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Technical specifications for a baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs

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Abstract

The European Commission requested scientific and technical assistance in the preparation of a EU-wide baseline survey protocol for a European Union (EU) coordinated monitoring programme on the prevalence of methicillin-resistant *Staphylococcus Aureus* (MRSA) in pigs. The objective of the survey is to estimate the MRSA prevalence in batches of fattening pigs at slaughter at both European and national levels, with a 95% level of confidence and a level of precision of 10% considering an expected prevalence of 50%. The survey protocol defines the target population, the sample size for the survey, sample collection requirements, the analytical methods (for isolation, identification, phenotypic susceptibility testing and further genotypic testing of MRSA isolates), the data reporting requirements and the plan of analysis. The samples are to be analysed according to the laboratory protocols available on the European Union Reference Laboratory (EURL-AR) website. Generalised linear models will be used to estimate proportion (with 95% confidence intervals) of batches of slaughter pigs tested positive to MRSA. The necessary data to be reported by the EU Member States to support this baseline survey are presented in three data models. The results of the survey should be reported using the EFSA data collection framework.

Keywords: harmonisation, cross-sectional prevalence study, methicillin-resistant *Staphylococcus aureus*, antimicrobial resistance, fattening pigs

Requestor: European Commission

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Table of contents

1. Introduction

1.1. Background

Since 2014, AMR in certain bacterial organisms present in food or food-producing animals has been monitored under Commission Implementing Decision 2013/652/EU implementing Directive 2003/99/EC and the data collected during these years provide an important resource against which future trends can be evaluated. The AMR monitoring in food-producing animals and food was revised in 2020 by Commission Implementing Decision (EU) 2020/1729 of 17 November 2020, applicable as from 1 January 2021, which has laid down specific technical requirements, for the period 2021–2027 for AMR testing and reporting in representative isolates deriving from randomised sampling in food-producing animals performed at farm and/or at slaughter and derived meat performed at retail and at border control posts.

In addition to the routine monitoring performed on a biennial basis, the undertaking of complementary cross-sectional baseline surveys (BS) was suggested by the EFSA Scientific Report on the technical specifications on harmonised monitoring of AMR in zoonotic and indicator bacteria from food-producing animals and food,[1] in order to assess specifically the situation on certain AMR issues, such as MRSA, AMR in bacteria from sea food and AMR in bacteria from the environment, over the period of validity of the upcoming Commission Implementing Decision (EU) 2020/1729 in 2021 onwards.[2]

In accordance with the agreement reached during the decision-making process on Commission Implementing Decision (EU) 2020/1729 between the Member States (MSs) and the Commission on complementing the routine monitoring of AMR with specific BS on AMR, an online survey of the EFSA Network on zoonoses/AMR monitoring was carried out to collect and better assess the views of the MSs regarding the scope, priorities and the timing of BSs on AMR and to gauge the potential for further harmonisation of procedures and the degree of support from MSs for further AMR monitoring. The outcome of the survey indicated that according of the order of priority of the MSs and reporting countries, the BS-AMR on methicillin-resistant *Staphylococcus aureus* (MRSA) from pigs should be performed first. Followed by the BS-AMR on Seafood-Environment and BS-AMR on Enterococci. Ideally, the timing of these surveys should be harmonised between MSs to optimise comparability of results.

It is therefore envisaged that a detailed harmonised protocol for a BS on the prevalence and AMR of MRSA in pigs and possibly in other relevant food-producing populations will be designed, taking account of recent data from MSs and the latest scientific developments. Ideally, the timing of these surveys should be harmonised between MSs to ensure comparability of results.

The survey should notably provide in relevant food-producing animal populations:

- The prevalence/occurrence of MRSA at the EU level;
- A comparison of the prevalence and occurrence of antimicrobial resistance of MRSA in the EU MSs; and
- Information on the genetic diversity (e.g. lineages/strains) and virulence factors of MRSA detected.

1.2. Terms of Reference as provided by the requestor

In accordance with article 31 of Regulation (EC) No 178/2002, the Commission requests scientific and technical assistance from EFSA to provide technical and scientific support for the development of a BS as regards MRSA in pigs considering the most recent scientific literature and technological developments, epidemiological trends and relevance for public health.

In particular, EFSA is asked to propose harmonised approaches for the collection and the analysis of AMR data for MRSA in pigs by:

[1] EFSA (European Food Safety Authority), Aerts M, Battisti A, Hendriksen R, Kempf I, Teale C, Tenhagen B-A, Veldman K, Wasyl D, Guerra B, Liebana E, Thomas-Lopez D and Belœil P-A, 2019. Scientific report on the technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA Journal 2019;17(6):5709, 122 pp. https://doi.org/10.2903/j.efsa.2019.5709

[2] Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU. OJ L 387, 19 November 2020, pp. 8–21.

a) proposing a sampling framework (including the origin of bacterial isolates subject to AMR testing, the sampling design and the sample size) for the implementation of BS on MRSA in pigs;
b) proposing a harmonised protocol for isolation and characterisation of MRSA from pigs;
c) proposing a harmonised protocol for antimicrobial susceptibility testing of MRSA isolates and for the use of molecular typing methods (e.g. whole genome sequencing) to complement or replace the phenotypic methods and facilitate detection of relevant virulence factors;
d) providing guidance for technical reporting of the BS data to EFSA.
e) providing guidance for scientific analyses of reported data.

2. Rationale for the choices made in the proposal

2.1. The choice of the pig population to be surveyed

It is proposed that fattening pigs are mainly targeted because they account for a large share of the overall pig population. MRSA strains found in the breeding pig population are likely to be transmitted to fattening pigs. The situation in fattening pigs will to some extent mirror that in the breeding pig population. Previously published studies indicate that the fattening pig population is likely to be an appropriate and sensitive target group for detecting MRSA in pigs.

2.2. Rationale for the stage of the food chain to survey MRSA

The two stages primarily considered to monitor or survey MRSA were the farm or the slaughterhouse. Monitoring at farm level could facilitate investigation of risk factors and the influence of farm management practices on the occurrence of MRSA, though costs of sampling on farms were likely to be higher than sampling at slaughterhouses; the latter being considered to be more cost-effective (EFSA, 2009a,b, 2012). A degree of cross-contamination between batches of animals is considered likely to occur in abattoir-based monitoring and changes in MRSA status in finished pigs can occur during transport to abattoirs and also while held in the lairage prior to slaughter (Broens et al., 2011). Changes in the MRSA status of pigs have usually been assessed by monitoring through the collection of nasal and/or ear skin swabs and it is possible that animals are also carriers at other anatomical sites, such as the tonsil. Longitudinal studies also indicate that carriage of MRSA declines about 4 weeks after weaning in pigs fed with feeding supplemented with and without ZnO (Slifierz et al., 2015), and on entry to the fattening period (Bangerter et al., 2016). Nevertheless, studies carried out in Italy in 2008 have shown that the outputs obtained through population studies on MRSA in production pigs on the farm (survey performed in accordance with Decision 2008/55/EC[3]) or at the abattoir (Battisti et al., 2010) provide a broadly similar picture, with non-significantly different holding-level prevalence assessed at around 35% in both approaches and similar distributions of spa-types and sequence types (STs). In most MSs, monitoring MRSA at the slaughterhouse is comparatively more cost-effective to determine prevalence, particularly in the case of low to very low prevalence, or to assess the diversity of the MRSA subtypes prevalent in a production sector, as it has been demonstrated to be highly sensitive. A drawback relates to difficulties in interpreting the prevalence data, as cross-contamination is known to occur during transport and lairage (EFSA BIOHAZ Panel, in press), making it difficult to infer the original MRSA prevalence of the animals on farm (Broens et al., 2011). Linking the MRSA strains discovered at the slaughterhouse to any particular farm will also be complex, if at all possible. If data on the within-batch prevalence of MRSA are not needed and between-batch comparison is enough, then this disadvantage is less critical. It is proposed to perform the MRSA BS at the slaughterhouse. In developing this protocol, the aim was to build as much as possible on the AMR routine monitoring already in place adding as little as possible additional burden on the MSs.

2.3. Rationale for the expected prevalence of MRSA

There is limited information regarding prevalence of MRSA in pigs in Europe, and the information that do exist is scattered. It consists of a limited number of national surveillance programmes as well as research studies performed over a long time period. Concordantly, there is little consistency or

[3] Commission Decision 2008/55/EC of 20 December 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States. OJ L 14, 17.1.2008, p. 10–25.

harmonisation among studies as regards to sample selection and molecular methods. Although this hampers direct comparison between studies, some general conclusions can be drawn.

The results of studies performed at slaughterhouses in Europe, investigating the prevalence of MRSA in fattening pigs using either nasal swabs and/or ear skin swabs and published in peer-reviewed journals have been summarised in Table 1. Information regarding the prevalence of MRSA in pigs assessed on the farm is also available, both from the same and additional countries. From these reports, it can be concluded that within Europe, there is a marked variability in the prevalence of MRSA in pigs, ranging from rare to extremely high. Moreover, increasing trends can be seen in countries that over time and in a consistent way have monitored the prevalence of MRSA in pigs. For example, the prevalence of MRSA among fattening pigs sampled at slaughter in Switzerland has increased from 5.1% in 2011 to 52.8% in 2019.

Table 1: Studies investigating prevalence of MRSA in pigs at the slaughterhouse in Europe

Reference	Study year	Country	Sampling stage	Sample type	Slaughter batch size (#animals)	Sample batch size (#animals)	MRSA prevalence (%)			
							Animal level	Batch level	Within batch	Farm level
De Neeling et al. (2007)	2005–2006	NL	AF	NS	NR	10	39	81	0–100	80[(a)]
Horgan et al. (2011)	2007 + 2009	IE	AF	NS	NR	1	0	0	0	0
Tenhagen et al. (2009)	< 2009	DE	AF	NS	NR	10	71	98	NR	NR
Tenhagen et al. (2009)	< 2009	DE	AF	NS	NR	1–18	49	82	NR	NR
Battisti et al. (2010)	2008	IT	AF	NS	NR	10	NR	14	NR	38
Broens et al. (2011)	2008–2009	NL	AF	NS	27–65	27–30	59.8	100	6.7–100	100
Agersø et al. (2013)	2009	DK	BS	NS	NR	1	13	NR	NR	14
Beneke et al. (2011)	2011	DE	AF	NS	NR	1	64.7	NR	NR	70.9
Normanno et al. (2015)	< 2015	IT	AF	NS	NR	1	37.6	NR	NR	NR
Dierikx et al. (2016)	2015	NL	AF	NS	NR	9–11	99.5	100	40–100	100
Smith et al. (2021)	2016	UK	AF	NS	NR	1 and 20	NR	34.6	NR	NR
Smith et al. (2021)	2016	UK	AF	ESS	NR	1 and 20	NR	29.5	NR	NR
Smith et al. (2021)	2016	UK	AF	NS + ESS	NR	1 and 20	NR	43.8	5–70	NR

AF: after stunning; BS: before scalding; ESS: ear skin swabs; NS: nasal swabs; NR: not reported.
(a): Neither the exact nor an approximate number was reported in the publication. However, an approximate number could be calculated.

2.4. Rationale for the choice of the sampling stage at the slaughterhouse and sample type

When sampling at the slaughterhouse, the most commonly reported sampling stage is post-stunning (before bleeding). Some studies still also report sampling at several other stages of the slaughter line (Broens et al., 2011; Smith et al., 2021). These studies show that batches of animals tested negative for MRSA when leaving the farm may test positive during transport, at lairage or at stunning. Sampling post-stunning is, however, perceived as easier and furthermore, does not inflict any additional stress on the animals.

The sampling method most often used in pigs is based on collecting nasal swabs: either a single swab per individual or one to several pools of four to six swabs per herd. Ear swabs (swabbing the skin behind the ears of pigs) have also been used (Agersø et al., 2013; Stewart-Johnson et al., 2019; Sasaki et al., 2021; Smith et al., 2021). When sampling at the slaughterhouse, the most commonly reported sample type is anterior nasal swabs. In Stewart-Johnson et al. (2019) and Smith et al. (2021), a higher prevalence of positive samples was detected with nasal swabs, whereas Sasaki et al. (2021) detected a higher prevalence of positive samples using skin swabs. A combination of both sample types rendered the highest prevalence of positive samples in all the three studies. Still, collecting nasal swabs has been found to be more cost-effective and also simplify the isolation phase at the laboratory.

Yet another aspect to consider when designing the sampling scheme is the number of animals per slaughter batch to be sampled as well as if and how these samples should be pooled. One study from Germany reports that all sample batches including seven or more animals were positive for MRSA (Tenhagen et al., 2009). Furthermore, of the batches with four or more animals per batch, 53 of 55 (96%) sampled batches tested positive for MRSA. In an Italian study (Battisti et al., 2010), six pools of 10 anterior nares swabs per farm from 118 holdings were analysed. In total, 45 (38.1%) of the holdings tested positive, but about half of them (22/45) had only one positive pool.

2.5. Rationale for monitoring antimicrobial susceptibility of MRSA

The monitoring of antimicrobial susceptibility patterns can be used, in conjunction with strain typing data to provide useful information on the evolution and dissemination of strains of MRSA. The antimicrobial susceptibility of MRSA isolates can provide useful information that is particularly relevant for LA-MRSA CC398 where livestock-associated strains are typically resistant to tetracyclines, providing one of the markers which assists in distinguishing between the livestock and human clades of CC398 (Kinross et al., 2017). Resistance to linezolid, a critically important last resort antimicrobial in human medicine, has been detected in *Staphylococcus* from livestock in Europe (EFSA and ECDC, 2018). Resistance to this antimicrobial may be conferred by chromosomal point mutations in 23S or by acquisition of resistance genes (*cfr*, *optrA* and *poxtA*), some of which also confer resistance to antimicrobials frequently used in veterinary medicine, with the consequence that use of certain veterinary medicines may co-select for linezolid resistance. Resistance to vancomycin (another critically important antimicrobial in human medicine) has not been detected in MRSA from livestock but is included in the voluntary monitoring performed by those MSs which undertake it. The degree of resistance to those antimicrobials which are particularly important in the treatment of humans should be assessed.

3. Objectives

The first objective of this BS is to assess the prevalence of MRSA in fattening pigs in the EU and in the different EU MSs. The investigation of the BS on MRSA in pigs should target slaughtered fattening pigs; monitored through a harmonised EU-wide baseline/cross-sectional survey at the slaughterhouse; and should provide comprehensive, comparable and reliable information on the development and spread of MRSA in slaughtered pigs across the EU.

The second objective is to assess the genetic diversity and antimicrobial resistance of MRSA in pigs in the EU MSs by performing detailed characterisation of MRSA strains and lineages, virulence and host-adaptation factors, and other genetic markers (e.g. phages) associated with certain animal hosts. Recent developments, including the increasing use of molecular monitoring through whole genome sequencing (WGS), are acknowledged and it is proposed to be incorporated into the BS. The detailed characterisation of isolates at the molecular level facilitates comparison of AMR in humans and animals

at several levels, including the occurrence and types of resistance genes, resistance plasmids and virulence factors. The technical specifications set out proposals whereby molecular monitoring in MRSA can be implemented in the BS across MSs, while retaining comparability with the phenotypic monitoring.

4. Survey design

4.1. Definitions

The objective is to estimate two probabilities for each MS:

- The **prevalence π:** The probability that a batch is MRSA positive; a batch is defined positive if at least one unit in the batch is positive.
- The **proportion of resistance p_r**: The probability that an MRSA isolate is resistant to a define set of tested substances.

4.2. Sampling frame

It is proposed that the BS on MRSA is based on the representative/random collection of samples from healthy fattening pig carcases at the slaughterhouse. Sampling performed at the slaughterhouse is envisaged, as in many of the MSs, it will be most cost-effective way to collect the samples. It is recommended that at least 60% of the domestic animal population in a MS are included in the sampling frame, meaning that slaughterhouses processing at least 60% of the domestic animals of the relevant animal category (starting with the slaughterhouses of largest throughput) are eligible for sampling.

The epidemiological units defining the sampling frame are the slaughter batches of fattening pigs, defined as a group of animals of the same age raised together under the same conditions and exposed to the same risk factors regarding MRSA prevalence/AMR and sent together to the slaughterhouse at the same moment, from slaughterhouses, including slaughterhouses accounting for at least 60% of the national production in each MS.

The sampling plan should be typically stratified per slaughterhouse. The total number of slaughter batches to be randomly sampled within a particular MS (see further section on sample sizes) is proportionately allocated to the slaughterhouses (stratified sampling), using proportions reflecting the relative throughput of the different slaughterhouses. An approximately equal distribution of the sampled slaughter batches over the year enables the different seasons to be covered.

The design follows the generic proportionate stratified sampling approach already implemented for the harmonised monitoring of AMR, as presented in the technical specifications on harmonised AMR monitoring (EFSA, 2019).

4.3. Sample sizes

4.3.1. Standard calculation

The determination of the required number of slaughter batches (sample size) in an MS is based on:

- The required number n_i of MRSA isolates to achieve an effective estimation of the proportion of resistance p_i, with preassigned level of confidence and accuracy.
- An upward adjustment to obtain the required number n_b of slaughter batches for the estimation of the prevalence p.

Following the technical specifications on harmonised AMR monitoring (EFSA, 2019), the required number of MRSA isolates is $n_i = 97$,[4] guaranteeing an overall accuracy of at least 0.1 with a confidence level of 0.95. The upward adjustment to obtain n_b depends on the unknown prevalence π by a factor, the inverse of the prevalence 1/p (EFSA, 2019). For example, if the (unknown) prevalence was 90%, $n_b = 97/0.9 \approx 108$. If an initial estimate $\hat{\pi}$ of the prevalence for a particular MS is available, this estimate can be used for the adjustment $97/\hat{\pi}$, with a maximal number of batches to be sampled of 194. This number of batches is expected to lead to the required number of 97 isolates only if the

[4] For comparison, the R command (package MKpower)
ssize.propCI(prop = 0.5, width = a*2, conf.level = 0.95, method = 'wald-cc')$n
with unknown proportion taken as 0.5 (worst case, leading to largest required sample size) and confidence level 0.95, leads to a required sample size of 106 for an accuracy of a = 0.10. Using method = 'jeffreys', the required sample size is 96.

estimated prevalence $\hat{\pi} \geq 0.5$. Table 2 shows the required number of slaughter batches in the first row, for prevalence decreasing from 100% to 50% in steps of 10%.

4.3.2. Accounting for possible missing data and loss during storage

As discussed in the technical specifications for harmonised AMR monitoring (EFSA, 2019), the required number of slaughter batches n_b should be further inflated by

5% in order to take into account a 5% occurrence of missing data,
2% in order to account for loss of strains during storage.

This leads to the required number of batches

$$n_b = \min\left(\frac{97}{\hat{\pi}}, 194\right) \times 1.05 \times 1.02,$$

with a maximum required number of slaughter batches $194 \times 1.071 \approx 208$.

Table 2 shows the required number of slaughter batches after this 'loss adjustment' (LA) in the second row, for prevalence decreasing from 100% to 50% in steps of 10%.

4.3.3. Finite population correction factor for small MSs

If the sample of batches with sample size n_b comprises more that 5% of the total population size N_b of batches of an MS, a correction factor resulting in a downward adjustment can be applied (see Appendix I of EFSA, 2019):

$$n_b^f = n_b \times \frac{N_b}{(N_b + n_{b.})}$$

For instance, if N_b = 688 (the number for Iceland as mentioned in the table of Appendix I in EFSA, 2019), the maximum required number of slaughter batches 208 is adjusted to $208 \times 688/(688 + 208) \approx 160$.

The total size of the population of batches of an MS is unknown but can be determined based on the total throughput of all selected slaughterhouses (SH).

Table 2 shows the required number of slaughter batches after the loss adjustment (LA) and with the finite population correction (FPC) from the third row onwards, for a range of population sizes and for prevalence decreasing from 100% to 50% in steps of 10%. The last column of Table 2 shows the maximum required number of slaughter batches, to be achieved in case the prevalence is 50% or less, or is unknown. In case the prevalence is unknown, an MS can opt for a quarterly adjusted sequential procedure, as explained and illustrated in the next section.

Table 2: Required number of slaughter batches (sample size). First row: required sample sizes for varying prevalence without loss adjustment (LA, see Section 4.3.2). Second row: required sample sizes for varying prevalence with loss adjustment (LA, see Section 4.3.2). Subsequent rows 3–18: required sample sizes after the finite population correction (FPC) for different population sizes (in first column) (see Section 4.3.3)

	Population size	Prevalence					
		$\hat{\pi}$ = 1	$\hat{\pi}$ = 0.9	$\hat{\pi}$ = 0.8	$\hat{\pi}$ = 0.7	$\hat{\pi}$ = 0.6	$\hat{\pi} \leq$ 0.5
without LA	**Inf**	97	108	122	139	162	194
with LA	**Inf**	104	116	130	149	174	208
with FPC[(a)]	**100**	51	54	57	60	64	68
with FPC	**200**	69	74	79	86	93	102
with FPC	**300**	78	84	91	100	110	123
with FPC	**400**	83	90	99	109	121	137
with FPC	**500**	87	94	104	115	129	147
with FPC	**600**	89	97	107	119	135	155
with FPC	**700**	91	100	110	123	139	161

	Population size	Prevalence					
		$\hat{\pi}$ = 1	$\hat{\pi}$ = 0.9	$\hat{\pi}$ = 0.8	$\hat{\pi}$ = 0.7	$\hat{\pi}$ = 0.6	$\hat{\pi} \leq$ 0.5
with FPC	**800**	92	101	112	126	143	165
with FPC	**900**	94	103	114	128	146	169
with FPC	**1,000**	95	104	115	130	148	173
with FPC	**2000**	99	110	122	139	160	189
with FPC	**3,000**	101	112	125	142	164	195
with FPC	**4,000**	102	113	126	144	166	198
with FPC	**5,000**	102	113	127	145	168	200
with FPC	**10,000[(b)]**	103	115	129	147	171	204

LA: loss adjustment; Inf: infinitely large population size; FPC: finite population correction.
(a): The finite population correction is applied on the original size in row 2, with loss adjustment.
(b): For very large finite populations, the sample sizes get closer to those shown in row 2, with loss adjustment.

4.3.4. Proportional allocation of sample size per strata and per quarter

As discussed in EFSA (2019), the number of batches to be taken from a particular SH is proportional to the throughput of that SH. Denote the fraction of throughput of an SH by f_t.

The total number of batches to be collected at an SH in a year equals $n_b^f \times f_t$ and thus, the number of batches to be collected per quarter should therefore equal $(n_b^f \times f_t)/4$. For instance, if $n_b^f = 160$ and $f_t = 0.08$, the quarter-based number of batches equals 4.

In case the prevalence is unknown, the required number of slaughter batches can be adjusted by a sequential quarterly procedure, based on all data from previous quarters. A quarter is referring to the point in time where 1/4 of the total required number of slaughter batches has been reached, to be expected after 3 months (if sampling is done throughout the whole period from 1 January 2023 to 31 December 2023) or after 2.5 months (if sampling starts at 1 March 2023, until 31 December 2023). Of course, the adjustment needs to be performed after each quarter and can go in both directions.

If an MS opts for this sequential procedure, it is desirable to document it by providing relevant data of the previous quarter, and a short report to EFSA with the calculations leading to the adjusted sample size. If, for any reason, the sequential procedure needs to be abandoned, the required number of slaughter batches turns back to the maximum (last column of Table 2).

Some examples of calculations are shown in Table 3.

Table 3: Two scenarios illustrating the sequential adaptation of the required number of slaughter batches

	Example 1	Example 2
Starting point	Unknown prevalence finite population of size 500 required sample size for the 1st quarter equals 147/4 = 37	Unknown prevalence finite population of size 500 required sample size for the 1st quarter equals 147/4 = 37
After 1st quarter	Prevalence estimate (based on the 37 slaughter batches of quarter 1) equals 0.70, implying that the sample size for the 2nd quarter decreases to 115/4 = 29	Prevalence estimate (based on the 37 slaughter batches of quarter 1) equals 0.70, implying that the sample size for the 2nd quarter decreases to 115/4 = 29
After 2nd quarter	Prevalence estimate (based on the 37 + 29 = 66 slaughter batches of quarter 1 and 2) equals 0.80, implying that the sample size for the 3rd quarter decreases further to 104/4 = 26	The member state has no longer the capacity to analyse the data and estimate the prevalence based on the 37 + 29 = 66 slaughter batches of quarter 1 and 2. The sample size for the 3rd quarter increases again to that of the starting situation: 147/4 = 37
After 3rd quarter	Prevalence estimate (based on the 37 + 29 + 26 = 92 slaughter batches of quarter 1 to 3) reduces again to 0.70, implying that the sample size for the 4th quarter increases again to 115/4 = 29	The sample size for the 4th quarter remains at 147/4 = 37

	Example 1	Example 2
Total sample size	37 + 29 + 26 + 29 = 121 instead of 147	37 + 29 + 37 + 37 = 140 instead of 147

4.3.5. Batch sensitivity (required number of units of a batch)

A batch is defined to be positive if at least one unit is positive. The probability that a truly positive batch is detected to be positive (batch sensitivity BSe) depends on the batch size M, the number of truly positive batch units $D > 0$, the (unit-) test sensitivity TSe, the (unit-) test specificity TSp, and the number m (out of M) of units tested. Following Cannon (2002),[5] we assume there are no false positives (TSp very high), leading to the batch sensitivity

$$BSe = 1 - \left(1 - \frac{m \times TSe}{M - \frac{1}{2}(D \times TSe - 1)}\right)^{D}$$

Setting a required batch sensitivity BSe, having a value for the test sensitivity TSe, the batch size M, and the number of truly positive units D, the required number m of units of the batch to be tested can be determined.

As an example, the analysis of an exemplary data set provided by Anses (Jouy et al., 2009) shows that batch sizes M vary in this example from 1 to 370, with median 100 (see Figure 1). About 98% of the batches has a size M larger than 25.

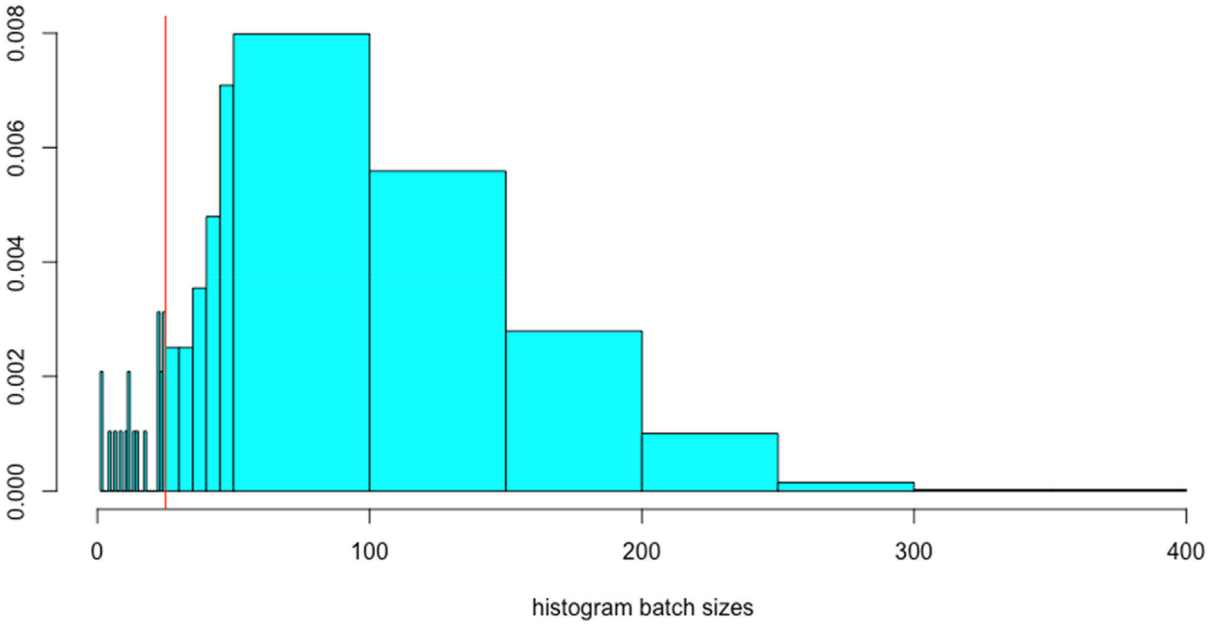


Figure 1: Distribution of batch sizes provided by a MS (Jouy et al., 2009)

Assume that the number D of truly MRSA-positive pigs in the batch is 10% of the batch size M, the test sensitivity for testing an individual pig is TSe = 0.9 and that for a pooled sample, a pool test can detect one infected unit in a pool of size 5 with probability 0.80, and two or more infected animals in the pool with probability 1 (as in Section 3.2 in Cannon, 2002).

The batch sensitivity obtained when selecting 25,20,15,10,5 animals, tested individually or in 5,4,3,2,1 pools of size 5 is shown in Table 4.

[5] Cannon RM. Demonstrating disease freedom-combining confidence levels. Prev Vet Med. 2002 Jan 22;52(3–4):227–249. https://doi.org/10.1016/s0167-5,877(01)00262-8. PMID: 11849719.

Table 4: Sensitivity of detection of MRSA in a batch of slaughter pigs characterised with a 10% contamination among the slaughter pigs of the batch when samples collected from sampled pigs are tested separately and when samples are pooled by 5 units

# of animals selected per batch	Sensitivity of detection of MRSA at the batch level	
	All samples tested individually	Pools of size 5 tested
25	0.905	0.878
20	0.848	0.815
15	0.757	0.718
10	0.611	0.570
5	0.376	0.344

With 20 animals sampled per batch, the batch sensitivity is still above 80%, and with 15 animals, the batch sensitivity is above 70%.

The required number *m* of randomly selected units from a batch of size M to be tested is: all units for batches up to size 20 and m = 20 for batches larger than 20, or

$$m = \min(M, 20).$$

Units can be pooled in groups of at most size 5, so with a maximum of four pooled samples of 5 units.

4.4. Sampling plan

The simple and robust randomised sampling procedure currently in place for routine monitoring of AMR in bacteria from slaughter pigs, mostly relying on a stratified sampling approach with proportional allocation of the sample numbers per strata, is proposed to be applied for the MRSA BS. The general characteristics of the proportional stratified sampling approach are briefly presented in Table 5. It illustrates stratified sampling concepts, such as strata, proportional allocation, epidemiological unit, to the sampling plans proposed. Similar functional procedures as those presented in detail in Appendix J of EFSA (2019) can be applied.

Table 5: General characteristics of the stratified sampling approach

Sampling concept	Sampling of nasal samples at slaughter
Target populations	Domestically produced – Fattening Pigs[(a)]
Strata (1st stage)	Slaughterhouses[(b)]
Proportional allocation	Sample size proportionate to the slaughterhouse throughput
2nd stage	Batches of fattening pig carcases originating from the same herd of pigs
Epidemiological Unit	Slaughter batches of fattening pigs
Sample/Isolate	Four pooled samples of 20 nasal swabs per epidemiological unit[(c)]

NA: not applicable.
(a): The source population of fattening pigs covers that domestically produced and slaughtered in the slaughterhouses representing at least 60% of fattening pigs slaughtered in the Member State.
(b): Those slaughterhouses that accounted for at least 60% of all fattening pigs domestically produced in the previous years, according to the most recent statistics.
(c): The 20 nasal swabs derive from 20 carcases per batch of carcases originating from the same herd of pigs are intended to be pooled by 5.

5. Sample collection

5.1. Type of samples and sample information

For each randomly selected epidemiological unit (slaughter batch), 20 nasal swabs (from 20 different pigs) are taken and pooled into four composite samples of five nasal swabs for further testing.

Each sample should be labelled with a unique number which should be used from sampling to testing. The use of unique numbering system is recommended.

5.2. Transport of samples

The EURL-AR provides general recommendations on collection and storage of samples for monitoring of MRSA.[6] Nasal swabs are obtained from individual slaughter pigs immediately after stunning by inserting a cotton tip into the anterior nares of each pig and rotating it five times. It is recommended to use commercially available transport swabs (which include suitable transport media) in accordance with the manufacturer's recommendations. In general, swab samples should be stored at 4–8°C, and isolation of MRSA should be initiated within seven working days of collection.

6. Laboratory analytical methods

6.1. Overview

To detect MRSA in a given sample, several successive analytical steps are needed. The first step is to isolate presumptive MRSA. Confirmation of MRSA is then obtained by polymerase chain reaction (PCR). Complementary analytical steps, antimicrobial susceptibility testing and WGS are performed to further characterise isolates.

6.2. Isolation method of MRSA

To ensure that the assessed MRSA prevalence is comparable between the MSs, the isolation procedure needs to be harmonised. The EURL-AR has recently updated the advised protocol for isolation of MRSA from animals and the environment.[6] The EURL-AR can be contacted for technical support. The previous technical specifications (EFSA, 2012) were based on the scientific results available at that time and recommended a two-stage isolation method rather than that currently recommended by the EURL-AR, which reflects recent findings and adopts a one-stage method (Larsen et al., 2017). The one-stage method has been shown to have increased relative sensitivity when applied to pigs (Larsen et al., 2017), though differences between the methods were not apparent in studies on cattle or poultry (Nemeghaire et al., 2013, 2014). The one-stage method has already been adopted by several EU MSs, as shown elsewhere (EFSA, 2019), and recently confirmed by a survey performed by the EURL-AR among NRL-AR in spring 2022.

The EFSA WG has also assessed the relative intrinsic characteristics (sensitivity, Se and specificity, Sp) of the one-stage (vs. two stage method) cited above when applied to pig samples and demonstrated that the one-stage method has a higher sensitivity that the two-stage method (0.924 vs. 0.825), while the specificity was slightly lower for the one-stage method when compared with the two-stage method (0.979 vs. 0.985).

The updated one-stage method recommended by the EURL-AR includes a pre-enrichment step followed by incubation on a chromogenic MRSA-selective and indicative agar plate:

- Samples (i.e. pools of five nasal swabs) are covered in 10 mL of Mueller–Hinton broth containing 6.5% sodium chloride (NaCl) and incubated at 35–37°C for 16–24 h.
- A 10-µL loopful of pre-enrichment culture is spread on a Brilliance MRSA 2 plate or an equivalent chromogenic screening plate for MRSA and incubated at 35–37°C for 16–24 h. Presumptive MRSA colonies appear as denim blue colonies after overnight incubation.

One presumptive MRSA colony from each chromogenic screening plate is then subcultured on a blood plate at 35–37°C for 24–48 h to look for characteristic morphology and haemolysis, and to perform catalase testing on doubtful isolates. MRSA colonies on blood agar are greyish or yellowish and usually surrounded by a zone of haemolysis. The catalase test can be used to distinguish staphylococci from enterococci, which sometimes produce similar colony morphology on Brilliance MRSA 2 agar.

The workflow for isolation of MRSA is illustrated in Figure 2.

[6] https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/430_mrsa-protocol-final-19-06-2018.pdf

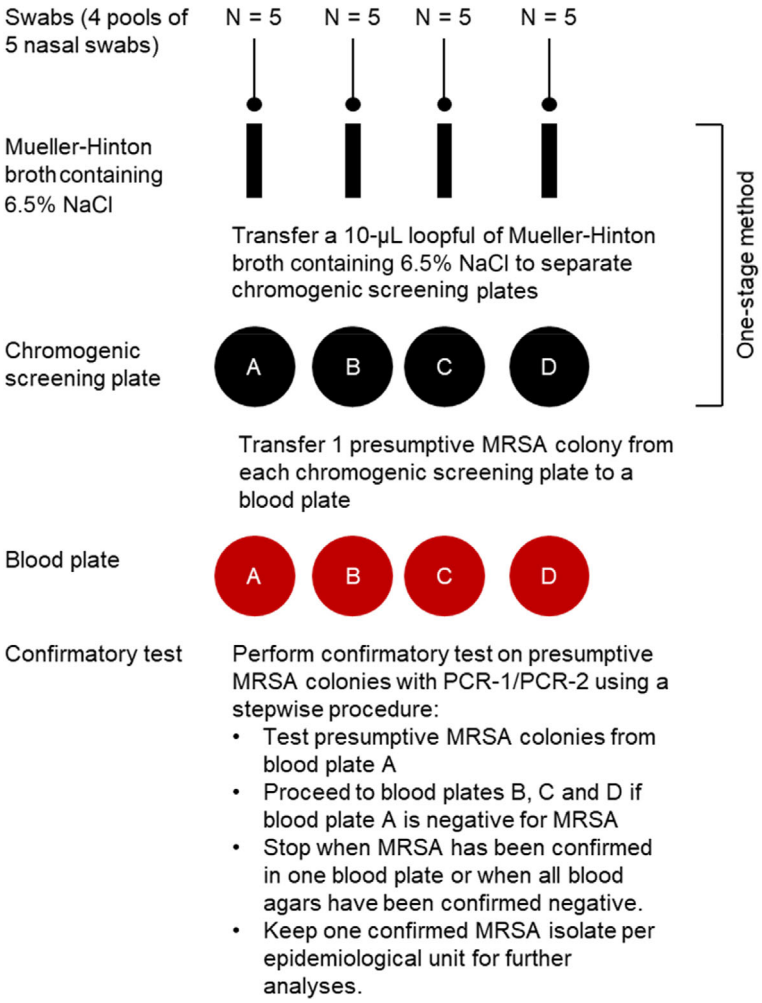


Figure 2: Workflow for isolation of MRSA

6.3. Confirmatory testing and typing of MRSA

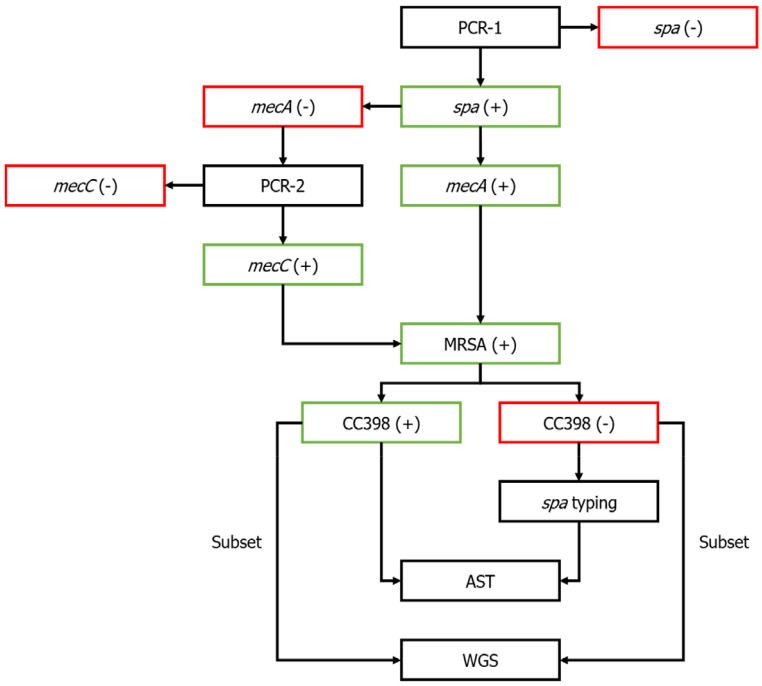
The chromogenic screening plates available may produce to some extent false-positive results, so presumptive MRSA colonies need to be confirmed by molecular testing of MRSA.

The EURL-AR has provided protocols for DNA extraction and PCR-based confirmation of MRSA through detection of the *spa*, *mecA*, *mecC* and *lukF-PV* genes[7] (hereafter referred to as PCR-2) followed by *spa* typing of all isolates.[8] Identification of the *spa* type can be used to assign isolates into clonal complexes (CCs).[9] Isolates for which no CC can be inferred from the *spa* type should be subjected to multi locus sequence typing (MLST).[10] It is expected that the vast majority of MRSA from pigs will be positive for the *mecA* and *spa* genes and belong to CC398.

To limit the costs of performing *spa* typing, presumptive MRSA isolates identified during the BS will be subjected to PCR-1 assay developed at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institute (SSI) in Denmark for surveillance purposes, rather than that currently recommended by the EURL-AR, as it allows simultaneous confirmation of presumptive MRSA colonies and differentiation between *S. aureus* CC398 and *S. aureus* non-CC398 (hereafter referred to as PCR-1). The detailed protocol of PCR-1 is available on the EURL-AR homepage, and the EURL-AR can be contacted for technical support.

[7] https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/636_protocol-for-spa-pvl-meca-mecc-pcr-25-05-2022.pdf
[8] https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/289_7-protocols-for-spa-typing.pdf
[9] *Spa* typing is primarily relevant for assigning *S. aureus* isolates into putative CCs based on previous knowledge on *spa* type/CC associations. As PCR-2 includes a marker for CC398, there is no need to perform *spa* typing on these isolates (see also Section 6.5).
[10] https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/284_8-protocols-for-mlst-typing.pdf

The different analytical steps and corresponding methods are summarised in Figure 3.



AST: antimicrobial susceptibility testing; MRSA: methicillin-resistant *Staphylococcus aureus*; WGS: whole genome sequencing.

Figure 3: Flowchart for confirmatory testing, typing, antimicrobial susceptibility testing and whole-genome sequencing of MRSA

The goal is to determine the number of MRSA-positive epidemiological units, and it is therefore sufficient to identify MRSA in one of the four blood plates from each epidemiological unit. Due to the high specificity of the one-stage method, it is expected that most of the presumptive MRSA isolates growing on the blood plates will be confirmed as MRSA. To further limit the costs, it is therefore recommended to initially perform confirmatory tests on one of the four blood plates containing unique presumptive MRSA isolates. It is consequently only necessary to perform confirmatory tests of the remaining blood plates if the first blood plate is negative for MRSA. The protocol will therefore only lead to the isolation of one MRSA isolate per epidemiological unit and will therefore not be able to detect the occurrence of more than one MRSA variant within each epidemiological unit, but this limitation has been balanced against cost and available resources.

PCR-1 shares the following features with the PCR-2 (Table 6):

- Species identification of *S. aureus* by detection of the *spa* gene. It should be noted that the *spa* gene can also be present in *S. argenteus* and *S. schweitzeri*, but these species can be distinguished by their unique *spa* types.
- Detection of the *mecA* gene, which is the dominant methicillin resistance determinant in MRSA from pigs.
- Detection of the *lukF-PV* gene, a marker of the phage-encoded Panton–Valentine leukocidin (PVL).

In addition, PCR-1 allows (Table 6):

- Detection of the *S. aureus* CC398-specific variant of the *sau1-hsdS1* genes (Stegger et al., 2011). It should be noted that the variant can also be present in *S. aureus* CC88, but this clone is not associated with pigs.
- Detection of the *scn* gene, a marker of the phage-encoded immune evasion cluster 1 (IEC1) associated with human adaptation (Sieber et al., 2020).

Isolates that are positive for the *spa* gene but negative for the *mecA* gene are subjected to PCR-1 in order to determine the presence or absence of the *mecC* gene (Table 6). Isolates that are positive

for the *spa* gene, the *mecA* or *mecC* gene and the *S. aureus* CC398-specific variant of the *sau1-hsdS1* genes are designated as MRSA CC398 and subjected to antimicrobial susceptibility testing (AST). Isolates that are positive for the *spa* gene, the *mecA* or *mecC* gene but negative for the *S. aureus* CC398-specific variant of the *sau1-hsdS1* genes are designated as MRSA and subjected to *spa*-typing and AST. A subset of the MRSA CC398 and all MRSA non-CC398 isolates will be selected based on their genetic diversity and subjected to WGS.

It is proposed that a proportion of 20% of isolates within each clone/spa type detected should be sequenced, with the additional rule that, within each clone, at least 1 isolate and a maximum of 20 isolates should be sequenced. It could also be relevant to sequence isolates that are resistant to certain antimicrobials (linezolid and vancomycin) or carry certain host-adaptive/virulence genes (*lukF*-PVL and *scn*). As the genetic diversity is unknown a priori, it would be desirable to take stock of the diversity after the first quarter of the survey to know more about the number of MRSA CC398 and the number of MRSA non-CC398 of each *spa* types.

Table 6: PCR assays

Gene	Primer name	Primer sequence (5′ to 3′)	Product Size (bp)	PCR-1	PCR-2
spa	*spa*-1113F	TAAAGACGATCCTTCGGTGAGC	200–600	+	+
	spa-1514R	CAGCAGTAGTGCCGTTTGCTT		+	+
mecA	mecA P4	TCCAGATTACAACTTCACCAGG	162	+	+
	mecA P7	CCACTTCATATCTTGTAACG		+	+
*lukF-PV*L	pvl-FP	GCTGGACAAAACTTCTTGGAATAT	85	+	+
	pvl-RP	GATAGGACACCAATAAATTCTGGATTG		+	+
sau1-hsdS1	FP2sau1	GAGAATGATTTTGTTTATAACCCTAG	106	+	–
	CC398r1	CAGTATAAAGAGGTGACATGACCCCT		+	–
scn	scnF1	TACTTGCGGGAACTTTAGCAA	130	+	–
	scnR1	AATTCATTAGCTAACTTTTCGTTTTGA		+	–
mecC	mecALGA251 MultiFP	GAAAAAAAGGCTTAGAACGCCTC	138	–	+
	mecALGA251 MultiRP	GAAGATCTTTTCCGTTTTCAGC		–	+

For those MSs performing it on a voluntary basis, an option could be to perform WGS of all presumptive MRSA isolates from the blood plates, each of which being seeded with a single colony isolated from the chromogenic screening agar for MRSA. It is believed that selection at this stage comes with a low risk of sequencing false-positive isolates. However, the presence/absence of genes that are detected in PCR-1 and PCR-2 should be reported for the sake of harmonisation of data reporting.

6.4. Antimicrobial susceptibility testing

For this BS, it is proposed that MRSA isolates are susceptibility tested using micro-dilution at least to a harmonised panel of antimicrobial substances. AST should be performed according to the EURL-AR protocol. Broth microdilution is the recommended method and that European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/) epidemiological cut-off values (ECOFFs) should be used as interpretative criteria to define microbiological resistance (except where indicated differently). The concentration ranges to be included in the panel of antimicrobials used should ensure that both the ECOFF and the clinical breakpoints (CBPs) are included so that comparability of results with human data is made possible. It is recommended to perform phenotypic AST on all MRSA isolates, including those for which WGS is performed, since WGS may not perform well for all antibiotics (Bortolaia et al., 2020).

The 2019 EFSA technical specifications (EFSA, 2019) reviewed and revised the concentration ranges for testing the susceptibility of MRSA by broth microdilution and described a recommended set (Table 2) of antimicrobials for inclusion in the susceptibility testing panel and suggested concentration ranges to be tested.

The recommended panel includes two antimicrobial compounds used in human medicine which may be last resort compounds for the treatment of MRSA (linezolid and vancomycin). The other antimicrobials have been included based on their therapeutic applications or use in veterinary medicine, as well as because they can provide useful epidemiological information. Subsequently, a commercial microtitre plate

has become available which covers the EFSA recommendation. It is proposed to use the recommended set of substances for the purpose of the BS on MRSA in pigs (Table 7).

Table 7: Core panel of antimicrobial substances, ECOFFs, CBPs and concentration ranges (in mg/L) for *S. aureus*

Antimicrobial	ECOFF 2022	CBP 2022	Suggested range, mg/L	Currently available plate format, mg/L[(a)]
Cefoxitin	> 4	> 4[(b)]	0.5–16	0.5–16
Chloramphenicol	> 16	> 8	4–64	4–64
Ciprofloxacin	> 2	> 1	0.25–8	0.25–8
Clindamycin	> 0.25	> 0.25	0.12–4	0.12–4
Erythromycin	> 1	> 1	0.25–8	0.25–8
Gentamicin	> 2	> 2	0.5–16	0.5–16
Linezolid	> 4	> 4	1–8	1–8
Mupirocin	> 1	NA	0.5–2 + 256	0.5–2 + 256
Quinupristin/Dalfopristin	> 1	> 2	0.5–4	0.5–4
Sulfamethoxazole	> 128	NA	64–512	64–512
Tetracycline	> 1	> 2	0.5–16	0.5–16
Tiamulin	> 2	NA	0.5–4	0.5–4
Trimethoprim	> 2	> 4	1–16	1–16
Vancomycin	> 2	> 2	1–8	1–8

NA: Not available; ECOFF: epidemiological cut-off value; CBP: clinical breakpoint.
(a): The currently commercially available plate also includes Fusidate (0.25–4), Kanamycin (4–32), Penicillin (0.06–1), Rifampin (0.015–0.5) and Streptomycin (4–32).
(b): Not given as a clinical breakpoint by EUCAST, but rather stated that *S. aureus* with cefoxitin MIC values > 4 are methicillin resistant.

6.5. Whole-genome sequencing

WGS will be performed on a subset of the MRSA CC398 and all MRSA non-CC398 isolates, representing the geographic and genetic diversity within each country. The primary objective is to perform strain typing and to search for genetic determinants involved in AMR, host adaptation and virulence. The secondary objective is to infer the population structure and dynamics of the dominant MRSA clones. Previously, *spa* typing has revealed geographic clustering among MRSA CC398 isolates in Europe (EFSA, 2009 and 2010), but the small number of common *spa* types within CC398 and the potential for homoplasy within the *spa* gene limits its phylogenetic utility (Nübel et al., 2008).

The EURL-AR has provided protocols for DNA extraction, DNA quality and quantity assessment, library preparation, library quality and quantity assessment, WGS and assembly.[11] The protocols to be used specifically in the BS will be proposed by the EURL-AR.

Quality assurance should address a number of mandatory quality checks[12] (Table 8).

Table 8: Mandatory quality checks

Measure	Description
Mean read length	Mean value of the number of base pairs sequenced from a DNA fragment. This element contains integer.
Q30 rate	The percentage of bases with a quality score[(a)] of 30 or higher across the whole read length. This element contains decimal.
Total bases	Absolute number of bases sequenced. This value should be calculated after trimming. This element contains integer.
Assembly coverage	Average of number of nucleotide bases sequence aligned to a specific locus in a reference genome. This should be calculated after mapping the sequencing raw reads against the assembly. This element contains decimal.

[11] https://www.eurl-ar.eu/CustomerData/Files/Folders/34-wgs/628_protocol-for-wgs-v2-2.pdf
[12] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2022.EN-7413

Measure	Description
Assembly N50	The sequence length of the shortest contig at 50% of the total genome length. This element contains integer.
Assembly total length	Absolute number of bases in the assembly. This element contains integer.
Assembly Number of contigs	Number of contigs of the assembly. Contigs are continuous stretches of sequence containing bases without gaps. This element contains integer.

(a): A quality score is an estimate of the probability of a base being called wrongly by the sequencer and it is calculated as: $q = -10 \times \log_{10}(p)$.

In addition, species validation and check for contamination should be performed to verify that the FASTQ files contain sequences from a single species and that the species is equal to the one indicated by the user. Assigned reads to an unexpected organism should be less than 5%.

The bioinformatics tools and settings used to obtain mandatory quality check measures and perform species validation and check for contamination, and the accompanying results, should be provided.

6.6. Bioinformatics analysis

Bioinformatics analyses include determining a number of mandatory genotypic characteristics such as multi-locus sequence typing (MLST), staphylococcus cassette chromosome *mec* (SCC*mec*) typing, detection of AMR genes and point mutations and detection of other genes associated with host adaptation and virulence (Table 9).

Table 9: Mandatory genotypic characteristics

Measure	Description	Tool[(a)]/database[(b)]	Settings[(c)]
MLST	–	MLST	
mec class	–	SCCmecFinder	%ID: 90% Min. length: 60% Database: Referenced
ccr type(s)	–		
SCC*mec* type	–		
SCC*mec* subtype	–		
AMR genes	–	ResFinder 4.1 or newer	%ID: 90% Min. length: 60%
AMR point mutations	–		
czrC	Cadmium and zinc resistance	MyDbFinder	%ID: 90% Min. length: 60%
scn	Staphylococcal complement inhibitor (SCIN)		
chp	Chemotaxis inhibitory protein of S. aureus (CHIPS)		
sak	Staphylokinase (SAK)		
sea	Staphylococcal enterotoxin A (SEA)		
sep	Staphylococcal enterotoxin P (SEP)		
IEC1	Prophage-borne immune evasion cluster encoding SCIN (genetic marker of IEC1) and different combinations of CHIPS, SAK, SEA and SEP (genetic marker of human adaptation)		
IEC1 type	A schematic representation of the different IEC1 types are provided by van Wamel et al. (2006)		
tarP	Prophage-encoded wall teichoic acid glycosyltransferase (TarP)		

Measure	Description	Tool[(a)]/database[(b)]	Settings[(c)]
vwb$_{SaPI}$	Staphylococcal pathogenicity island (SaPI)-encoded von Willebrand factor-binding protein (genetic marker of ruminant adaptation		
lukS-PV	Prophage-encoded Panton–Valentine leukocidin (PVL)		
lukF-PV	Prophage-encoded Panton–Valentine leukocidin (PVL)		
seb	Staphylococcal enterotoxin B (SEB)		
sec	Staphylococcal enterotoxin C (SEC)		
tsst	Staphylococcal pathogenicity island (SaPI)-encoded toxic shock syndrome toxin 1 (TSST-1)		

(a): Tools recommended by the EURL-AR are available from the CGE website (https://cge.cbs.dtu.dk/services/).
(b): Links to tools and predefined databases will be made available on the EURL-AR homepage.
(c): To ensure harmonised monitoring, it is important to use predefined databases and settings.

The bioinformatics tools, settings and the type of sequence used to obtain mandatory genotypic characteristics, and the accompanying results, should be provided.

6.7. Storage of strains

Isolates verified as MRSA should be saved and stored under conditions not allowing changes in their properties at −80°C at least for 5 years. This is to allow, for instance, later testing for antimicrobial susceptibility or other types of characterisations if requested by the EFSA or for research or other purposes at a national or European level. Isolates sent to the EURL-AR will also be stored for a minimum of 5 years.

7. Data reporting from the EU MSs/participating countries to EFSA

7.1. Overall description on the implementation of the survey

Three sets of data will be reported to cover all information collected during sampling and analysis:

- Prevalence sample-based: Detailed analytical results of all samples taken (positive and negative) reported using the EFSA standard for reporting laboratory results: Standard Sample Description version 2 (SSD2).
- AMR isolate-based: Isolate-level quantitative antimicrobial resistance and WGS data reported according to the antimicrobial resistance (AMR) data model of EFSA.
- Population data: Annual throughput of slaughterhouses involved in the survey and pig population size of the farms of origin.

The laboratory isolate code will be used to link the data reported in the prevalence sample-based (SSD2) data model with those reported in the AMR isolate-based data model. The slaughterhouse identification code and the holding identification code will be used to link the analytical results of sampled to population data.

Potential slight alterations to the data reporting model may still occur while implementing it at EFSA and they will be communicated to the reporting countries considering the appropriate time for implementing them.

7.2. Data to be reported

7.2.1. Prevalence sample-based

The EU MSs/participating countries can use the SSD2 data model to report sample-based zoonoses and zoonotic agent data to the Data Collection Framework (DCF) of EFSA. Specific guidance to report

information under the framework of Directive 2003/99/EC, Regulation (EU) 2017/625, Commission Implementing Regulation (EU) 2019/627 and of Commission Delegated Regulation (EU) 2018/772 is published annually by EFSA (EFSA, 2022a). Analytical results for MRSA samples can already be reported to the DCF following the instructions provided there. However, some additional information will be requested in the context of the BS (Table 10).

The full list of data elements relevant for the MRSA BS is presented below together with the default (fixed) value to be used in the context of the BS, where applicable. The detailed description of all the data elements can be found in the latest prevalence sample-based guidance (EFSA, 2022a).

The sampling unit for the BS is the slaughter batch, and therefore, all elements at the sampling unit level (e.g. sampEventId) refer to a slaughter batch sampled at a given time in a given slaughterhouse. The data will be reported at the level of the individual analytical results, e.g. if one pooled sample undergoes only one test for screening in the laboratory, one line will be reported for this sample, but if the screening test is positive and the samples undergo further testing, one additional line will be reported for each additional test. As a result, several lines will be reported per slaughter batch (at least one line per pooled sample) and possibly several lines will be reported per pooled sample. In this context, each analytical results can be reported with the exact date on which it was performed.[13]

[13] For example, if the first isolation test of presumptive MRSA is performed on 19 September 2023 and the first PCR is performed on 23 September 2023, two separate lines will be reported with the two different analysis dates.

Table 10: SSD2 data model for reporting data on prevalence of zoonotic agents, adapted for the needs of the MRSA BS

Data element	Description	Constraint	Data type	Catalogue/hierarchy	Default value
sampStrategy	Underlying strategy of selecting samples from the population	Mandatory	xs:string (5)	SAMPSTR/zooSampstr	ST10A (Objective sampling)
progType	Type of programme	Mandatory	xs:string (5)	PRGTYP/zooSampContext	K027A (EU baseline survey)
sampMethod	Sampling method	Mandatory	xs:string (5)	SAMPMD	N002A (Pooled/batch)
sampler	Type of organisation performing the sampling	Mandatory	xs:string (5)	SAMPLR	CX02A (Official sampling)
sampPoint	Point in the food chain where the sample was taken	Mandatory	xs:string (5)	SAMPNT/zooss	E311A (Slaughterhouse)
sampEventId	Sampling event identification code, in this case referring to the identification code of the sampled slaughter batch	Mandatory	xs:string (100)	–	–
sampUnitType	Sampling unit	Mandatory	xs:string (5)	SAMPUNTYP	G200A (Slaughter batch)
sampUnitSize	Size of the sampling unit, i.e. in this case the number of animals of the sampled slaughter batch	Mandatory	xs:double	–	–
sampUnitSizeUnit	Unit of measurement of the size of the sampling unit	Mandatory	xs:string (5)	UNIT	G199A (Animal)
sampUnitIds.batchId	Slaughter batch identification code. For the data reported in the context of the BS, the sampling unit is always the batch and, therefore, for each record, the value reported here should be equal to the value reported in sampEventId.	Mandatory	xs:string (250)	–	–
sampUnitIds.slaughterHouseId	Slaughterhouse identification code	Mandatory	xs:string (250)	–	–
sampUnitIds.sampHoldingId	Sampling holding identification code	Optional	xs:string (250)	–	–
sampEventInfo.durationTransport	Duration of transportation before arrival of the slaughter batch to the slaughterhouse	Optional	xs:double	–	–
sampEventInfo.durationLairage	Duration of lairage	Optional	xs:double	–	–
sampEventInfo.washingLairage	Misting of the sampled slaughter batch during lairage	Optional	xs:string (1)	YESNO	–

Data element	Description	Constraint	Data type	Catalogue/hierarchy	Default value
sampEventInfo.stunningMethod	Method of stunning of the sampled slaughter batch	Optional	xs:string	Potential values: electrical, carbon dioxide, other	–
sampEventInfo.prevSlaughteredBatches	Number of batches slaughtered on the same day before the sampled slaughter batch	Optional	xs:integer	–	–
sampId	Identification code of the collected sample	Mandatory	xs:string (100)	–	–
repCountry	Reporting country	Mandatory	xs:string (2)	COUNTRY/ EUSRrepCountry	–
sampCountry	Country of sampling	Mandatory	xs:string (2)	COUNTRY	–
sampArea	Area of sampling	Optional	xs:string (5)	Nuts Gaul/ nuts2021	–
repYear	Reporting year (i.e. year for which the data are reported)	Mandatory	xs:integer (4)	–	2023
sampY	Year of sampling	Mandatory	xs:integer (4)	–	2023
sampM	Month of sampling	Mandatory	xs:integer (2)	–	–
sampD	Day of sampling	Mandatory	xs:integer (2)	–	–
sampSize	Size of sample taken, i.e. in this case, the number of swabs taken from different animals and pooled together in the same pooled sample	Mandatory	xs:double	–	–
sampSizeUnit	Sample taken size unit	Mandatory	xs:string (5)	UNIT	G199A (Animal)
sampMatType	Type of matrix	Mandatory	xs:string (5)	MTXTYP	S000A (Animal sample)
sampMatCode.base	Matrix of the sample taken	Mandatory	xs:string (5)	MTX	A0C9Y (Fattening pigs)
sampMatCode.part	Matrix part	Mandatory	xs:string (5)	MTX	A16HC (Nasal swab)
sampMatCode.prod	Organic or conventional production	Optional	xs:string (5)	MTX	–
origCountry	Country of origin of the sample taken	Mandatory	xs:string (2)	COUNTRY	–
origArea	Area of origin of the sample taken	Optional	xs:string (5)	Nuts Gaul/ nuts2021	–
analysisY	Year of the individual analysis performed in the laboratory	Mandatory	xs:integer (4)	–	–
analysisM	Month of the individual analysis performed in the laboratory	Mandatory	xs:integer (2)	–	–

Data element	Description	Constraint	Data type	Catalogue/hierarchy	Default value
analysisD	Day of the individual analysis performed in the laboratory	Mandatory	xs:integer (2)	–	–
anPortSeq	The sequence of the portion of the sample analysed. Different portions of the same analysed with different methods sampled can be differentiated using a sequence of numbers (e.g. 1, 2, 3, etc.).[(a)]	Mandatory	xs:string (100)	–	–
labId	Laboratory identification code	Mandatory	xs:string (50)	–	–
paramType	Type of parameter	Mandatory	xs:string (5)	PARAMTYP	P001A (Individual)
paramCode, base	Coded description of the parameter	Mandatory	xs:string (15)	PARAM	RF-00003853-MCG (MRSA)
paramCode.t	*spa*-type	Dependent	xs:integer	–	–
paramCode.st	Multi locus sequence	Dependent	xs:integer	–	–
paramCode.cc	Clonal complex	Dependent	xs:integer	–	–
paramCode.genes	Reporting the genes identified by the PCR tests	Dependent	xs:string	–	–
anMethRefCode	Analytical method reference code	Mandatory	xs:string (5)	ANLYREFMD	R049A (EURL method)
anMethType	Type of analytical method type	Mandatory	xs:string (5)	ANLYTYP	AT01A (Detection)
anMethCode	Analytical method	Mandatory	xs:string (5)	ANLYMD	–
resId	Unique identification code of the analytical result	Mandatory	xs:string (100)	–	–
resQualValue	Result qualitative value	Mandatory	xs:string (3)	POSNEG	–
resType	Type of result	Mandatory	xs:string (3)	VALTYP	BIN
isolId	Identification code of the isolate	Dependent	xs:string (100)	–	–
evalLimitType	Type of limit for the result evaluation	Mandatory	xs:string (5)	LMTTYP	W012A (Presence)
evalCode	Evaluation of the result	Mandatory	xs:string (5)	RESEVAL	–
evalInfo.sampTkAsses	Sample taken assessment, containing the final assessment of the sample, i.e. 'Satisfactory' if no MRSA was detected in the pooled sampled or 'Unsatisfactory' if MRSA was detected in the pooled sample.	Mandatory	xs:string (5)	RESEVAL	–

Data element	Description	Constraint	Data type	Catalogue/hierarchy	Default value
evalInfo.sampEventAsses	Sampling event assessment, containing the final assessment at the level of the sampling unit, which in this case is the slaughter batch, i.e. 'Satisfactory' if no MRSA was detected in any pooled sample of the slaughter batch or 'Unsatisfactory' if MRSA was detected in at least one pooled sample of the slaughter batch.	Mandatory	xs:string (5)	RESEVAL	–

(a): For a positive sample, anPortSeq should have different values for the first isolation test of presumptive MRSA and for all the subsequent tests. For example, the anPortSeq for the screening test can be equal to 1 and for the first PCR can be equal to 2. This data element is required in such cases for technical reason and EFSA is investigating the possibility of generating its values automatically.

7.2.2. AMR isolate-based data

MSs can use the AMR data model to report isolate-level quantitative antimicrobial resistance and WGS data to the DCF of EFSA. Guidance to report such data under the framework of Directive 2003/99/EC and Commission Implementing Decision 2020/1729/EC is published annually (EFSA, 2021). Isolate-level quantitative antimicrobial resistance results for MRSA samples can already be reported to the DCF following the instructions provided there. In the context of the BS, the data model will be extended to receive WGS results of MRSA isolates.

The full list of data elements is presented below together with the default (fixed) value to be used in the context of the BS, where applicable (Table 11). The detailed description of all the data elements can be found in the latest guidance (EFSA, 2021).

Table 11: EFSA data model for isolate-based antimicrobial resistance data reporting

Data element	Description	Constraint	Data type	Catalogue/ hierarchy	Default value
resultCode	Unique result identifier	Mandatory	xs:string (100)	–	–
repYear	Reporting year	Mandatory	xs:integer (4)	–	–
repCountry	Reporting country	Mandatory	xs:string(2)	COUNTRY	–
zoonosis	Zoonotic agent	Mandatory	xs:string (4000)	PARAM/serovaramr	MRSA + Info about MLST, CC, spa
zoonosis.t	Spa type	Dependent	xs:integer		
zoonosis.st	Multi locus sequence	Dependent	xs:integer		
zoonosis.cc	Clonal complex	Dependent	xs:integer		
matrix	Matrix from which the tested isolate derives	Mandatory	xs:string (4000)	ZOO_CAT_MATRIX	A006701A (Fattening pigs)
totUnitsTested	Total number of epidemiological units of interest (i.e. in this case slaughter batch) investigated in the context of the BS, i.e. it should be the total number of batches sampled for the BS.	Mandatory	xs:integer (10)	–	–
totUnitsPositive	Total number of epidemiological units of interest (i.e. in this case slaughter batch) investigated in the context of the BS and tested positive for MRSA.	Mandatory	xs:integer (10)	–	–
sampUnitType	Type of sampling unit	Mandatory	xs:string(5)	UNIT	G200A (Slaughter batch)
sampStage	Stage along the food chain at which the sample has been collected	Mandatory	xs:string(5)	SAMPNT	E311A (Slaughterhouse)
sampOrig	Country of origin of the sample	Mandatory	xs:string(2)	COUNTRY	–
sampType	Matrix part	Mandatory	xs:string(5)	ZOO_CAT_SMPTYP	S015A (nasal swab)

Data element	Description	Constraint	Data type	Catalogue/ hierarchy	Default value
sampContext	Type of programme in the framework of which the sample was collected	Mandatory	xs:string(5)	PRGTYP	K027A (EU BS)
sampler	Type of organisation performing the sampling	Mandatory	xs:string(5)	SMPLR	CX02A (Official sampling)
progCode	Basis of the programme in the framework of which the sample/isolate has been collected/analysed	Mandatory	xs:string(7)	AMRPROG	AMR MON
progSampStrategy	Underlying strategy of selecting samples from the population	Mandatory	xs:string(5)	SAMPSTR	ST10A (Objective sampling)
labCode	Laboratory identification code	Mandatory	xs:string (100)	–	–
labIsolCode	Identification code of the isolate	Mandatory	xs:string (20)	–	–
sampY	Year of sampling	Mandatory	xs:integer (4)	–	–
sampM	Month of sampling	Mandatory	xs:integer (2)	–	–
sampD	Day of sampling	Mandatory	xs:integer (2)	–	–
isolY	Year of isolation	Mandatory	xs:integer (4)	–	–
isolM	Month of isolation	Mandatory	xs:integer (2)	–	–
isolD	Day of isolation	Mandatory	xs:integer (2)	–	–
analysisY	Year of susceptibility test	Dependent	xs:integer (4)	–	–
analysisM	Month of susceptibility test	Dependent	xs:integer (2)	–	–
analysisD	Day of susceptibility test	Dependent	xs:integer (2)	–	–
seqY	Year of sequencing	Dependent	xs:integer (4)	–	–
seqM	Month of sequencing	Dependent	xs:integer (2)	–	–
seqD	Day of sequencing	Dependent	xs:integer (2)	–	–
anMethCode	Method used for AMR testing	Mandatory	xs:string(5)	ANLYMD/amram	–
substance	Antimicrobial substance against which the isolate was tested	Dependent	xs:string (15)	PARAM/AMRsub	–
cutoffValue	Cut-off value for the dilution method	Dependent	xs:double		–

Data element	Description	Constraint	Data type	Catalogue/ hierarchy	Default value
lowest	The lowest concentration of the concentration range used to test AMR in the laboratory	Dependent	xs:string(5)	ZOO_CAT_FIXMEAS/ number	–
highest	The highest concentration of the concentration range used to test AMR in the laboratory	Dependent	xs:string(5)	ZOO_CAT_FIXMEAS/ number	–
MIC	The MIC value (by default reported in mg/L) resulting from the susceptibility testing of the isolate in question	Dependent	xs:string(5)	ZOO_CAT_FIXMEAS/ mic	–
Genotype	Reporting the genes identified by the WGS tests included in Table 5	Dependent	xs:string (100)	PARAM	–
perCC	Performed CC MRSA characterisation	Mandatory	xs:string(1)	YESNO	–
perMLST	Performed MLST MRSA characterisation	Mandatory	xs:string(1)	YESNO	–
seqTech	Instrument used for sequencing	Dependent	xs:string(8)	INSTRUM	–

7.2.3. Population data

To improve the analysis that will be performed with the BS data, MSs are requested to provide information about the pig population in the involved slaughterhouses and in the farms of origin of the sampled slaughter batches. In particular, they should report the annual throughput of each slaughterhouse and the number of pigs in each farm of origin following the data model in the table below. This data model described below (Table 12) is a simplified version of a data model developed by EFSA to report animal population at the establishment level (EFSA, 2022b).

Table 12: Data model to report population of animals in the farm of origin and annual slaughterhouse throughput

Data element	Description	Constraint	Data type	Controlled terminology
recordId	Unique identifier of the record	Mandatory	xs:string (100)	–
recordCensusY	Year of extraction of census data	Mandatory	xs: integer (4)	–
recordCensusM	Month of extraction of census data	Mandatory	xs: integer (2)	–
recordCensusD	Day of extraction of census data	Mandatory	xs: integer (2)	–
estabId	Identification code of the slaughterhouse or the farm. The 'establishment' describes any	Mandatory	xs:string (200)	–

Data element	Description	Constraint	Data type	Controlled terminology
	premises, structure, or, in the case of open-air farming, any environment or place, where animals or germinal products are kept, on a temporary or permanent basis, except for households (where pet animals are kept) and veterinary practices or clinics. This information is protected by the EU General Data Protection Regulation (EU GDPR).			
estabType	Type of establishment	Mandatory	xs:string (5)	E311A (Slaughterhouse) OR E101A (Farm)
estabArea	Area of the establishment	Optional	xs:string (5)	Nuts Gaul
subUnitId	Identification code of the subunit of the slaughterhouse or the farm (or repeating estabId). The 'subunit' is the smallest management group of animals of the same species, sharing the same geographical location and the same rearing purpose in the context of a given establishment. This information is protected by the EU General Data Protection Regulation (EU GDPR).	Mandatory	xs:string (200)	–
subUnitSpecies	Subunit species	Mandatory	xs:string (5)	A0C9Y (Fattening pigs)
subUnitPurpType	Type of farm (e.g. farrow-to-finish, weaner-to-finish, finisher)	Mandatory for farms, not applicable for slaughterhouses	xs:string (5)	MTX
subUnitCapacity	Theoretical capacity of the farm. It refers to the number of registered fattening places. The information is mandatory for the farms and not applicable for the slaughterhouses.	Mandatory for farms, not applicable for slaughterhouses	xs: integer (9)	–
subUnitActualNumber	Actual number of animals in each subunit. In the case of farms, it refers to the number of animals at a given point in time, i.e. a snapshot of the pig population at the farm level close to the time of sampling. In the case of slaughterhouses, it refers to the annual throughput expressed in tonnes of carcase equivalent during the calendar year.	Mandatory	xs: integer (9)	–
subUnitArea	Area of the subunit	Optional	xs:string (5)	Nuts Gaul

8. Analytical approach to data

8.1. Data validation

The submitted data will be checked to ensure that the mandatory elements are completed, the correct data type is used and for compliance with the controlled terminologies. A set of data validation criteria will be set up to identify and exclude non-valid, non-consistent and non-plausible data in the MRSA data set submitted by the MSs to EFSA. The MSs will be given the opportunity to consider and correct data not fulfilling the data validation criteria. A report listing the identified errors will be sent to the data providers with advice on required modifications. The data providers must resubmit the data until the data set passes the validation checks and is considered as valid. The validated data sets at the level of batches of slaughtered pigs will be used to perform the phylogenetic analyses and assess MRSA prevalence.

8.2. Sample summary statistics and protocol-sample comparison

Descriptive analysis of all data collected will be carried out, considering simple statistics, such as proportions, means, standard deviations, frequency tables and graphs in order to provide a general overview of the data submitted. A comparison between the BS protocol and the collected sample, notably in terms of sample size, stratification by slaughterhouse, stratification by month and time elapsed between sampling and testing, will be carried out using frequency tables and graphs.

8.3. Assessment of the observed prevalence of MRSA

A batch of slaughtered pigs will be considered positive if MRSA is detected in any of the four pooled samples of five nasal swabs, and negative otherwise. The prevalence of certain lineages of MRSA of particular public health interest may be specifically assessed.

The prevalence of batches of slaughtered pigs will be estimated for each MS through the slaughter batch positivity ratio (proportion of tested positive slaughter batches out of the total number of slaughter batches tested). Within each MS, the slaughterhouses will be considered as strata, for which the proportion of sampled batches of slaughtered pigs is not constant across slaughterhouses. The MRSA prevalence will be assessed by accounting for unequal sampling fractions among the slaughterhouses. At the EU level, the prevalence is going to be assessed while considering MSs as strata and accounting for unequal sampling fractions among MSs. Point estimates of the observed MRSA prevalence at the MS and EU level will be complemented with 95% confidence interval estimates.

Factors considered to be potentially related to the sensitivity positivity of the testing slaughtered batches and to have a potential impact on the probability of the detection of MRSA isolates in samples will be accounted for in the prevalence analysis investigated. To investigate such potential impact, logistic models predicting batch positivity as a function of country and those factors will be fitted. In the case of impact, the estimated prevalence figures will be adjusted by further logistic regression analyses.

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Abbreviations

AMR	antimicrobial resistance
AST	antimicrobial Susceptibility Testing
BS	baseline surveys
BSe	batch sensitivity
CBP	clinical breakpoint
CC	clonal complex
dCF	data collection framework
ECOFF	epidemiological cut-off value
EURL-AR	EU Reference Laboratory for Antimicrobial Resistance
MIC	minimum inhibitory concentration
MLST	multi locus sequence typing
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	EU Member States
PCR	polymerase chain reaction
SH	slaughterhouse
SSD2	standard sample description version 2
SSI	statens Serum Institute
TSe	test sensitivity
TSp	test specificity
WGS	whole genome sequencing

Annex A – Protocol for designing technical specifications for a baseline survey on MRSA in pigs

Annex A can be found in the online version of this output ('Supporting information' section of the online version of this scientific report).