Development of the MyDBFinder database with all relevant genes for the EU-wide baseline survey on the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in pigs

Genes relevant to the PT (PCR-1 and PCR-2):

1. ***mecA*** – gene encoding methicillin-resistance.
2. ***scn*** – human association (host relationship).
3. **CC398** – pigs association (host relationship).
4. **PVL** - Panton-Valentine Leucocidin.
5. ***spa*** – confirmation of *Staphylococcus aureus* species. The *spa* fragment resulting from the amplification is variable in size and ranges depending on the *spa* type present and this fragment should be amplified from all *S. aureus* strains (no amplification of the *spa* fragment indicates the isolate is not an *S. aureus* and further identification procedures might be necessary to determine the species, in case this is necessary).
6. ***mecC*** - presumptive MRSA isolates, which are negative for *mecA*, can additionally be screened for *mecC* by the MRSA Multiplex PCR-2.

PRIMERS PCR-1:

Gene name	Primer name	Sequence (5' to 3')	Amplicon size (bp)	EURL positive controls[1]
lukF-PVL	PVL-F	GCTGGACAAAACTTCTTGGAATAT	85	EURL-ST-12.7
	PVL-R	GATAGGACACCAATAAATTCTGGATTG		
mecA	mecA P4	TCCAGATTACAACTTCACCAGG	162	EURL-ST-11.3
	mecA P7	CCACTTCATATCTTGTAACG		
scn	scnF1	TACTTGCGGGAACTTTAGCAA	130	EURL-ST-12.7
	scnR1	AATTCATTAGCTAACTTTTCGTTTTGA		
spa[2]	spa-1113F	TAAAGACGATCCTTCGGTGAGC	Variable 180-600	EURL-ST-12.7 EURL-ST-11.3
	spa-1514R	CAGCAGTAGTGCCGTTTGCTT		
sau1-hsdS1	FP2sau1	GAGAATGATTTTGTTTATAACCCTAG	106	EURL-ST-11.3
	CC398r1	CAGTATAAAGAGGTGACATGACCCCT		

---

[1] *S. aureus* EURL-ST-12.7 is an MSSA; *S. aureus* EURL-ST-11.3 and *S. aureus* 50A2047 are MRSA. *S. aureus* 50A2047 can also be used as positive control for *spa*, *mecA*, and *scn* (alternative control from PCR-2).

[2] A tool in CGE already exists for *spa* typing, so these variants were not included in MyDB

PRIMERS PCR-2:

Gene Name	Primer Name	Sequence (5’ to 3’)	Amplicon Size (bp)	EURL Positive controls
mecC	mecA$_{\mathrm{LGA251}}$ MultiFP	GAAAAAAAGGCTTAGAACGCCTC	138	EURL-ST-17.7
	mecA$_{\mathrm{LGA251}}$ MultiRP	GAAGATCTTTTCCGTTTTCAGC		

Mandatory genotypic characteristics

Gene Name	Gene Description	Reference Accession	EURL Positive controls
czrC	Cadmium and zinc resistance gene C	Y00688.1	S. aureus 171
chp	Chemotaxis inhibitory protein of S. aureus (CHIPS)	DQ530361.1	
***lukS*-PV**	Prophage-encoded Panton-Valentine leukocidin (PVL)	AB006796.1	
***lukF*-PV**	**Prophage-encoded Panton-Valentine leukocidin (PVL)**	**AB006796.1**	
scn	**Staphylococcal complement inhibitor (SCIN)**	**DQ530361.1**	
sak	Staphylokinase (SAK)	DQ530361.1	
sea	Staphylococcal enterotoxin A (SEA)	DQ530361.1	
seb	Staphylococcal enterotoxin B (SEB)	AF410775.1	
sec	Staphylococcal enterotoxin C (SEC)	X05815.1 AF217235.1 CP001996.1	
sep	Staphylococcal enterotoxin P (SEP)	BA000018.3	
tarP	Prophage-encoded wall teichoic acid glycosyltransferase (TarP)	BA000018.3	

Gene Name	Gene Description	Reference Accession	EURL Positive controls
tsst	Staphylococcal pathogenicity island (SaPI)-encoded toxic shock syndrome toxin 1 (TSST-1)	U93688.2 AF217235.1 CP001996.1	
vwbSaPI	Staphylococcal pathogenicity island (SaPI)-encoded von Willebrand factor-binding protein (genetic marker of ruminant adaptation	HM211303.1 CP001996.1 HM228920.1	
IEC1	Prophage-borne immune evasion cluster encoding SCIN (genetic marker of IEC1, encoded by scn) and different combinations of CHIPS, SAK, SEA, and SEP (genetic marker of human adaptation)	This cluster consist of scn, chp, sak, sea and sep, which are all in the database	
IEC1 type	A schematic representation of the different IEC1 types is provided by van Wamel et al. (2006)	This cluster consist of scn, chp, sak, sea and sep, which are all in the database	

Additional Genes in Virulence Finder Database:

Please see the **VirulenceFinder database** (in bold blue are genes already added to MyDB) - https://bitbucket.org/genomicepidemiology/virulencefinder_db/src/master/

The database includes the following genes:

- **ACME** – stands for arginine catabolic mobile element, which enhances the survival of S. aureus on the skin and mucosal surfaces.
- ***aur*** – encodes aureolysin, a metalloprotease that degrades host proteins and aids in infection.
- ***edinABC*** – encodes epidermal cell differentiation inhibitors that disrupt skin cell function.
- ***etAB*** – encodes exfoliative toxins A and B, which cause the skin to blister and peel (seen in staphylococcal scalded skin syndrome).

- **Enterotoxins A-E, G-O, R, U, Q** –are different types of staphylococcal enterotoxins that cause food poisoning and are superantigens that stimulate massive immune responses.
- ***hlb*** - encodes beta-hemolysin, a toxin that lyses red blood cells.
- ***hlgABC*** – encodes gamma-hemolysins, which are toxins that also lyse red blood cells but are composed of three components (HlgA, HlgB, HlgC) that form a pore in the cell membrane.
- ***lukED*** – encodes leukocidin ED, a toxin that targets and kills white blood cells, affecting the immune response.
- ***lukFS-PV*** (also known as lukSF-PV or Panton-Valentine leukocidin, PVL) – encodes another leukocidin that specifically targets and kills leukocytes and is associated with severe skin infections and pneumonia.
- ***splABE*** – encodes serine proteases, which degrade host proteins to aid bacterial invasion and immune evasion.
- ***scn*** – encodes staphylococcal complement inhibitor, which helps the bacteria evade the host immune system.
- ***sak*** – encodes staphylokinase, which breaks down blood clots and aids in the spread of infection.
- ***tst*** – Encodes toxic shock syndrome toxin (TSST-1), a superantigen causing toxic shock syndrome.

Flowchart for confirmatory testing, typing, antimicrobial susceptibility testing, and whole-genome sequencing of MRSA: