Outcomes of the MRSA PCR PT

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18th EURL-AR Workshop 2024

PT for confirmation and characterisation of MRSA by PCR (or other genotypic testing method)

- Aim
  - to assess the laboratory setup for confirmation and characterisation of presumptive MRSA isolates,
  - assessment of positive (and negative) controls selected for the multiplex PCR methods

- Objectives
  - participating laboratories can use this PT to ensure that their setup is prepared for the MRSA baseline survey in 2025
  - to evaluate and improve the comparability of surveillance data on MRSA reported to EFSA by different laboratories

MRSA PCR PT - overview

- Sent out in April/May
  - Protocol
  - 3 control strains
  - 10 test strains
  - MyDBFinder Database file

  – Perform PCR using the suggested PCR assays (PCR-1 and -2)
  – Report the presence/absence of genes (online survey)
  – Used for test or validation of PCR setup
  – Overall evaluation report with expected results

  – Can also be performed by WGS
    - Reporting of the **PCR-relevant genes**
      – Presence/absence of genes
        » ID 90%, coverage 60%
- Report of results June/July

Table 2: Codes for the test strains

Test strains:
EURL MRSA-PT-01
EURL MRSA-PT-02
EURL MRSA-PT-03
EURL MRSA-PT-04
EURL MRSA-PT-05
EURL MRSA-PT-06
EURL MRSA-PT-07
EURL MRSA-PT-08
EURL MRSA-PT-09
EURL MRSA-PT-10

PCR control strains – expected results

Three *Staphylococcus aureus* isolates– two are MRSA – one is not!

Previously sent out as EQAS strains

	Gene:	***spa***	***mecA***	***mecC***	***pvl***	***scn***	**CC398**
	Product size:	200-600 bp	162	138	85	130	106
ID Control Strains	Control for:						
PCR-1-C1 EURL ST-12.7	*spa, pvl, scn*	+	-	-	+	+	
PCR-1-C2 EURL ST-11.3	*spa, mecA,* CC398	+	+	-	-	-	+
PCR-2-C3 EURL ST 17.7	*spa, mecC*	+	-	+	-	-	-

Test strains – expected results

ID	Test Strains	Gene:	*spa*	*mecA*	*mecC*	*pvl*	*scn*	CC398
		Product size:	200-600 bp	162	138	85	130	106
EURL MRSA-PT-01	*S. aureus*		+	-	-	-	-	+
EURL MRSA-PT-02	*S. aureus*		+	-	+	-	-	-
EURL MRSA-PT-03	*S. aureus*		+	+	-	-	+	-
EURL MRSA-PT-04	*S. epidermidis*		-	+	-	-	-	-
EURL MRSA-PT-05	*S. aureus*		+	+	-	-	-	+
EURL MRSA-PT-06	*S. aureus*		+	-	-	+	+	-
EURL MRSA-PT-07	*S. aureus*		+	+	-	-	-	+
EURL MRSA-PT-08	*S. aureus*		+	-	-	-	-	+
EURL MRSA-PT-09	*S. aureus*		+	-	+	-	-	-
EURL MRSA-PT-10	*S. aureus*		+	+	-	-	-	-

Reference materials still available (or again…)

Please let us know if you inquire the control and/or test strains (jetk@food.dtu.dk)

Outcomes of the PT

- 27 countries participated
  - 13 used PCR
    - One country used only partial set of primers (no primers for *scn* and CC398)
    - Two countries did not report the *spa*
  - 14 used WGS or combination
    - Reported genes based on WGS tools

Concordance with expected results

- Overall **99.6 % concordance** with expected results (n = 1560)

For *S. aureus*:

- Two false positive CC398
- One false positive *mecA*

1620	Possible number of results
54	Two participants not reporting spa
6	One participant missing primers
1560	Reported results

Results for *S. epidermidis*

- Did not grow on OXOID Brilliance MRSA2 agar
- No hemolysis on blood agar
- Mixed colony morphology or poor growth

- Two false positive *spa* genes identified
- One false positive *scn* gene identified
- All reported *mecA* (one lab did not analyse)

- Sequence analysis:
  - Kraken 2 show contamination: 84 % *S. epidermidis,* 16 % *S. aureus*
  - Kmerfinder identified it as mixed (*S. epidermis* and *E. faecalis*)
    - Database issues?

Tools used by participants

- Ridom SeqSphere
- MyDBFinder (CGE)
- BLAST+ search on command line agains MyDBFinder_MRSA_Baseline.fasta
- Kraken
- SpaTyper
- SCCmec typing
- VirulenceFinder (CGE)
- MLST
- AMRFinder plus
- BioNumerics
- ResFinder
- Clonal Complex (PubMLST)
- Kmerfinder
- Genefinder

Report on the way

- Expected results and report to be distributed later this month
  - Also including subtyping results (MLST, *spa*) and MyDBfinder results

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2024

The EURL-AR Proficiency Test for confirmation and characterisation of methicillin-resistant *Staphylococcus aureus* (MRSA) by PCR or other genotypic testing methods

Thank you for participation!

Questions or comments?