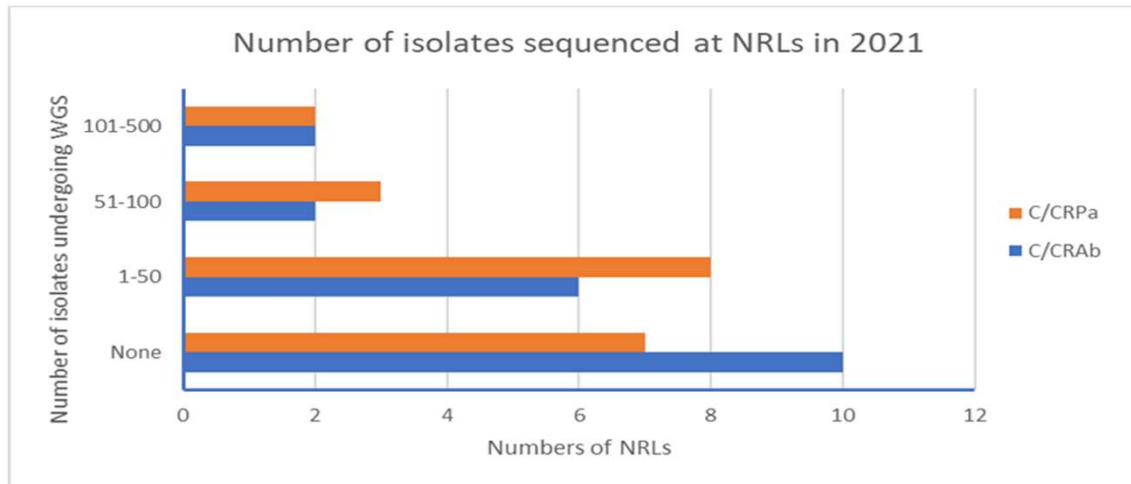
Referral patterns of C/CRAb and C/CRPa isolates to the NRL

- **A subset of isolates** were referred to the NRL in 14 countries,
- **All isolates** were referred to the NRL in 5 countries: **Denmark, Iceland, Malta, Netherlands and Norway.**
- Referral patterns were the same for both WS2 pathogens in all countries.
(Albania and Slovenia: no reply; Croatia and Cyprus: N/A)

NRL Services

- **C/CRAb:** 18 countries had NRLs for in service 6-10 years. Netherlands had an NRL service for 0-1 year Kosovo* and Slovakia for 2-5 years. **Croatia and Cyprus** had not established an NRL.
- **C/CRPa:** 19 countries had NRLs for 6-10 years
- Netherlands and Kosovo* had an NRL for 2-5 years **Croatia and Cyprus** had not established an NRL
- **Croatia:** > 95% of *A. baumannii* strains are resistant to carbapenems, and the mechanism of carbapenem resistance in *P. aeruginosa* is mostly caused by efflux and porin loss together with AmpC and **not by (acquired) carbapenemases**
- **Cyprus** has planned to establish NRLs for C/Crab and C/CRPa in 2023-2024.

1.2 WGS for C/CRAb and C/CRPa at the NRLs in 2021

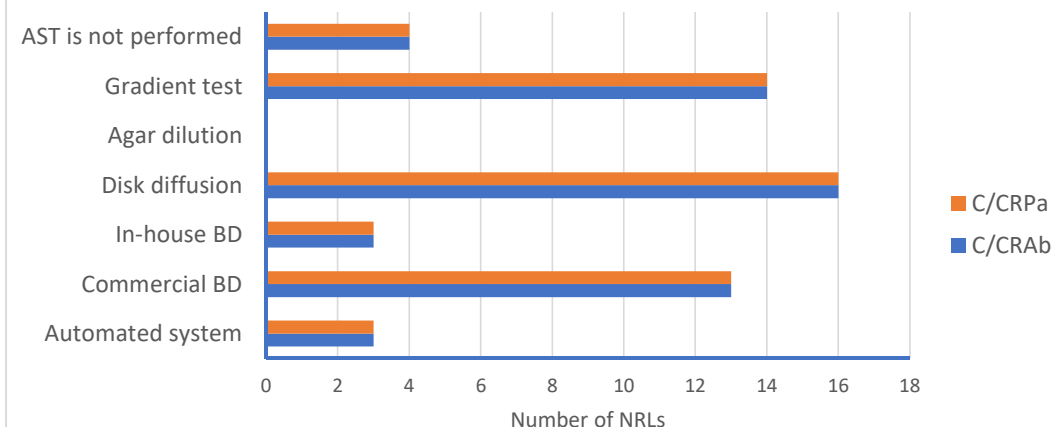


WGS activity in 2021

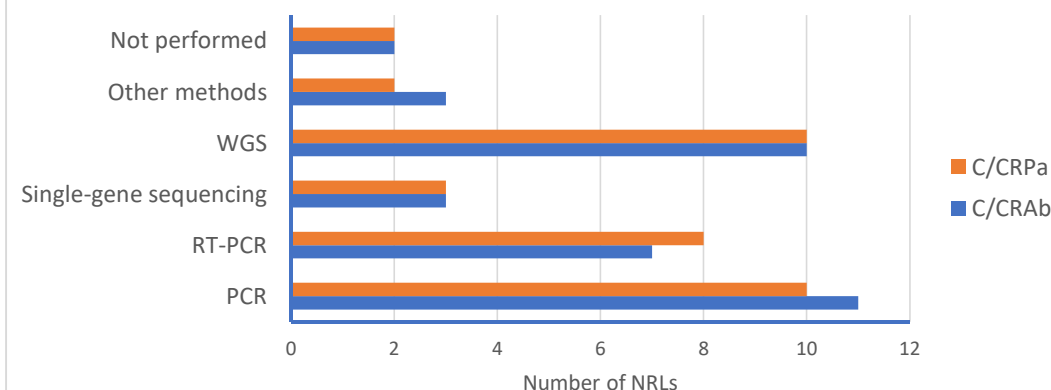
- **Few NRLs** sequenced > 50 isolates WS2 pathogens in 2021
- **C/CRAb:**
France and Spain sequenced 51-100 isolates
Germany and Czechia sequenced 101-500 isolates
- **C/CRPa:**
Spain and Sweden sequenced 51-100 isolates
Czechia and France sequenced 101-500 isolates

2.1 Reference diagnostics and typing (AST and AMR Genes)

Methods used for phenotypic AST at NRLs



Methods used for characterization of AMR genes in C/CRAb and C/CRPa



Phenotypic AST

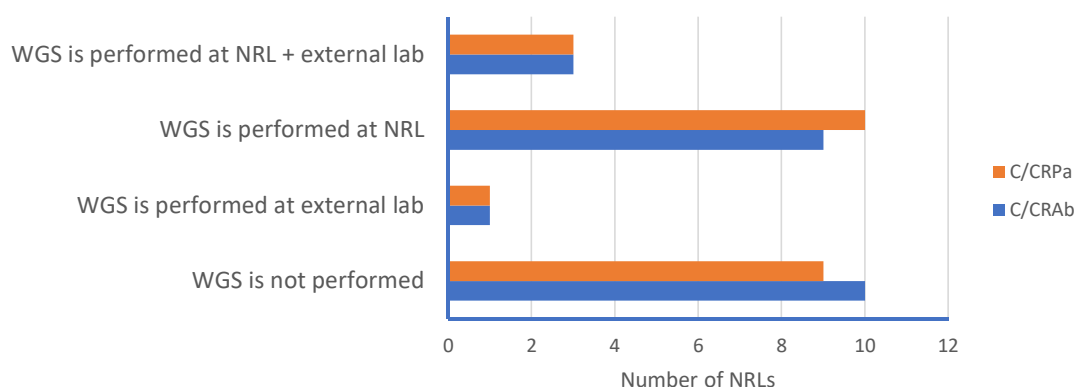
- 18 NRLs performed phenotypic AST (incl. Cyprus)
- 4 NRLs did not routinely perform **phenotypic AST (Denmark, Finland, Netherlands and Sweden)** (1 N/A)
- Relying on AST data from the clinical laboratories requires that testing methodology is standardised and quality controlled.
- Microbroth dilution (MBD) is recommended method for colistin testing (EUCAST) – 15 out of 18 NRLs included MBD

Genotypic characterization of AMR Genes

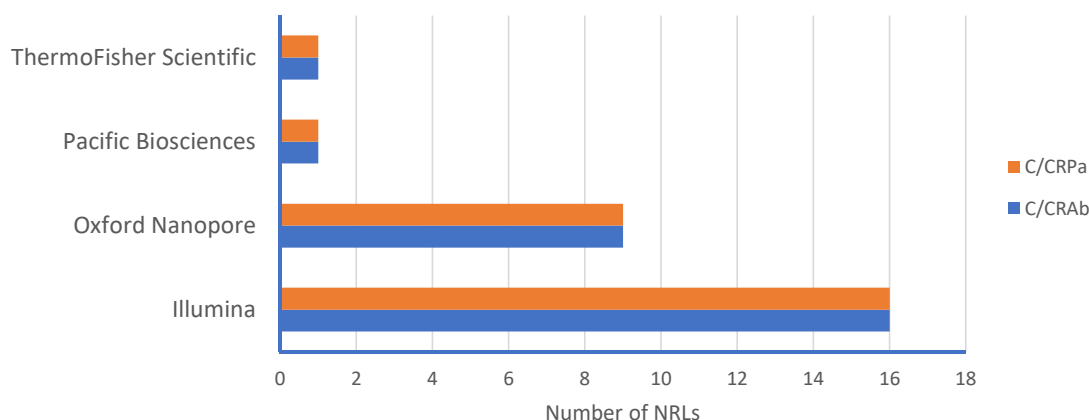
- 18 NRLs characterised AMR genes in both WS2 pathogens (14 used combinations of methods, 4 used WGS alone)
- 2 NRLs (Cyprus, Malta) did not perform **genotypic characterisation** of AMR genes in C/CRAb and C/CRPa (3 N/A)
- Overview of genes detected are in the report
- 22/23 used EUCAST 2017 guidelines (others: NordicAST, CLSIM100, CA-SFM)

2.2 Implementation of WGS

Performance of WGS and location



Use of WGS technology by manufacturer



Implementation of WGS

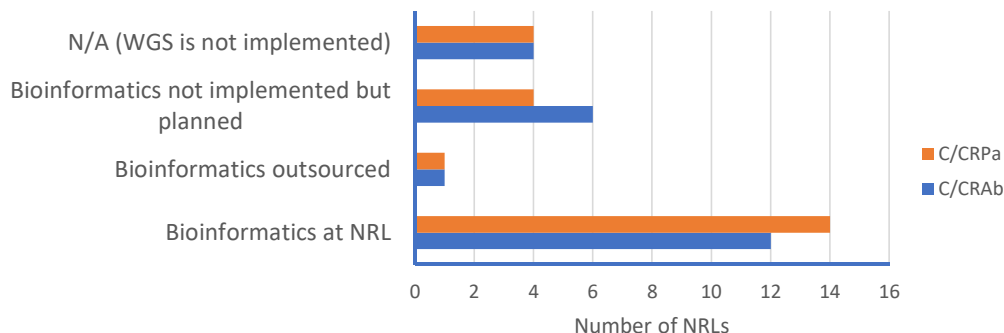
- WGS had been implemented in **13 countries for C/CRAb** and **14 countries for C/CRPa**.
- 9 countries had not yet **implemented WGS** at the NRL or externally (both WS2 pathogens): **Albania, (Croatia, Cyprus), Estonia, Greece, Kosovo*, Malta, Slovakia and Slovenia**. Most NRLs had planned the implementation of WGS in 2023-2024.
- Bulgaria performed WGS for C/CRPa only.**

WGS platforms used

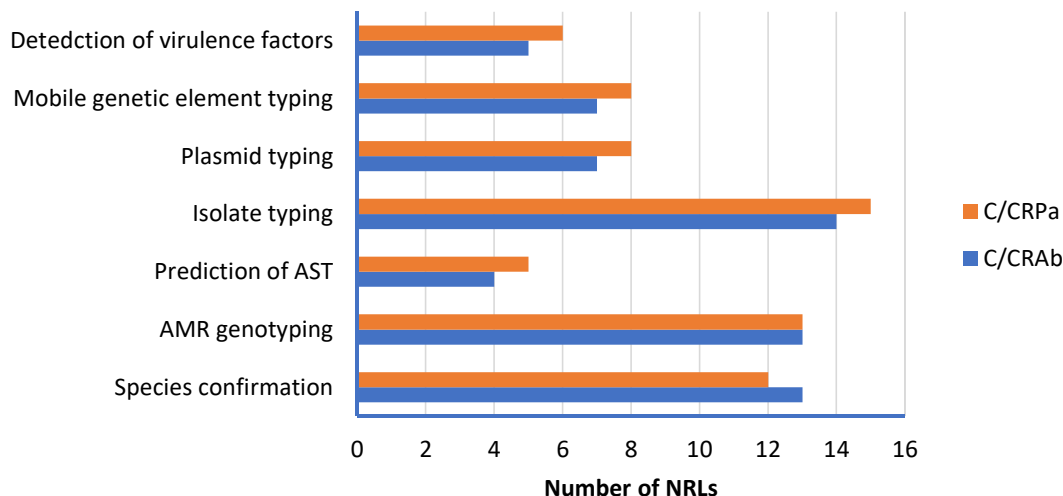
- NRLs in 7 countries used **Illumina only** for C/CRAb and C/CRPa: Austria, Denmark, Finland, Iceland, Lithuania, Malta and the Netherlands.
- A combination of Illumina and Oxford Nanopore** was used in 8 countries: Bulgaria, Croatia, France, Germany, Norway, Poland, Slovenia and Spain
- NRL in Czechia used Illumina and Pacific Biosciences
- NRL in Estonia used the Oxford Nanopore only
- NRL in Sweden used ThermoFisher Scientific only

2.3 Bioinformatic analysis

Implementation of bioinformatic analysis



Analytical purposes of WGS data analysis



Implementation of bioinformatic analysis

- Bioinformatic analysis was implemented in 13/13 NRLs for C/CRAb, and in 15/14 for C/CRPa (incl. Slovenia)
- NRLs performed bioinformatic analysis for **surveillance, outbreak/cluster** and **research purposes**, respectively (data not shown)

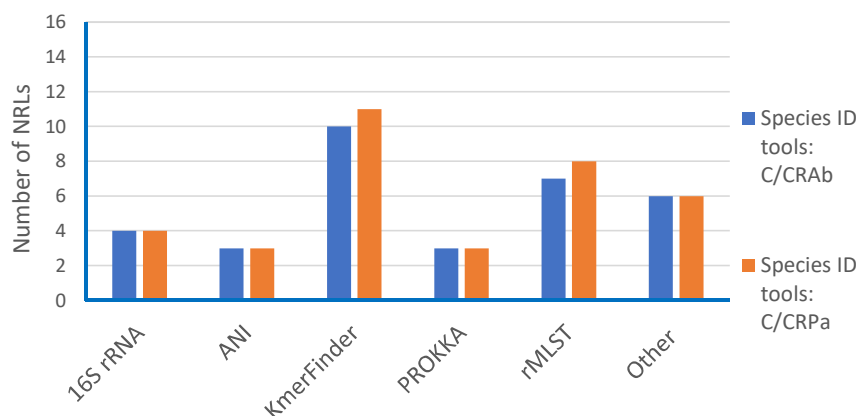
Analytical purposes of WGS data analysis

- Most frequent analytical purposes of WGS data analysis: **confirmatory species ID, isolate typing, AMR genotyping** and more infrequently prediction of AST patterns
- More infrequently: plasmid typing, mobile genetic element and virulence factor typing
- The complete picture obtained by analysing all genetic structures may not be needed in all routine investigations

2.4 Bioinformatic methods and tools used for C/CRAb and C/CRPa



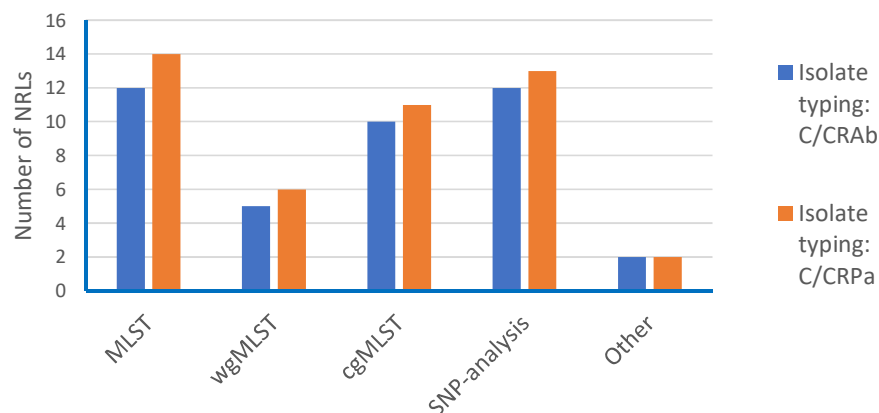
Use of bioinformatic tools for Species ID



Species ID

- **KmerFinder** (in 10: C/CRAb; 11: C/CRPa NRLs) and **rMLST** (7; 8 NRLs) were most frequently used for confirmatory species identification

Isolate typing methods used



Isolate typing

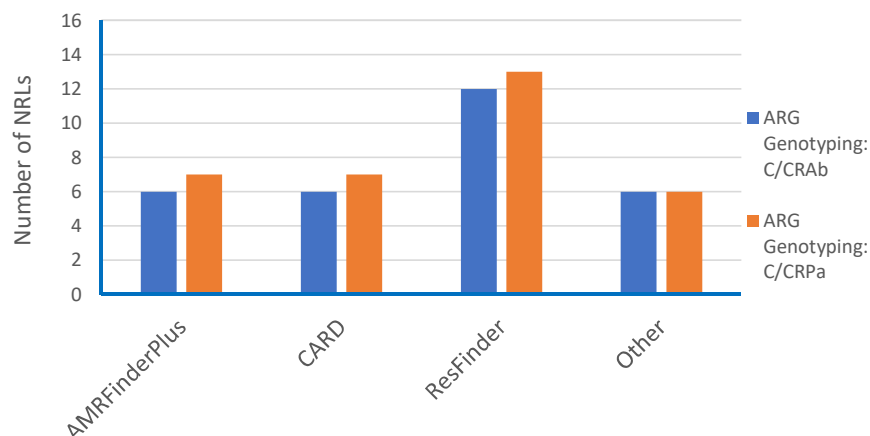
- **MLST** used (in 12: C/CRAb; 14: C/CRPa NRLs), **cgMLST** in 10; 11 NRLs and **wgMLST** in 5; 6 NRLs
- **SNP-analysis** in 12; 13 NRLs
- **All NRLs used at least 2 typing methods** for both pathogens

SNP-analysis: data comparability between laboratories or countries requires that the same references are used.

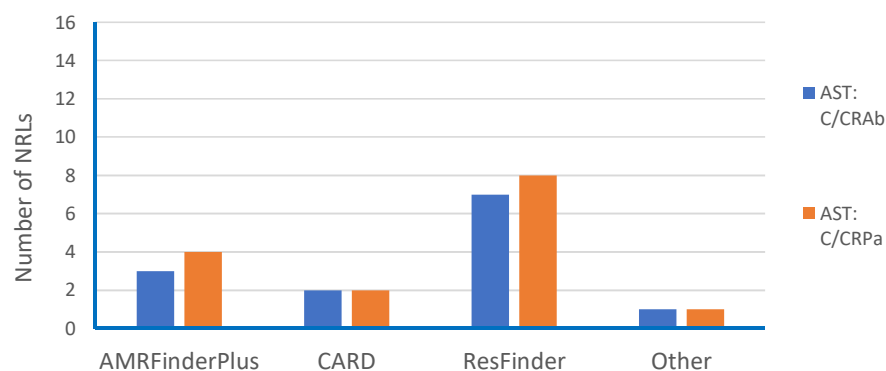
2.4 Bioinformatic methods and tools used for C/CRAb and C/CRPa



Use of bioinformatic tools for AMR genotyping



Use of bioinformatic tools for Prediction of Antimicrobial Susceptibility Patterns



Antimicrobial resistance genes typing

- **ResFinder** was used in 12; 13 NRLs
- **CARD** used in 6; 7 NRLs
- **AMRFinderPlus** used in 6; 7 NRLs

Prediction of AST patterns

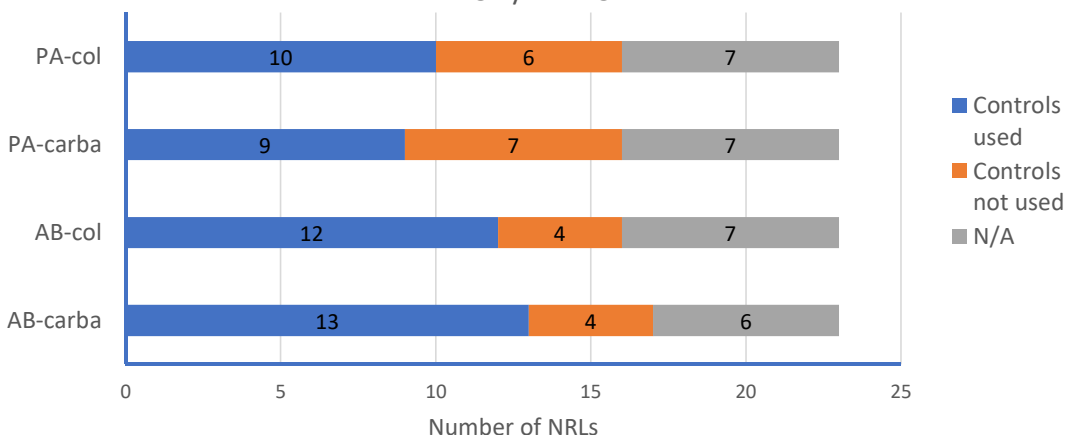
- ResFinder used in 7; 8 NRLs
- AMRFinderPlus used in 3; 4 NRLs
- CARD used in 2; 2 NRLs

Complex genotypes behind the resulting phenotypes

- ***A. baumannii***: overexpression of intrinsic carbapenemase OXA-51 via upstream ISAba1; acquired carbapenemases; porin alteration; efflux pumps; modified PBP
- ***P. aeruginosa***: overexpression of intrinsic genes, increased expression of efflux pumps and changed permeability and acquired carbapenemases
- *EURGen-RefLabCap WGS-based methods protocols are in development (consultation May 2023)*

3.1 Quality assurance and proficiency

Use of controls for QC of genotypic testing in
PCR / RT-PCR



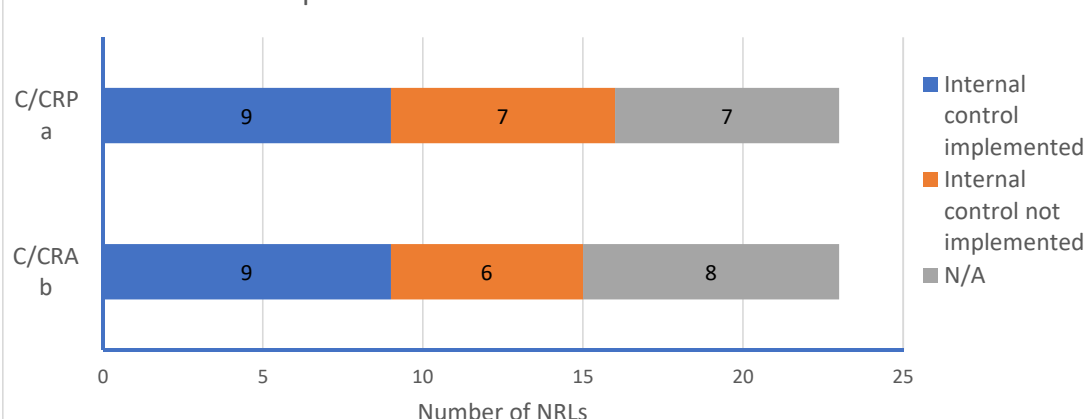
Use of controls in PCR/RT-PCR for QC purposes

- 4-7 NRLs do not **use controls for QC from a reliable source** for quality control testing of genotypic testing using other methods than WGS

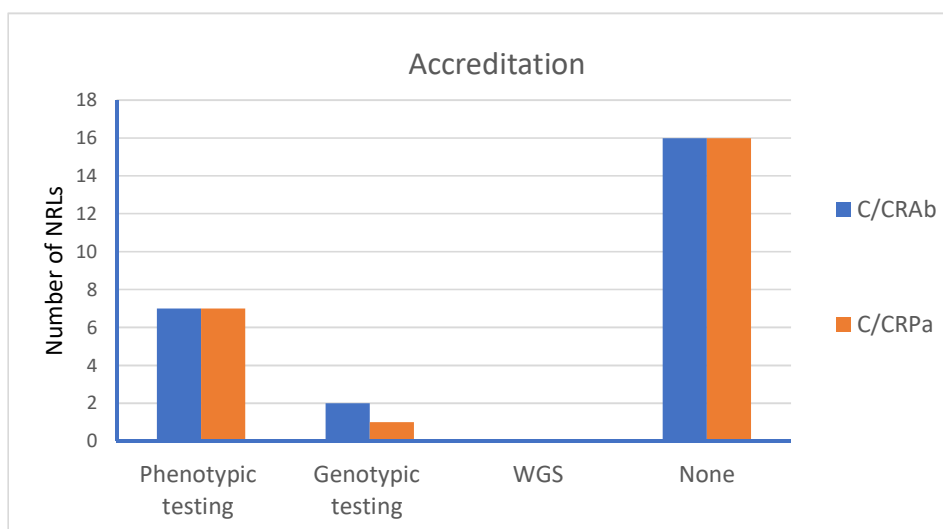
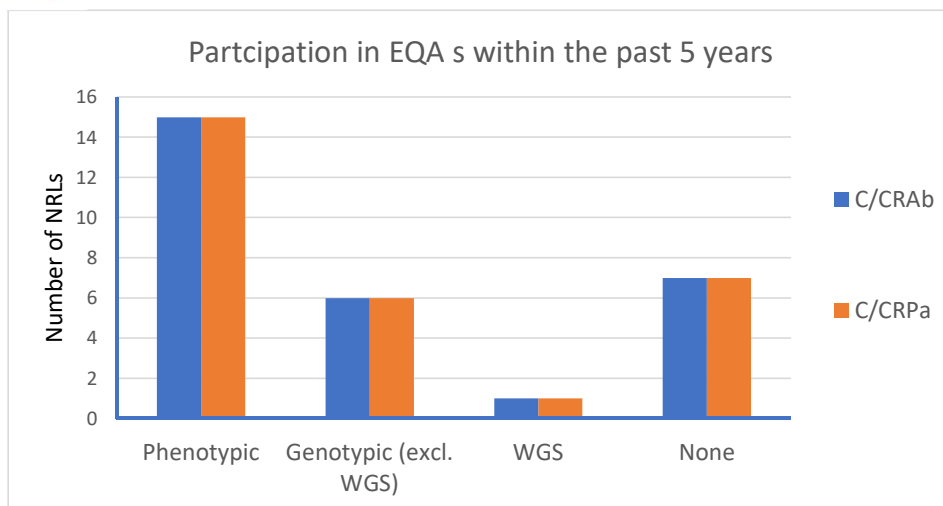
Internal control of WGS

- 6-7 NRLs among laboratories that use WGS have not implemented **Internal Control of WGS**
- Recommendations on quality control schemes are available in: **EURGen-RefLabCap Guidance document on internal quality control schemes** for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance
<https://www.eurgen-reflabcap.eu/resources/protocols-and-guidelines>
- New EURGen-RefLabCap document on internal quality control schemes for C/CRAb and C/CRPa is in development

Implementation of internal control for WGS



3.2 Quality assurance and proficiency cont.



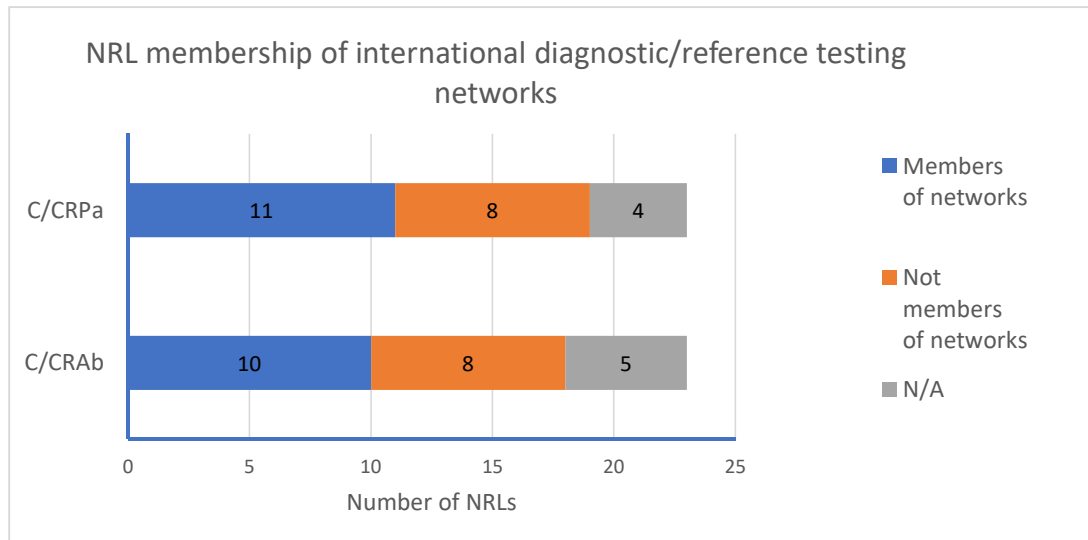
Participation in EQA (past 5 years)

- 7 NRLs did not **participate in any EQAs** for C/CRAb nor C/CRPa within the past 5 years
- A large number of EQAs are accesible in Europe (organised by ECDC, WHO CAESAR, NEQAS, QCMD, regional ring trials and EQAs)
- 2 EQAs on WGS are planned in EURGen-RefLabCap (2023, 2024)

Accreditation

- Few NRLs currently hold **accreditation for phenotypic and genotypic testing** of C/CRAb or C/CRPa, respectively.
 - **Phenotypic only (7; 7 NRLs)**: Cyprus, Czechia, France, Poland, Slovakia and Spain (for C/CRPa only)
 - **Genotypic (2; 1 NRLs)**: Iceland and Spain (for C/CRAb only)
- See also: <https://www.eurgen-reflabcap.eu/resources/protocols-and-guidelines> (Internal Control Schemes document)

3.3 Quality assurance and proficiency cont. (collaborations)

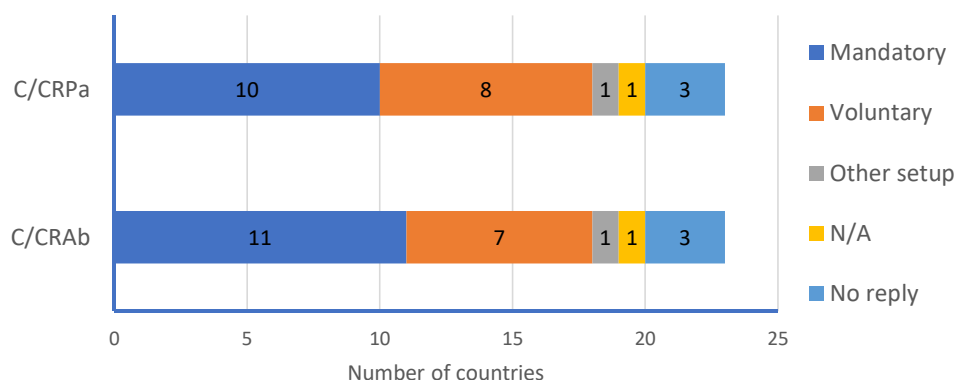


NRL membership of international collaborations/networks engaged in diagnostic and reference testing methods for C/CRAb and C/CRPa

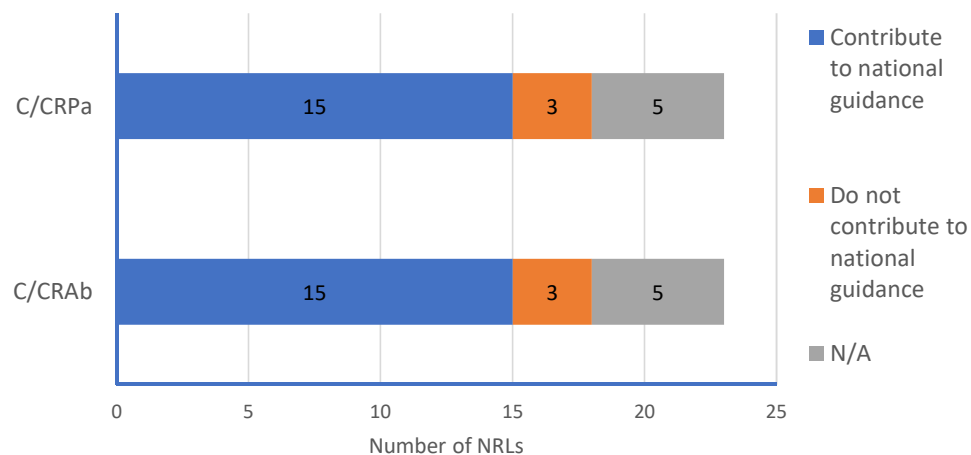
- **Only 10; 11 NRLs** replied they were **members of international collaborations/networks**
- **Most likely an underestimate** as known European networks have many more members from NRLs or national organisations (e.g. EARS-Net, EURGen-Net, EUCAST-AST, JPIAMR-ISARPAE network etc.)
- The question should be rephrased for clarity (in any subsequent surveys)
- The question should be replied to by the NRLs in consultation with other colleagues/collaborators

4.1 Surveillance and NRL support functions

National surveillance setup in participant countries



NRL contribution to national guidance



National surveillance

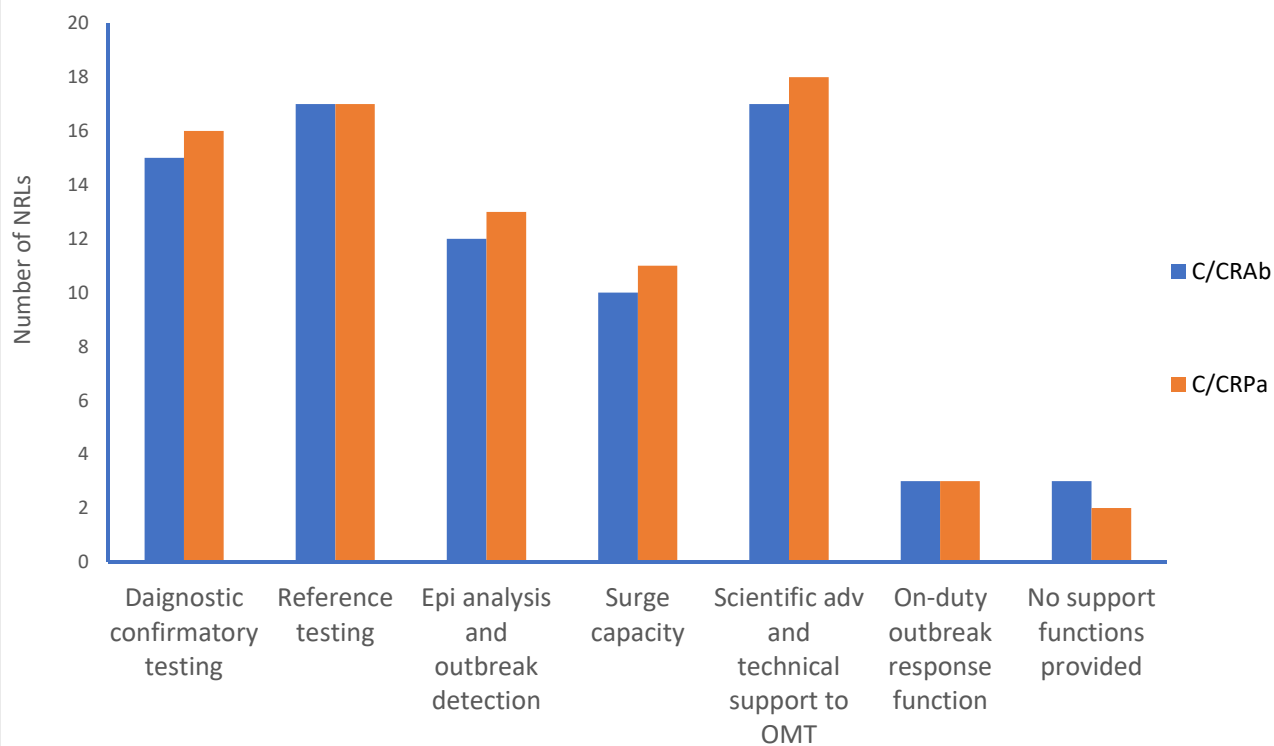
- **Surveillance** for C/CRAb and C/CRPa is in place in **19 (out 23) countries**
 - **C/CRAb**: 11 mandatory, 7 voluntary, 1 other setup
 - **C/CRPa**: 10 mandatory, 8 voluntary, 1 other setup
- **10 national systems** use a **case definition** (both pathogens)
- **6 national systems** use an **outbreak definition** (both pathogens)

NRL Advisory role

- **15 NRLs (out of 21) contribute** to the development of national guidance on testing/screening, phenotypic AST, molecular characterisation, infection prevention and control and/or a national containment for C/CRAb and/or C/CRPa

4.2 NRL Outbreak support functions

Outbreak support functions provided by NRLs



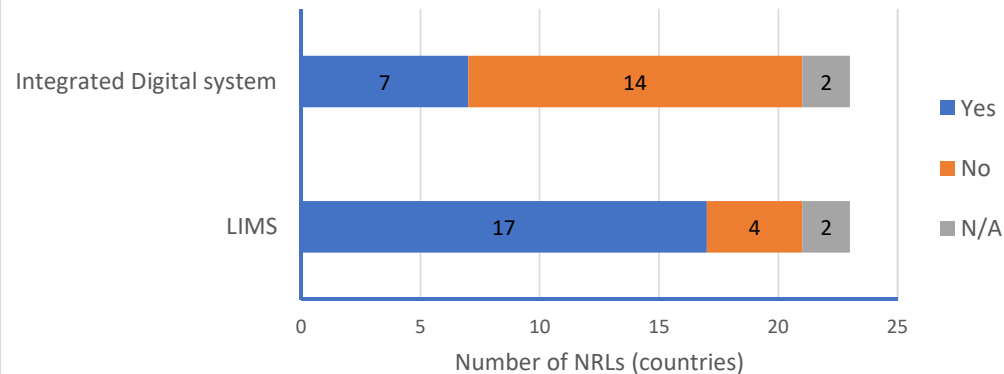
Outbreak support functions provided by NRLs

- **Scientific advice/technical support to "outbreak management team" (OMT)** provided by 17; 18 NRLs
- **Reference testing** - 17 NRLs (both pathogens)
- **Diagnostic confirmatory testing** – 15; 16 NRLs
- **Epidemiological analysis and outbreak detection** – 12; 13 NRLs
- **Surge capacity for diagnostic testing** – 10; 11 NRLs

5.1 Data management: monitoring, alert and response



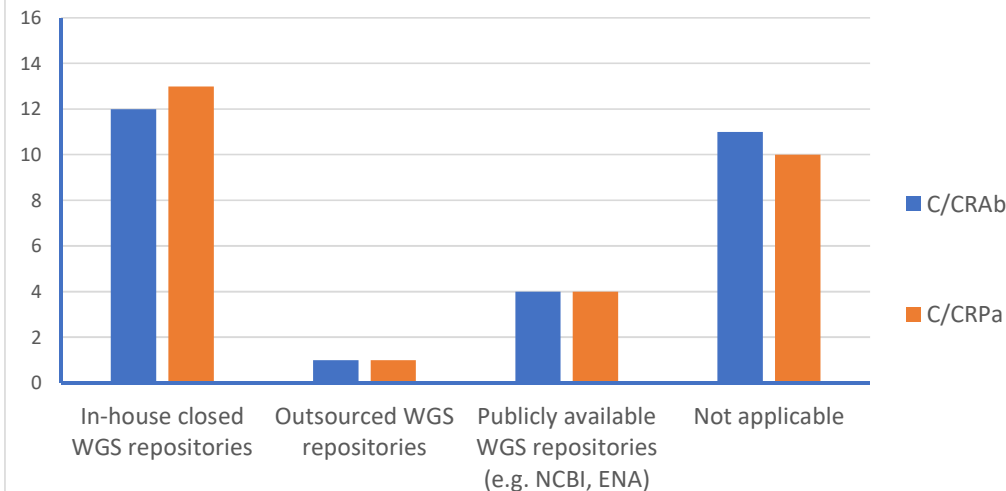
Access to LIMS and National Integrated Digital Systems



Laboratory data systems

- The NRLs in 17 countries had access to a LIMS or software application (e.g. WHONET) for collection, storage and reporting of test results
- Only 7 countries had a national integrated digital system that holds information on isolates, test results, NRL results and public health authorities allowing interoperability

Storage of WGS raw reads



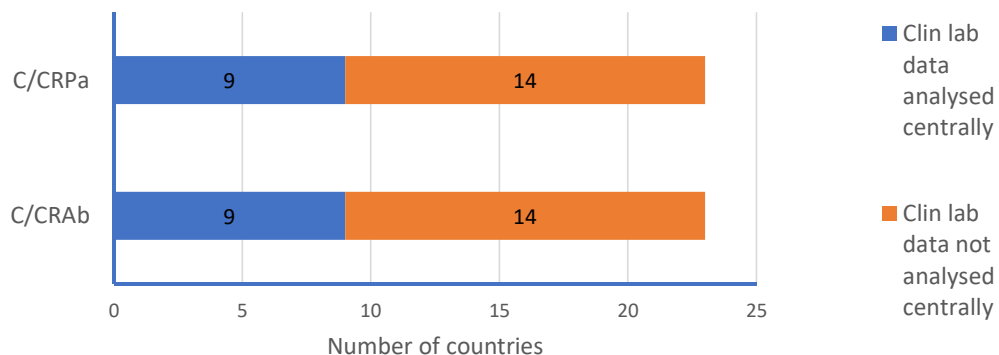
Storage of WGS data

- National WGS data were most commonly stored in in-house closed WGS repositories (CRAb: 12 countries; C/CRPa: 13 countries)
- WGS were uploaded to publicly available WGS repositories in 4 countries (both pathogens)

5.2 Data management: monitoring, alert and response



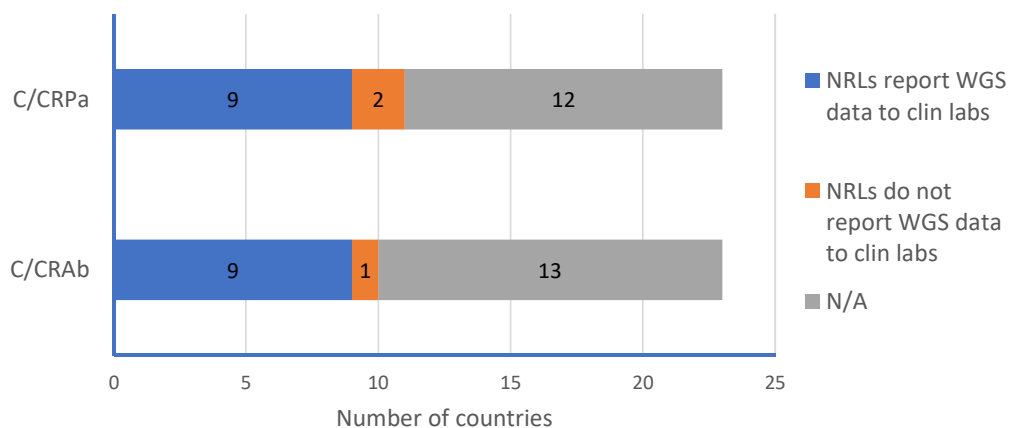
Central analysis of clinical laboratory data
(for outbreak detection)



Central (national) analysis of data from clinical laboratories by the NRLs

- **Clinical laboratory (diagnostic) data** were routinely analysed centrally in 9 countries by the NRL or public health authorities to detect outbreaks of C/CRAb and/or C/CRPa

Reporting of WGS data by NRLs to clinical laboratories

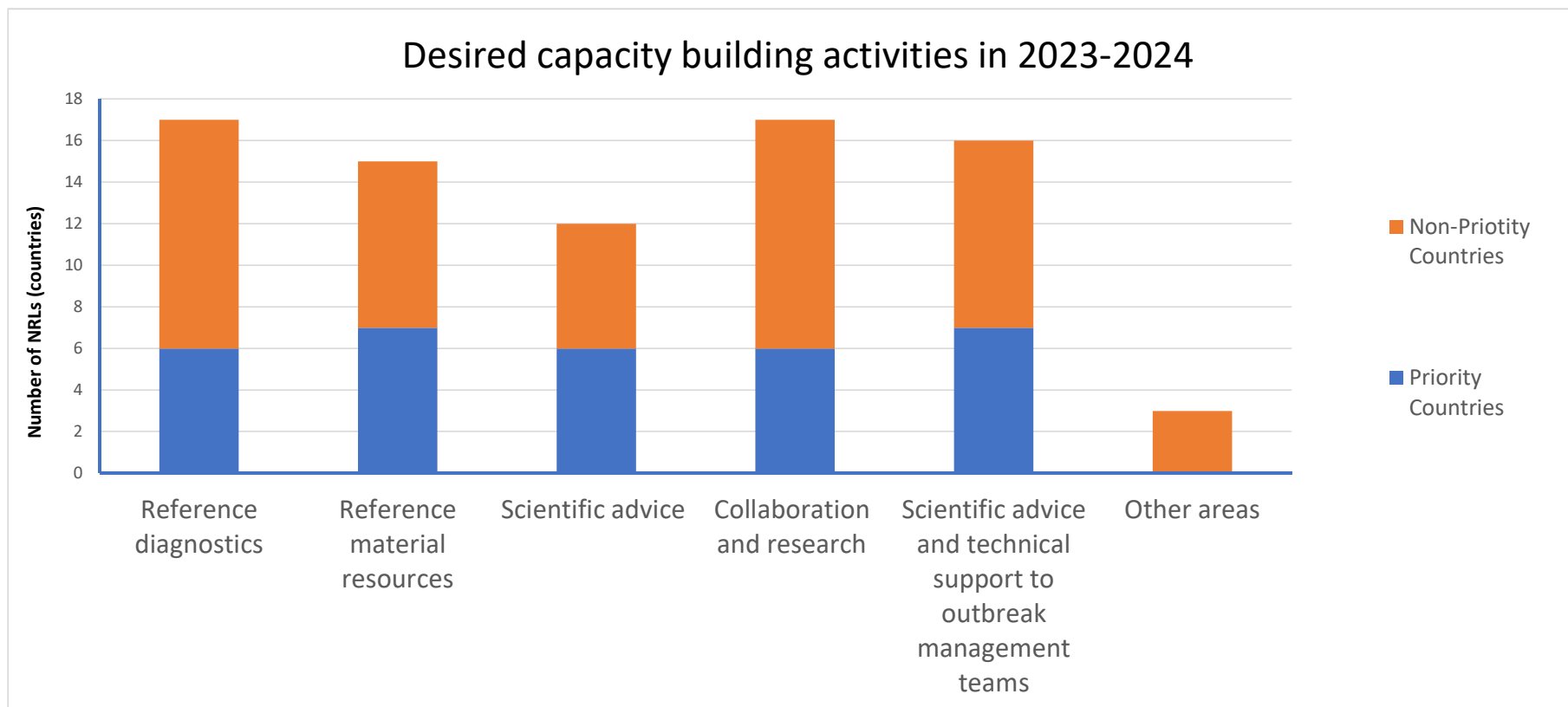


Reporting of WGS data from NRL to Clinical laboratories

- **NRLs in 9 countries** reported WGS data to the clinical laboratories



6. Further capacity building



Other suggested areas

- Quality control; International EQA;
- Business cases (to get more funding and resources for NRLs)

Key findings

- The survey results represents the situation in 23/37 (62%) countries in EURGen-RefLabCap
- 21/23 countries provide NRL services for WS2 pathogens (NRL in development in Cyprus, but not in Croatia)
- WGS has been implemented **for C/CRAb in 13 countries** and **for C/CRPa in 14 countries** – WGS activities were still low in most countries except for Czechia, France, Germany, Spain and Sweden. NRLs in further 10 countries are planning implementation of WGS
- Analytical purposes of WGS at the NRLs were most commonly: **confirmatory species ID, isolate typing and AMR genotyping** aimed at **surveillance, outbreak/cluster investigations and research**
- There **were gaps in Quality Control setups** for both for phenotypic and genotypic methods (PCR) and WGS and **National IT-systems**
- There were **national surveillance systems** for both WS2 pathogens in **19 out 23 countries**
- Up to 18 NRLs provide support functions to **outbreak management teams** mainly in the areas of: **Diagnostic confirmatory testing, Reference testing and Scientific advice/technical support**, and, but fewer NRLs providing **Epidemiological analysis and outbreak detection** (only provided by 12; 13 NRLs)
- NRLs expressed need for further capacity building activities in all areas (NRL core functions) addressed in WS1 for CCRE and CRE
- EURGen-RefLabCap protocols on WGS and internal control schemes aimed at supporting the NRLs **are available for WS1 and in development for WS2 pathogens**: <https://www.eurgen-reflabcap.eu/resources/protocols-and-guidelines>