SCIENTIFIC REPORT OF EFSA

Report
on the availability of molecular typing methods for *Salmonella, Campylobacter,* verotoxigenic *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and feedingstuffs in European Union Member States (and in some other reporting countries)

Prepared by the Unit on Zoonoses Data Collection

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Executive summary

The European Food Safety Authority carried out a questionnaire survey, in collaboration with the European Commission and the relevant Community Reference Laboratories, to make an inventory on the availability of molecular typing methods for the main food-borne pathogens in animals, food and feedingstuffs in the European Union's Member States. Also some other European countries participated in the survey. The questionnaire covered *Salmonella*, thermophilic *Campylobacter*, verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus.*

The questionnaire was distributed to all Member States and additionally to five non-Member States. In total, 26 Member States and four non-Member States submitted data, which resulted in an overall participation rate of 96.3% for Member States and 80% for non-Member States.

Out of the 26 Member States and four non-Member States replying, 20 Member States and three non-Member States reported performing molecular typing for *Salmonella*, 19 Member States and three non-Member States for thermophilic *Campylobacter*, 20 Member States and two non-Member States for verotoxigenic *Escherichia coli*, 15 Member States and three non-Member States for *Listeria monocytogenes* and 19 Member States and three non-Member States for *Staphylococcus aureus.*

The method most frequently used by Member States was Pulsed Field Gel Electrophoresis (PFGE) used by 20 Member States, followed by Multi Locus Variable-Number Tandem Repeat Analyses (MLVA), Multi Locus Sequence Typing (MLST) and Spa typing. The methods used varied between the pathogens. The isolates typed were mainly from animals and food and derived from official controls, outbreak investigations and research. Most Member States reported of carrying out the molecular typing occasionally.

For each pathogen in several Member States and other reporting countries at least one food/veterinary laboratory performs molecular typing of isolates from animals, food or feed. The National Reference Laboratory of each pathogen is involved in the molecular typing of isolates from animals, food or feed in most Member States.

Four Member States reported purchasing molecular typing analyses of food, feed or animals isolates from laboratories of another Member State/country.

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1. Introduction

The Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents lays down the Community system for the monitoring and collection of information on zoonoses, which obliges Member States (MSs) to collect relevant and, where applicable, comparable data of zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks.

In this context, the possibility of collecting data on molecular typing of the most relevant zoonotic agents has been addressed. In order to facilitate further discussion of this subject, it was agreed between the European Food Safety Authority (EFSA) and the European Commission (EC), in consultation with EFSA's Task Force on Zoonoses Data Collection, to carry out a questionnaire survey on the availability of the molecular typing methods for the main food-borne pathogens in animals, food and feed in MSs. The relevant Community Reference Laboratories (CRLs) were consulted in the preparation of the survey questionnaire.

2. Objectives

The objective of the questionnaire survey was to collect information from MSs and other reporting countries in order to make an inventory on the availability of molecular typing methods for *Salmonella*, verotoxigenic *Escherichia coli* (VTEC), thermophilic *Campylobacter*, *Listeria monocytogene*s and *Staphylococcus aureus* isolates from animals, food and feedingstuffs.

In addition, information was also gathered on the frequency of the typing carried out, origin of the isolates, the methods used and the laboratories involved in the typing.

The EC may wish to utilise the results when considering whether the collection of molecular typing data would be feasible at the Community level.

3. Definitions

Amplified fragment length polymorphism analysis (AFLP) is a genetic mapping technique that uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprint for a particular genome. The power of AFLP analysis derives from its ability to quickly generate large numbers of marker fragments for any organism, without prior knowledge of the genomic sequence.

Community Reference Laboratories (CRLs) are laboratories with scientific and technical expertise within the areas of animal health, public health and zootechnics, designated in different Community Decisions, Directives and Regulations. The Council Directives contain provisions that specify the functions and duties of each designated Community Reference Laboratory. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, lays down the general tasks, duties and requirements for CRLs for food and feed and for animal health.

DNA microarray consists of a small solid support (a membrane, a silicon chip or a glass slide) onto which sequences of DNA are fixed in an orderly display. It is also known as a DNA chip.

Molecular typing methods are based on the microbial genomic analysis and allow improvement of differentiation at subspecies level making possible molecular source tracking in epidemiological investigation.

Multi Locus Sequence Typing (MLST) is an unambiguous procedure for characterising isolates of bacterial species using the sequences of internal fragments of seven house-keeping genes.

Multi Locus Variable-Number Tandem Repeat Analyses (MLVA) is a method that takes advantage of the polymorphism of DNA sequences repeated in tandem used for the genetic analysis of particular microorganisms. In a MLVA assay, a number of selected and characterised loci are amplified by a Polymerase Chain Reaction enabling the size of each locus to be measured. From this measurement, the number of repeat units at each locus can be obtained. This information is a code which can then be compared to reference databases.

National Reference Laboratories (NRLs) are laboratories with scientific and technical expertise within the areas of animal health, public health and zootechnics at national level designated in different national laws.

Polymerase Chain Reaction (PCR) is a technique used to replicate segments of DNA by repeatedly splitting the DNA strands and duplicating them with a DNA polymerase enzyme.

Pulsed Field Gel Electrophoresis (PFGE) is a technique that allows for the separation of large fragments of DNA (> 50 kb), by rapid alternation of electrophoretic migration in agarose gels.

Restriction Fragment Length Polymorphism (RFLP) is a variation in the DNA sequence of a genome that can be detected by fragmenting DNA with restriction enzymes followed by the analysis of the size of the resulting fragments by gel electrophoresis**.**

Ribotyping is a technique involving the fingerprinting of genomic DNA restriction fragments containing all or part of the gene coding for the 16S and 23S rRNA.

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing. The staphylococcal cassette chromosome (SCC) is a gene cassette widely disseminated in *Staphylococci*. The staphylococcal cassette chromosome *mec* (SCC*mec*) is the most representative SCC encoding for methicillin-resistance. SCC*mec* typing, can be used to classify SCC*mec* elements based on their structural differences.

Spa typing is a technique consisting of the sequencing of the polymorphic X region of the protein A gene.

4. Materials and methods

4.1 Questionnaire

The questionnaire was prepared by EFSA, in collaboration with the CRLs on *Salmonella*, VTEC, *Campylobacter*, *Listeria monocytogenes* and *Staphylococcus aureus*. The questionnaire was sent to the members and observers of the Task Force on Zoonoses Data Collection on 25 September 2008 with a recommendation that they would consult their National Reference Laboratories (NRLs) for the agents in question. The replies from the countries were received by 31 October 2008.

The questions covered the availability of the molecular typing methods for isolates from food, animals and feed, the frequency of the typing, the typing methods used and the laboratories performing the analyses.

4.2 Analysis of the results

The replies to the questionnaire were analysed by EFSA. The data collected was entered in a MS Excel database. Bar graphs and frequency tables were used to summarise the results of the survey.

5. Results

In total, 26 MSs (all except Malta) replied to the questionnaire. In addition, Norway, Switzerland, the Former Yugoslav Republic of Macedonia and Turkey (hereafter called non-MSs) sent their replies. This resulted in an overall participation rate of 96.3% of MSs and 80% of non-MSs represented in the Task Force on Zoonoses Data Collection.

The completeness of data was generally very high (about 100%), except for information on technical protocols, which varied in relation to the pathogens and to the analytical methods.

Together 22 MSs and three non-MSs reported that they carry out molecular typing of the bacteria covered by the survey as regards isolates from food, animals or feed. Four MSs (BG, EE, GR and PT) and one non-MS (MK) reported that they did not carry out molecular typing for any of the agents selected for this report. However, Estonia informed that they purchase these analyses from other countries. The results are presented per bacterium in the following.

5.1 *Salmonella*

Twenty MSs and three non-MSs stated that they currently perform molecular typing for *Salmonella* isolates from food, animals or feed with different frequencies. Five MSs and one non-MS reported that they neither perform or purchase molecular typing of *Salmonella* isolates (Table 1).

Typing of animal isolates is carried out by 19 MSs, of which 16 do it occasionally and three on a routine basis. The three non-MSs carry out the typing only occasionally (Figure 1).

Together 18 MSs and three non-MS perform typing of food isolates. This typing is done occasionally by 16 MSs and three non-MSs, whereas two MSs do so on a routine basis (Figure 1).

Fewer countries reported typing of feed isolates. In total, 14 MSs and two non-MSs carry out this kind of typing. Only one MS does so on a routine basis, and the rest of the MSs and non-MSs do so occasionally (Figure 1).

Table 1. Countries performing molecular typing of *Salmonella* isolates

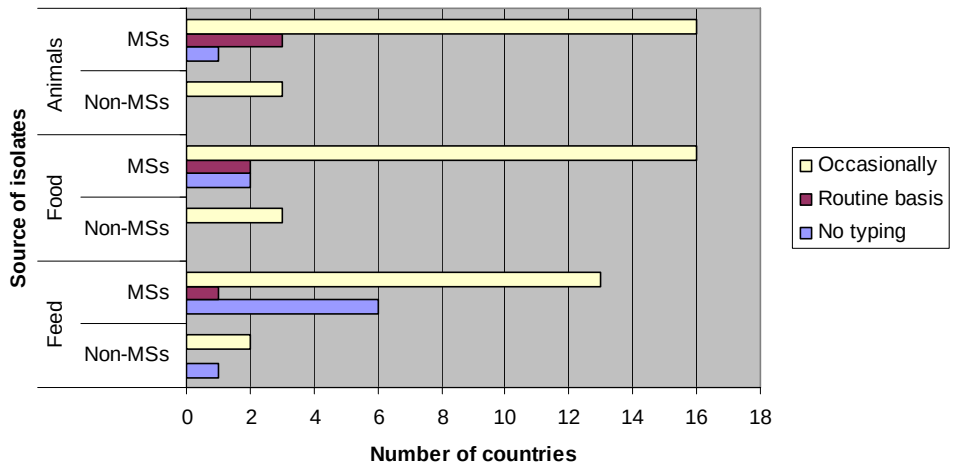
MSs	
AT	Yes
BE	Yes
BG	No
CY	Yes
CZ	No
DE	Yes
DK	Yes
EE	No*
ES	Yes
FI	Yes
FR	Yes
GR	No
HU	Yes
IE	Yes
IT	Yes
LT	Yes
LU	Yes
LV	No
NL	Yes
PL	Yes
PT	No
RO	Yes
SE	Yes
SI	Yes
SK	Yes
UK	Yes

Non-MSs	
CH	Yes
NO	Yes
MK	No
TR	Yes

* EE buys analyses abroad

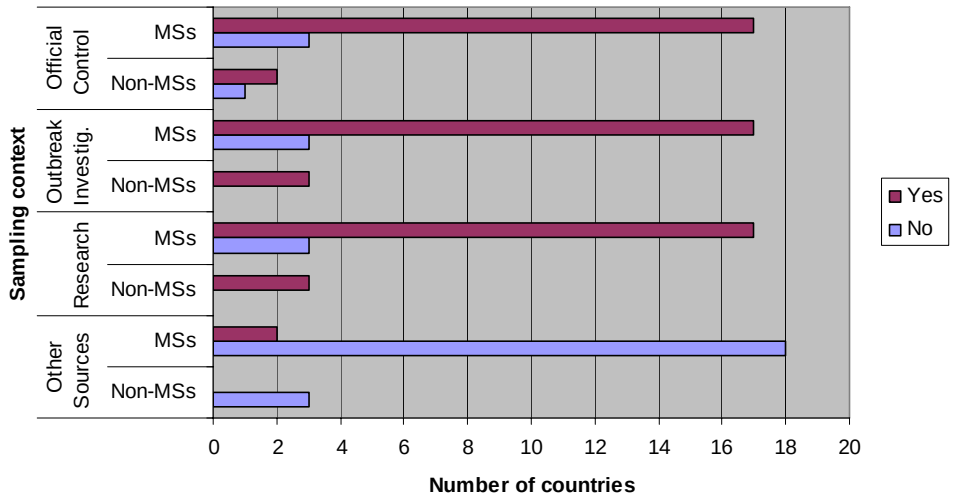
Details on typing frequency for reporting countries by source are presented in Annex I (Table SA1).

Figure 1. Frequency of molecular typing of *Salmonella* isolates by source of isolates



Together 17 MSs and two non-MSs reported that the isolates typed derive from sampling related to official controls, national controls or monitoring programmes or surveys carried out by competent authorities. In addition, 17 MSs and three non-MSs also type isolates coming from sampling related to outbreak investigations as well as from sampling related to research (Figure 2) (Annex I - Table SA2).

Figure 2. Sampling context of *Salmonella* isolates typed



Regarding the methods used in the typing, Pulsed Field Gel Electrophoresis (PFGE) is used by 19 of the 20 MSs typing *Salmonella* spp. isolates, all of them reporting some information on the protocol used. All 19 use the PulseNet Europe PFGE protocol, and two MSs specified the additional standardised protocol used (Biorad and Salm-gene protocol). Out of 19 MSs performing PFGE, 15 MSs use Bionumerics software for analysing the genomic profiles obtained (Annex I – Table SA3).

All three non-MSs performing molecular typing use the PFGE method, two of them using the PulseNet protocol and all three using Bionumerics software (Annex I - Table SA3).

Multi Locus Variable-Number Tandem Repeat Analyses (MLVA) is used by 11 MSs for typing *Salmonella* isolates, while no non-MSs use the method. Nine MSs reported the protocol used; all of them using the protocol Lindstedt *et al*. (2004). Moreover, four MSs reported the use of other protocols, those of Beranek *et al*. (2009); Malorny *et al*. (2008); Lindstedt *et al*. (2003); Lindstedt *et al*. (2007); Best *et al.* (2007); Nordic standard method for *S.* Typhimurium consisting of a combination of Lindstedt *et al*. (2003), Lindstedt *et al*. (2004) plus Hopkins *et al.* (2007) and a Trial method for *S*. Enteritidis based on Cho *et al*. (2008) (Annex I - Table SA4). All the 11 MSs using MLVA are also typing the isolates with the PFGE method.

Seven MSs perform typing of *Salmonella* isolates using other molecular methods, such as ribotyping (four MSs), plasmid profile analysis (three MSs), Multi Locus Sequence Typing (MLST) (two MSs), DNA microarray analysis (one) and IS200 typing (one). No non-MSs use these other methods (Annex I, Table SA5).

Fifteen MSs reported that at least one food/veterinary laboratory carries out molecular typing of *Salmonella* isolates from food, feed or animals, with a maximum of six laboratories in the United Kingdom. In four MSs the molecular typing is performed in laboratories of human health. Laboratory information was not submitted by two MSs performing molecular typing. In the three non-MSs performing molecular typing, at least one food/veterinary laboratory is involved (Table 2).

Table 2. Laboratories involved in molecular typing of *Salmonella* isolates

	No of food/veterinary laboratories performing molecular typing	NRL performing molecular typing of food, feed or animals isolates
MSs		
AT	0*	Yes
BE	4	Yes
CY	1	Some
DE	Unknown	Yes
DK	2	Yes
ES	4	Yes
FI	1	Yes
FR	4	Yes
HU	0*	No
IE	4	Yes
IT	3-4	Yes
LT	2	Some
LU	0*	No
NL	2	Yes
PL	1	Yes
RO	Unknown	Some
SE	1**	Yes
SI	3	Some
SK	2	No
UK	6	Yes
Non-MSs		
CH	2	Some
NO	1	Yes
TR	4	No

* Human health laboratories performing molecular typing

** Also one human health laboratory performing molecular typing

The NRL for *Salmonella* is involved in molecular typing of some or all isolates from food, feed or animals in 17 MSs and in two non-MSs (Table 2).

One MS, Estonia, reported that national laboratories do not perform molecular typing for *Salmonella*, but the analyses are bought from laboratories in other countries and the PFGE method is used in the analyses.

5.2 *Campylobacter*

Nineteen MSs and three non-MSs perform molecular typing for *Campylobacter* isolates from animals, food and feed. Six MSs and one non-MS reported that they neither perform or purchase molecular typing of *Campylobacter* isolates (Table 3).

Table 3. Countries performing molecular typing of *Campylobacter* isolates

MSs	
AT	Yes
BE	Yes
BG	No
CY	No
CZ	Yes
DE	Yes
DK	Yes
EE	No*
ES	Yes
FI	Yes
FR	Yes
GR	No
HU	Yes
IE	Yes
IT	Yes
LT	Yes
LU	Yes
LV	Yes
NL	Yes
PL	Yes
PT	No
RO	No
SE	Yes
SI	Yes
SK	No
UK	Yes*

Non-MSs	
CH	Yes
NO	Yes
MK	No
TR	Yes

* MSs buy analyses abroad

Typing of animal isolates is carried out by 18 MSs, of which 14 do so occasionally and four routinely. Three non-MSs carry out the typing, 2 occasionally and 1 routinely (Figure 3).

Together 18 MSs and three non-MSs perform typing of food isolates. This typing is done occasionally by 14 MSs and three non-MSs, whereas four MSs do so routinely (Figure 3).

Typing of feed isolates is performed by five MSs and by one non-MS. Only one MS does so on a routine basis, and the rest of the MSs and non-MSs do so occasionally (Figure 3).

Details about typing frequency for reporting countries by source are presented in Annex I (Table CA1).

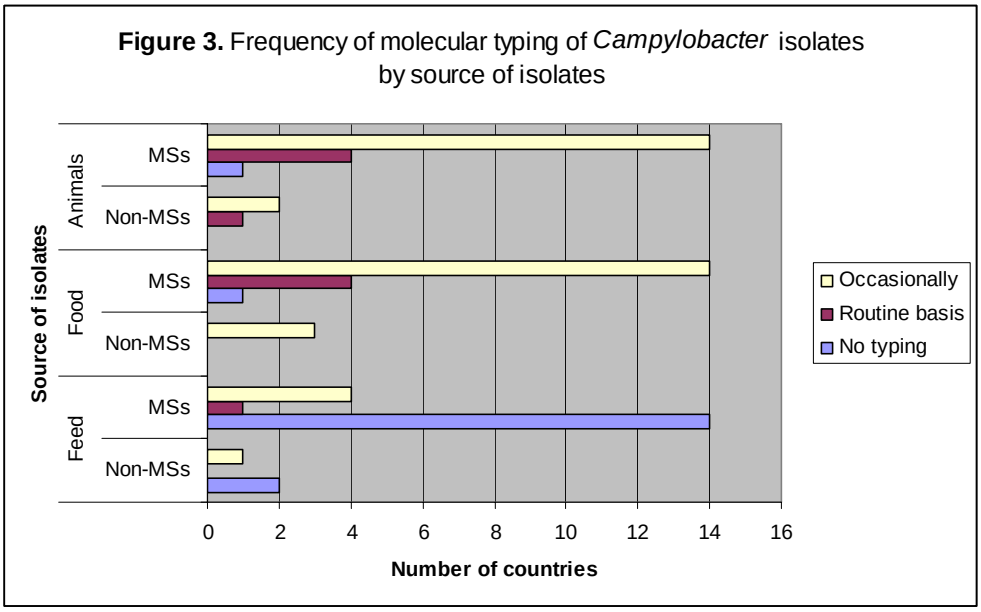


Figure 3. Frequency of molecular typing of *Campylobacter* isolates by source of isolates

Fifteen MSs and two non-MSs reported that the typed isolates derive from sampling related to official controls, national controls, monitoring programmes or surveys carried out by competent authorities. In addition 13 MSs and three non-MSs also type isolates coming from sampling related to outbreak investigations, and 17 MSs and three non-MSs type isolates from sampling related to research (Figure 4) (Annex I - Table CA2).

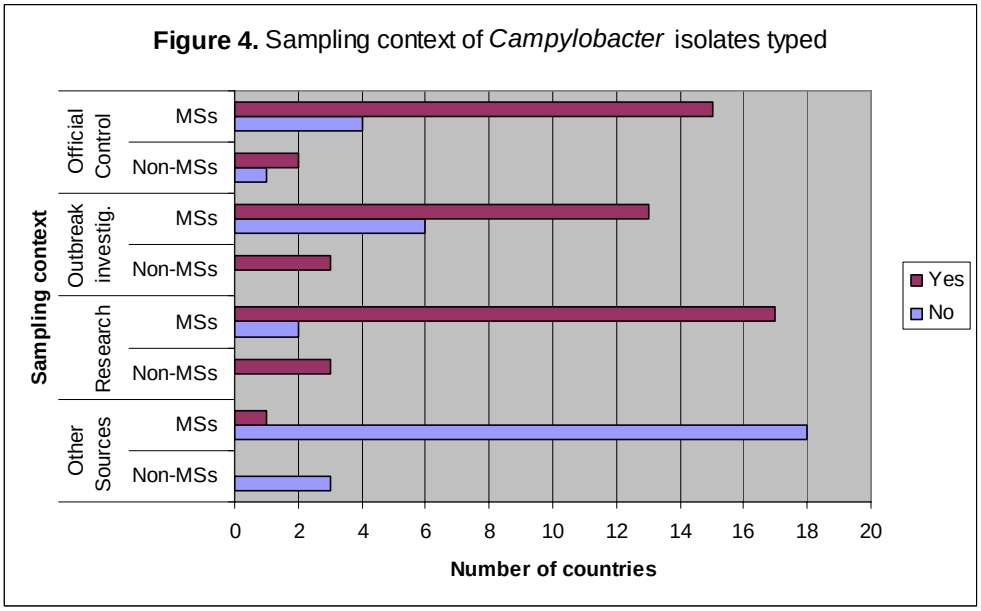


Figure 4. Sampling context of *Campylobacter* isolates typed

Regarding typing methods, PFGE is used by 12 of the 19 MSs typing *Campylobacter* isolates, but only eleven of these provided detailed information regarding the protocol used. Six of them use the PulseNet USA protocol, four the Campynet protocol (one MS in conjunction with the PulseNet USA protocol) and five MSs specified other protocols used (modification of Ribot *et al.*, 2001;; Rivoal *et al.*, 2005; modification of Michaud *et al.*, 2001 and Ribot *et al.*, 2001; On *et al.*, 1998;

Biorad). Out of 12 MSs performing PFGE, nine use Bionumerics software for analysing genomic profiles (Annex I - Table CA3).

One non-MS uses PFGE with a standardised protocol (not stated) and the Bionumerics software (Annex I - Table CA3).

MLST is used by eight of the 19 MSs typing *Campylobacter* spp. isolates. Five MSs reported the protocol; four use the protocol of Dingle *et al.* (2001), one of them together with two other protocols: the Oxford method based on a combination of Dingle *et al.* (2001) and Miller *et al.* (2005) protocols plus FlaA SVR according to Meinersmann *et al.* (1997) and Dingle *et al.* (2002). One MS uses the protocol of van Bergen *et al.* (2005). The only non-MS performing MLST did not report details on the protocol (Annex I - Table CA4).

Twelve MSs perform typing of *Campylobacter* isolates also using other molecular methods, such as FlaA typing (four MSs), Amplified Fragment Length Polymorphism (AFLP) (three MSs), Restriction Fragment Length Polymorphism (RFLP) (three MSs), ribotyping (one MS). Two non-MSs use other methods, one uses Fla typing and the other one AFLP (Annex I - Table CA5).

Sixteen MSs reported that at least one food/veterinary laboratory carries out molecular typing of *Campylobacte*r isolates from food, feed or animals, with a maximum of four laboratories in Spain and in the United Kingdom. In one MS molecular typing is performed in laboratories of human health. In the three non-MSs performing molecular typing at least two food/veterinary laboratories are actively involved in typing (Table 4).

The NRL for *Campylobacte*r is involved in molecular typing of some or all isolates from food, feed or animals in 16 reporting MSs and two non-MSs (Table 4).

Table 4. Laboratories involved in molecular typing of *Campylobacter* isolates

	No of food/veterinary laboratories performing molecular typing	NRL performing molecular typing of food, feed or animals isolates
MSs		
AT	1	Yes
BE	1	No
CZ	Unknown	Yes
DE	Unknown	Yes
DK	2	Yes
ES	4	Yes
FI	1	Yes
FR	2	Yes
HU	1	Yes
IE	2	No
IT	1**	Yes
LT	2	Some
LU	0*	No
LV	1	Yes
NL	2	Yes
PL	1	Yes
SE	3	Yes
SI	2	Some
UK	4	Yes
Non- MSs		
CH	2	Some
NO	2	Yes
TR	4	No

* Human health laboratories performing molecular typing
** At least the NRL; no information is available on other laboratories

Estonia reported that national laboratories do not perform molecular typing for *Campylobacter*, but the analyses are bought from laboratories in other countries that use the method recommended by the CRL. In the United Kingdom molecular typing analyses are carried out by the national laboratories and also are purchased from laboratories in other countries using the MLST and fla-SVR methods.

5.3 Verotoxigenic *Escherichia coli*

Twenty MSs and two non MSs currently perform molecular typing for VTEC isolates from animals, food and feed. Five MSs and two non-MSs reported that they neither perform or purchase molecular typing of VTEC isolates (Table 5).

Table 5. Countries performing molecular typing of VTEC isolates

MSs	
AT	Yes
BE	Yes
BG	No
CY	No
CZ	Yes
DE	Yes
DK	Yes
EE	No*
ES	Yes
FI	Yes
FR	Yes
GR	No
HU	Yes
IE	Yes
IT	Yes
LT	Yes
LU	Yes
LV	Yes*
NL	Yes
PL	Yes
PT	No
RO	No
SE	Yes
SI	Yes
SK	Yes
UK	Yes

Non-MSs	
CH	Yes
MK	No
NO	Yes
TR	No

* MSs buy analyses abroad

Typing of animal isolates is carried out in total by 17 MSs, of which 13 do so occasionally and four routinely. Two non-MSs carry out the typing occasionally (Figure 5).

Together 19 MSs and two non-MSs perform typing of food isolates. This typing is carried out occasionally by 13 MSs and the two non-MSs, whereas six MSs do so on a routine basis (Figure 5).

Fewer countries reported typing of feed isolates. In total, four MSs and two non-MSs carry out this typing occasionally (Figure 5).

Details about typing frequency for reporting countries by source are presented in Annex I (Table VT1).

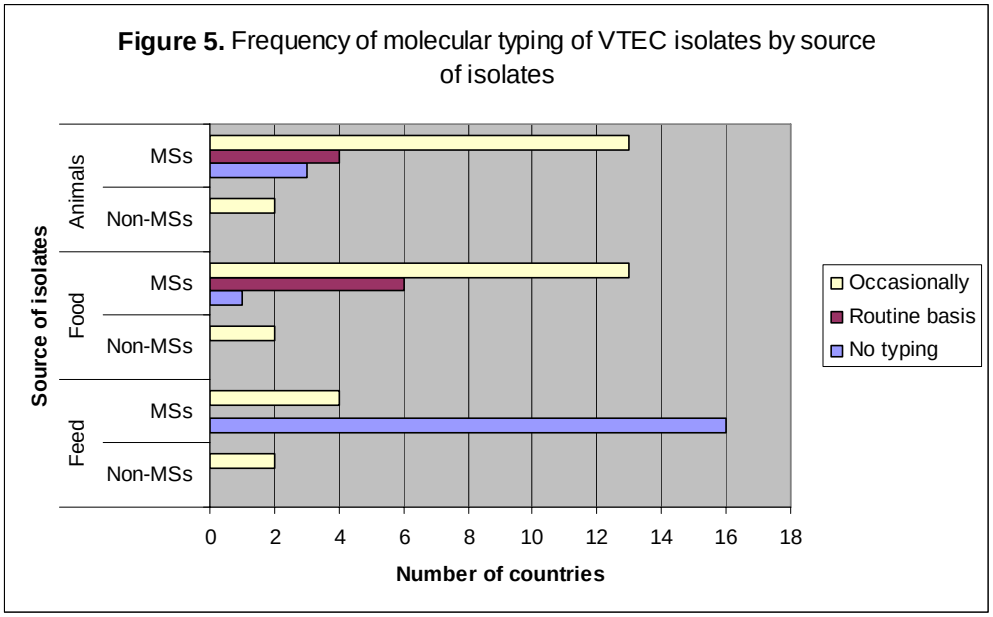


Figure 5. Frequency of molecular typing of VTEC isolates by source of isolates

Fourteen MSs and one non-MS reported that the isolates typed come from sampling related to official controls, national controls or monitoring programmes or surveys carried out by competent authorities. In addition 15 MSs and two non-MSs also type isolates from sampling related to outbreak investigations and 15 MSs and two non-MSs type the isolates from sampling related to research. (Figure 6) (Annex I - Table VT2).

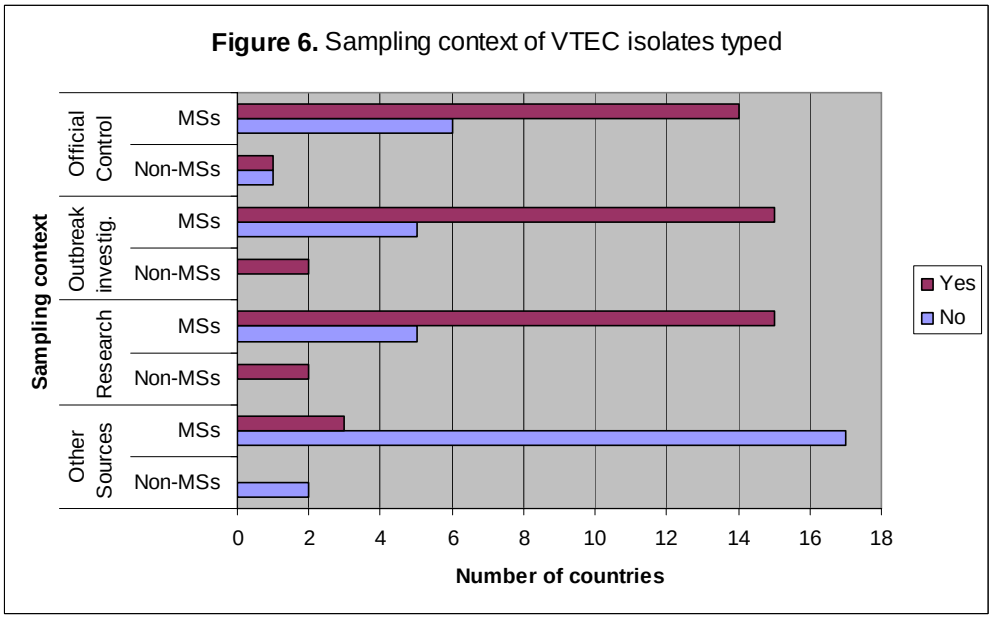


Figure 6. Sampling context of VTEC isolates typed

Regarding the methods used in the typing, PFGE is used by 17 of the 20 MSs typing VTEC isolates, but only 16 MSs provided some information regarding the protocol. In total 14 MSs use PulseNet Europe protocol and three of them specified the additional standardised protocol used (Vali *et al*., 1997, Biorad and Willshaw *et al.*, 1997 protocols). Out of 17 MSs performing PFGE, 12 currently use Bionumerics software for analysing genomic profiles (Annex I - Table VT3).

All two non-MSs performing molecular typing use the PFGE method, both using the PulseNet Europe protocol and the Bionumerics software (Annex I - Table VT3).

MLVA is used by seven MSs and one non-MS for typing VTEC isolates. Five MSs reported the protocol; two MSs use the protocol of Noller *et al.* (2003), one MS a modified PulseNet protocol, one MS uses the PulseNet USA protocol and one the protocol of Kawamori *et al*. (2008). The non-MS uses the protocol of Lindstedt *et al.*, (2004) (Annex I - Table VT4).

Eight MSs perform typing of VTEC isolates also using other molecular methods, such as PCR-based methods (five MSs), MLST (one MS), ribotyping (one MS). One non-MS uses MLST together with microarray (Annex I, Table VT5).

Seventeen MSs reported that at least one food/veterinary laboratory carries out molecular typing of VTEC isolates from food, feed or animals, with a maximum of four laboratories in Belgium, Germany, Ireland and the United Kingdom. In two MSs molecular typing is performed in laboratories of human health. In the two non-MSs performing molecular typing, two food/veterinary laboratories are involved (Table 6).

The NRL for VTEC is involved in molecular typing of some or all isolates from food, feed or animals in 19 MSs and two non-MSs (Table 6).

Estonia reported that national laboratories do not perform molecular typing for VTEC isolates, but the analyses are bought from laboratories in other countries using the method recommended by the CRL. In Latvia the analyses for molecular typing are carried out by the national laboratories, and also are purchased from laboratories in other countries using conventional PCR for the detection of verocytotoxins producing genes (vtx1, vtx2, eaeA).

Table 6. Laboratories involved in molecular typing of VTEC isolates

	No of food/veterinary laboratories performing molecular typing	NRL performing molecular typing of food, feed or animals isolates
MSs		
AT	0*	Some
BE	4	Some
CZ	Unknown	Yes
DE	4**	Yes
DK	1	Yes
ES	3	Yes
FI	2	Yes
FR	1	Yes
HU	2	Some
IE	4	Yes
IT	1***	Yes
LT	2	Some
LU	0*	No
LV	1	Yes
NL	1	Yes
PL	1	Yes
SE	2	Yes
SI	3	Some
SK	1	Some
UK	3-4	Yes
Non-MSs		
CH	2	Some
NO	2	Yes

* Human health laboratories performing molecular typing
** At least 4 laboratories
*** At least the NRL; no information is available on other laboratories

5.4 *Listeria monocytogenes*

Fifteen MSs and three non-MSs currently perform molecular typing for *Listeria monocytogenes* isolates from animals, food and feed. Ten MSs and one non-MS reported that they neither perform or purchase molecular typing of *Listeria monocytogenes* isolates (Table 7).

Table 7. Countries performing molecular typing of *Listeria monocytogenes* isolates

MSs	
AT	Yes
BE	Yes
BG	No
CY	No
CZ	Yes
DE	Yes
DK	Yes
EE	No*
ES	No
FI	Yes
FR	Yes
GR	No
HU	No
IE	Yes
IT	Yes
LT	No
LU	Yes
LV	No
NL	Yes
PL	No
PT	No
RO	No
SE	Yes
SI	Yes
SK	Yes
UK	Yes

Non-MSs	
CH	Yes
NO	Yes
MK	No
TR	Yes

* EE buys analyses abroad

Typing of animal isolates is carried out by 11 MSs, of which 10 do so occasionally and 1 on a routine basis. Two non-MSs carry out the typing only occasionally (Figure 7).

Together 15 MSs and two non-MSs perform typing of food isolates. This typing is done occasionally by 8 MSs and by two non-MSs, whereas seven MSs do so on a routine basis (Figure 7).

Fewer countries reported typing of feed isolates. In total, seven MSs and one non-MS carry out this typing. Only 2 MSs do so routinely, and the rest of the MSs and the non-MS do so occasionally (Figure 7).

Details about typing frequency for reporting countries by source are presented in Annex I (Table LI1).

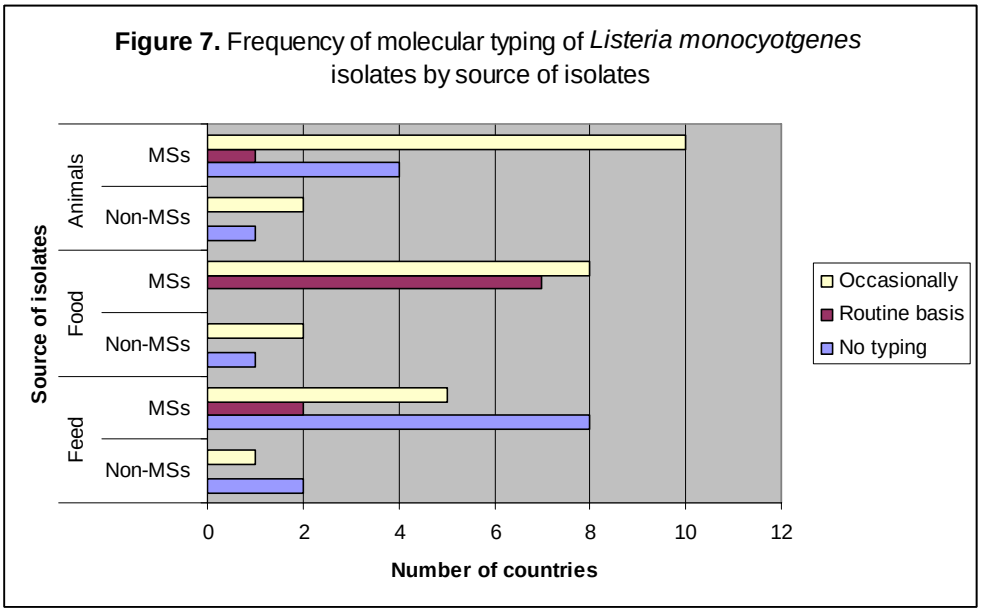


Figure 7. Frequency of molecular typing of *Listeria monocyotgenes* isolates by source of isolates

Fourteen MSs reported that the isolates typed derive from sampling related to official controls, national controls, monitoring programmes or surveys carried out by competent authorities. In addition, 12 MSs and two non-MSs type isolates from sampling related to outbreak investigations, and nine MSs and three non-MSs those from sampling related to research. (Figure 8) (Annex I - Table LI2).

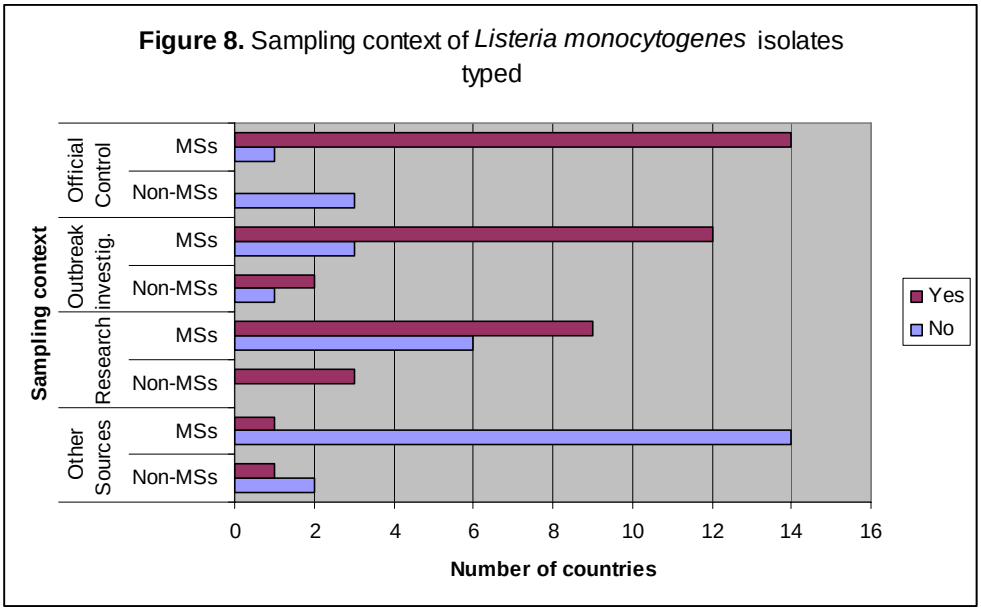


Figure 8. Sampling context of *Listeria monocytogenes* isolates typed

Regarding the methods used for typing, PFGE is used by 14 of the 15 MSs typing *Listeria monocytogenes* isolates. The 12 MSs providing information regarding the protocol, use the PulseNet Europe protocol. Out of 14 MSs performing PFGE, 11 currently use Bionumerics software for analysing the genomic profiles obtained (Annex I - Table LI3).

The two non-MSs which perform PFGE, use the PulseNet Europe protocol and Bionumerics software (Annex I - Table LI3).

MLVA is used by two of 15 MSs (Ireland and the Netherlands) typing *Listeria monocytogenes* isolates, while no non-MSs use the method. No MSs reported the actual protocol used.

Five MSs perform typing of *Listeria monocytogenes* isolates using other methods, such as ribotyping (two MSs), AFLP (one MS), PCR-based methods (one MS). Two non-MSs use PCR-based methods (Annex I - Table LI4).

Thirteen MSs reported that at least one food/veterinary laboratory carries out molecular typing of *Listeria monocytogenes* isolates from food, feed or animals, with a maximum of six laboratories in France. In one MS molecular typing is performed in laboratories of human health. In three non-MSs performing molecular typing at least one food/veterinary laboratory is involved (Table 8).

The NRL for *Listeria monocytogenes* is involved in molecular typing of some or all isolates from food, feed or animals in 14 MSs and in two non-MSs (Table 8).

Table 8. Laboratories involved in molecular typing of *Listeria monocytogenes* isolates

	No of food/veterinary laboratories performing molecular typing	**NRL performing molecular typing of food, feed or animals isolates**
MSs		
AT	1	Yes
BE	1	Yes
CZ	1	Some
DE	Unknown	Yes
DK	1	Yes
FI	1	Yes
FR	6	Yes
IE	3	Yes
IT	1**	Yes
LU	0*	No
NL	1	Yes
SE	2-3	Yes
SI	1	Some
SK	1	Some
UK	1	Yes
Non-MSs		
CH	2	Some
NO	1	Yes
TR	1	No

* Human health laboratories performing molecular typing
** At least the NRL; no information is available on other laboratories

Estonia reported that national laboratories do not perform molecular typing for *Listeria monocytogenes* isolates, but the analyses are bought from laboratories in other countries using the method recommended by the CRL.

5.5 *Staphylococcus aureus*

Nineteen MSs and three non MSs currently perform molecular typing for *Staphylococcus aureus* isolates from animals, food and feed. Six MSs and one non-MS reported that they neither perform or purchase molecular typing of *Staphylococcus aureus* isolates (Table 9).

Table 9. Countries performing molecular typing of *Staphylococcus aureus* isolates

MSs	
AT	Yes
BE	Yes
BG	No
CY	No
CZ	Yes
DE	Yes
DK	Yes
EE	No*
ES	Yes
FI	Yes
FR	Yes
GR	No
HU	Yes
IE	Yes
IT	Yes
LT	Yes
LU	Yes*
LV	Yes*
NL	Yes
PL	No
PT	No
RO	No
SE	Yes
SI	Yes
SK	Yes
UK	Yes

Non-MSs	
CH	Yes
MK	No
NO	Yes
TR	Yes

* MSs buy analyses abroad

Typing of animal isolates is carried out by 19 countries, of which 15 do so occasionally and four routinely. Three non-MSs carry out the typing occasionally (Figure 9).

Together 12 MSs and two non-MSs perform typing of food isolates, all occasionally (Figure 9).

Fewer countries reported typing of feed isolates. In total three MSs and one non-MS perform this typing occasionally (Figure 9).

Details about typing frequency for reporting countries by source are presented in Annex I (Table ST1).

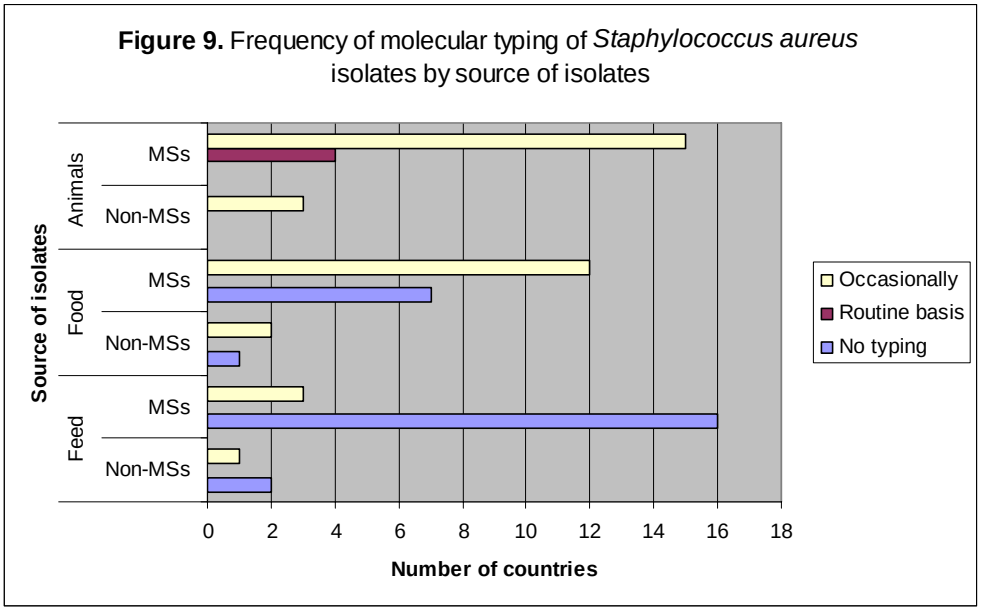


Figure 9. Frequency of molecular typing of *Staphylococcus aureus* isolates by source of isolates

Fifteen MSs reported that the isolates typed derive from sampling related to official controls, national controls, monitoring programmes or surveys carried out by competent authorities. In addition, ten MSs and one non-MS also type isolates coming from sampling related to outbreak investigations, and 13 MSs and three non-MSs those from sampling related to research (Figure 10) (Annex I - Table ST2).

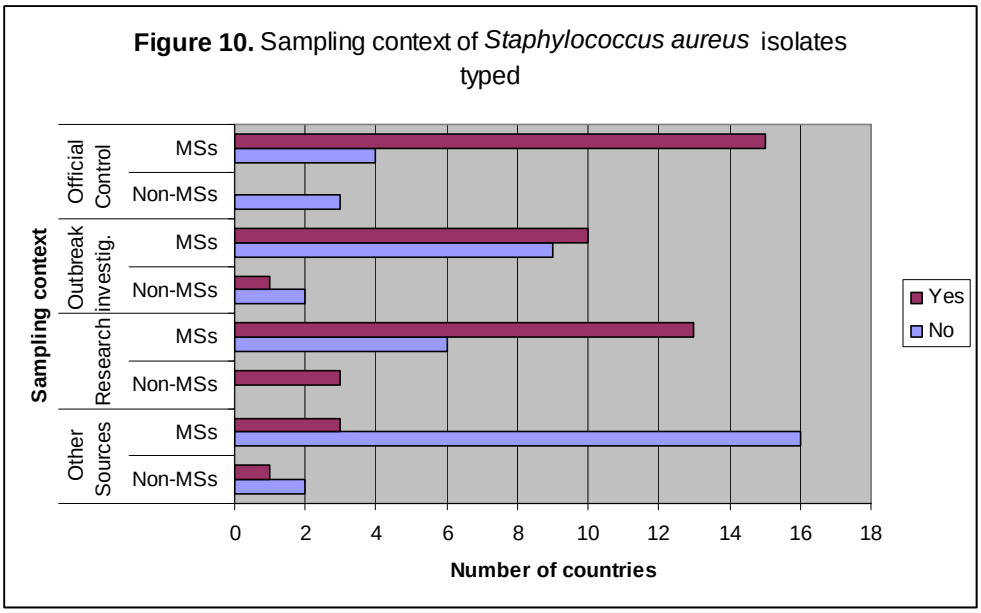


Figure 10. Sampling context of *Staphylococcus aureus* isolates typed

Regarding typing methods used, PFGE is used by 13 of the 19 MSs typing *Staphylococcus aureus* isolates, even though only 12 MSs provided some information regarding the protocol used. Two MSs use the PulseNet USA protocol, four MSs use the protocol of Murchan *et al.* (2003) and three other MSs specified the standardised protocol used; in particular one MS uses the Harmony protocol, one combines the Harmony protocol together with the protocol of Shimizu *et al.*, (1997) and another MS combines the Harmony protocol together with the protocol of Mulvey *et al.* (2001). Out of 13 MSs performing PFGE, eight use Bionumerics software for analysing genomic profiles (Annex I - Table ST3).

Two of three non-MSs performing molecular typing carry out the PFGE method and use the PulseNet USA protocol and the protocol of McDougal *et al.* (2003); one of them also uses the Bionumerics software (Annex I - Table ST3).

MLVA is used by three MSs (Ireland, Luxembourg and the Netherlands) of 19 MSs performing typing of *Staphylococcus aureus* isolates, while no non-MSs use this method. Two MSs reported information on the protocol; one uses the protocol of Sabat *et al.* (2003), another uses the protocol of Melles *et al.* (2009).

Ten of 19 MSs typing *Staphylococcus aureus* isolates, use MLST, while no non-MSs perform this typing method. Six MSs reported the protocol used; four of them use the protocol of Enright *et al.* (2000), one uses the protocol of Huijdens *et al.* (2006) and another uses the protocol of the CRL for Antimicrobial Resistance (CRL-AR) (Annex I - Table ST4).

Spa typing is used by 13 MSs, while no non-MSs perform this typing method. Six MSs reported the protocol used; two use the protocol of Shopsin *et al.* (1999), one uses that of Ruppitsch *et al.* (2006), one the protocol of De Neeling *et al.* (2007), one uses the protocol from the CRL-AR and another participates in a European network of excellence for sequence-based typing (SEQnet.org) (Annex I - Table ST5).

RFLP is performed by two non-MSs (Switzerland and Turkey), while no MSs use this typing method.

Ribotyping is used by one MS (Ireland) and also by one non-MS (Turkey).

Seven MSs use also other methods to perform molecular typing of *Staphylococcus aureus* isolates, such as PCR-based methods (four MSs), SCC*mec* typing (three MSs), microarray technique (one MS). One non-MS uses a PCR-based method (Annex I - Table ST6).

Table 10. Laboratories involved in molecular typing of *Staphylococcus aureus* isolates

	No of food/veterinary laboratories performing molecular typing	NRL performing molecular typing of food, feed or animals isolates
MSs		
AT	1	Yes
BE	2	Yes
CZ	Unknown	Yes
DE	Unknown	Yes
DK	2	Yes
ES	2	Some
FI	0*	No
FR	2	Yes
HU	2	Some
IE	2	No
IT	4	Yes
LT	2	Yes
LU	0*	No
LV	1	Yes
NL	2	Some
SE	2	Yes
SI	1	Some
SK	1	No
UK	2	Some
Non-MSs		
CH	1	No
NO	1	Yes
TR	2	No

* Human health laboratories performing molecular typing

Fifteen MSs reported that at least one food/veterinary laboratory carries out molecular typing of *Staphylococcus aureus* isolates from food, feed or animals, with a maximum of four laboratories in Italy. In two MSs typing is performed in laboratories of human health. In three non-MSs performing molecular typing, at least one food/veterinary laboratory is involved (Table 10).

The NRL for *Staphylococcus aureus* is involved in molecular typing of some or all isolates from food, feed or animals in 15 MSs and one non-MS (Table 10).

Estonia reported that national laboratories do not perform molecular typing for VTEC isolates, but the analyses are bought from laboratories in other countries using the method recommended by the CRL.

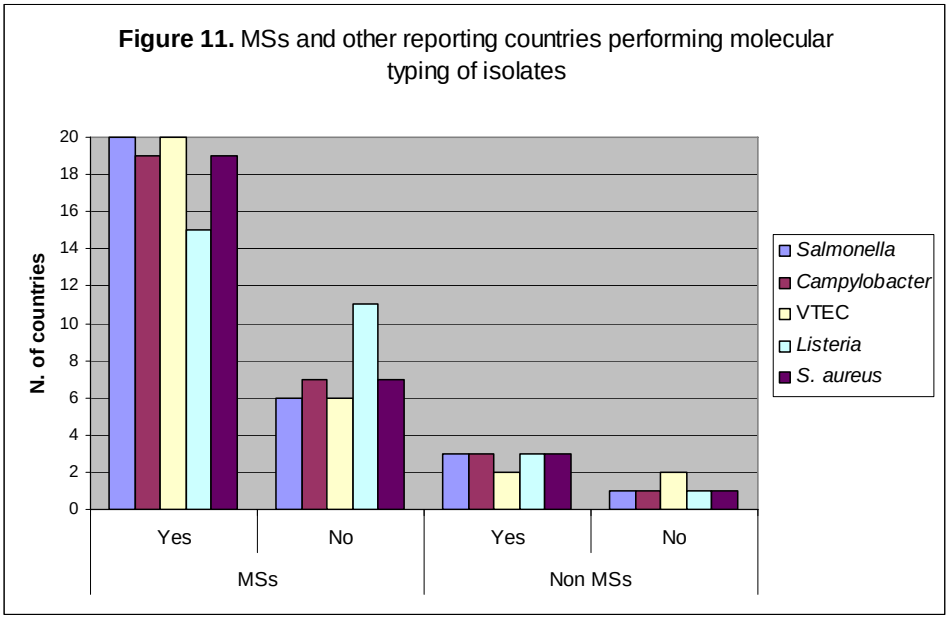
In Luxembourg and Latvia analyses for molecular typing are carried out by national laboratories, and are also purchased from laboratories in other countries using different methods (Spa typing and MLST for LU and conventional PCR for detection of the *mec*A gene for LV).

5. Summary

In total 22 MSs and three non-MSs perform molecular typing of isolates of one or more pathogens covered by the survey. Four MSs (BG, EE, GR, PT) reported that they do not perform molecular typing on isolates of any of the food-borne pathogens selected for this report. However, Estonia informed that they purchase these analyses from other countries.

In particular 20 MSs and three non-MSs type *Salmonella* isolates from animals, food and feed; 19 MSs and three non-MSs perform molecular typing of *Campylobacter* isolates; 20 MSs and two non-MSs perform molecular typing of VTEC isolates; 15 MSs and three non-MSs type *Listeria monocytogenes* isolates and finally 19 MSs and three non-MSs perform typing of *Staphylococcus aureus* isolates. *Salmonella* is the pathogen most frequently typed (Figure 11).

Figure 11. MSs and other reporting countries performing molecular typing of isolates



Generally both in MSs and non-MSs the isolates typed derive mainly from animals and food. Fewer countries (MSs and other countries) type isolates coming from feed. However variability is relative to the pathogen concerned (Figure 12).

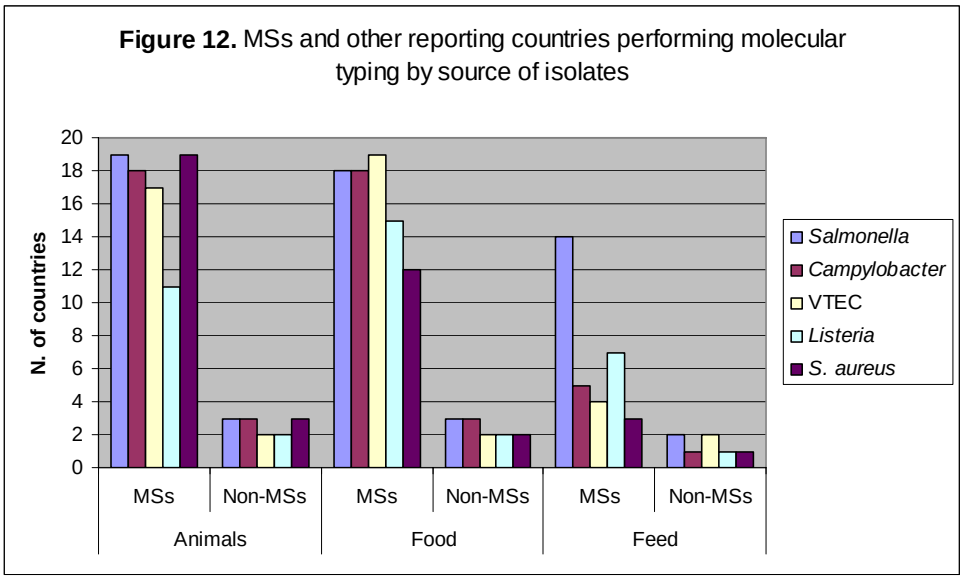


Figure 12. MSs and other reporting countries performing molecular typing by source of isolates

Typing is performed mainly occasionally for all the pathogens; only few countries type isolates on a routine basis (three MSs for *Salmonella*; four MSs for *Campylobacter* spp. and *Staphylococcus aureus*; six MSs for VTEC; seven for *Listeria monocytogenes*). However, there is some variability in the typing frequency between isolates from different sources, the food isolates appearing to be typed more often on a routine basis (Figure 13).

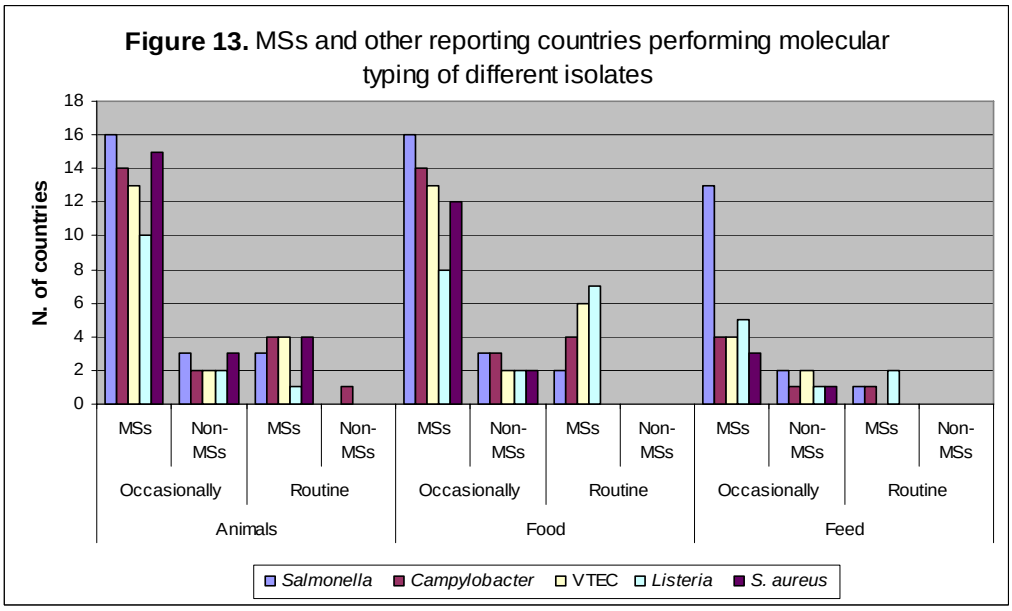
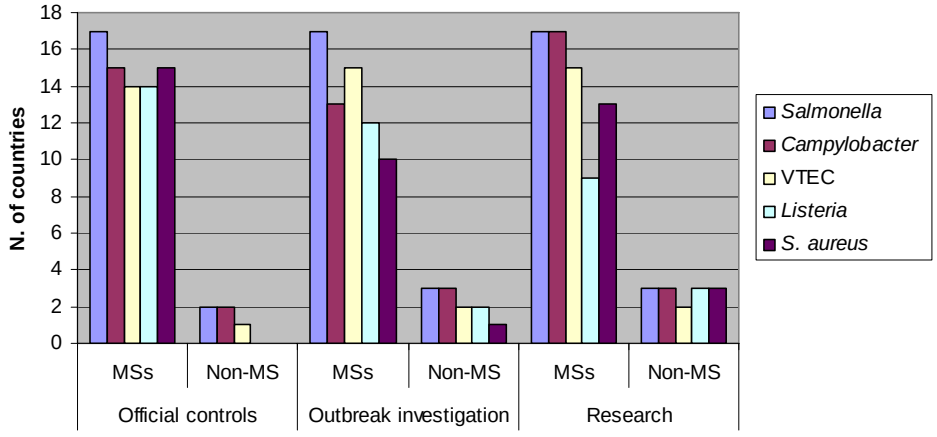


Figure 13. MSs and other reporting countries performing molecular typing of different isolates

As regards the sampling context, MSs type isolates coming most often from official controls, but also the typing of isolates from sampling related to research and isolates related to outbreak

investigations was common. Non-MSs type most often isolates deriving from sampling related to research. There is some variability between the pathogens (Figure 14).

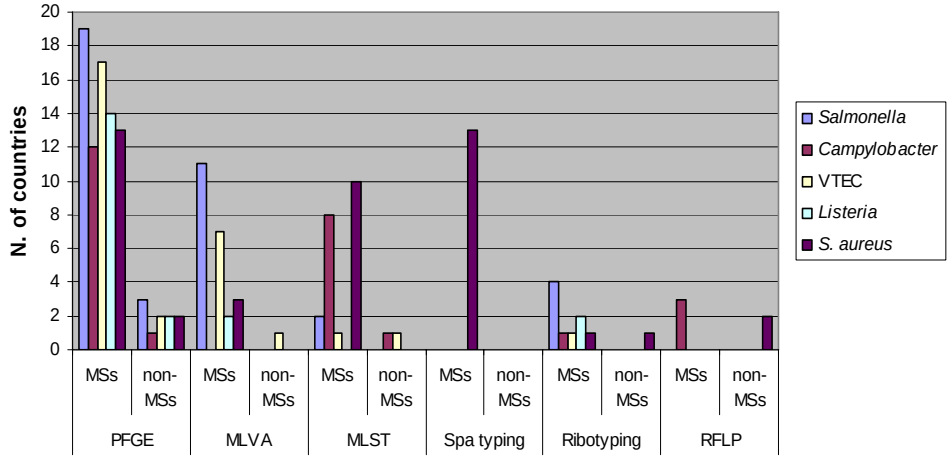
Figure 14. MSs and other reporting countries performing molecular typing of isolates by sampling context



The PFGE is used by most countries for typing all pathogens concerned (19 MSs and 3 non-MSs for *Salmonella*, 12 MSs and 1 non-MS for *Campylobacter*, 17 MSs and 2 non-MSs for VTEC, 14 MSs and 2 non-MSs for *Listeria monocytogenes*, 13 MSs and 2 non-MSs for *Staphylococcus aureus*).

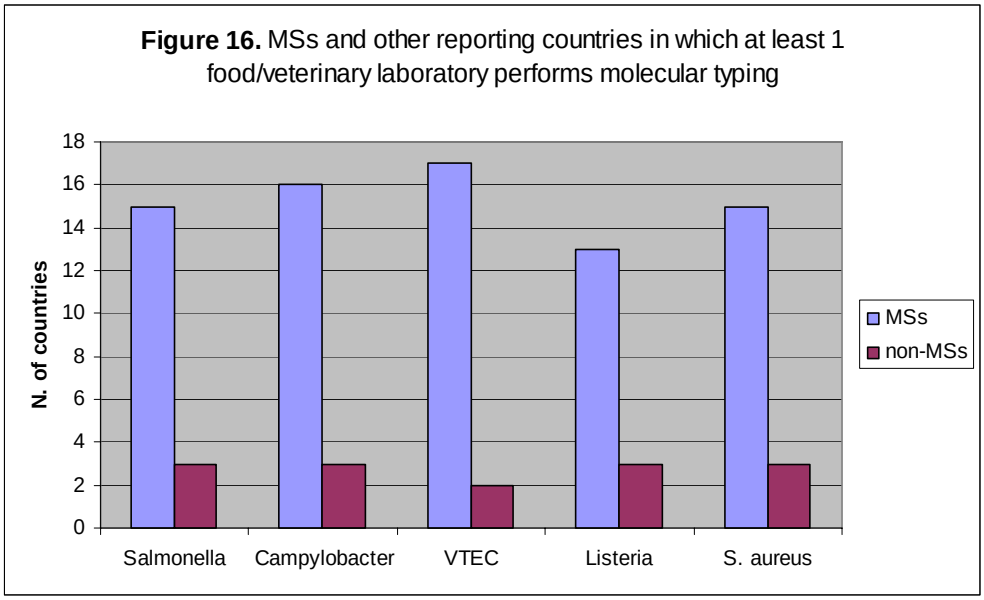
Fewer countries use other methods, such as MLVA, MLST, Spa typing, ribotyping, RFLP, AFLP, Fla typing, SSC*mec* (Figure 15).

Figure 15. The main molecular methods used by MSs and other reporting countries



For each pathogen in several MSs and other reporting countries at least one food/veterinary laboratory performs molecular typing of isolates from animals, food or feed, according to the replies received (MSs: 15 for *Salmonella* and *Staphylococcus aureus*; 13 for *Listeria*; 16 for *Campylobacter* and 17 for VTEC; non-MSs: 2 for VTEC, 3 for other pathogens) (Figure 16).

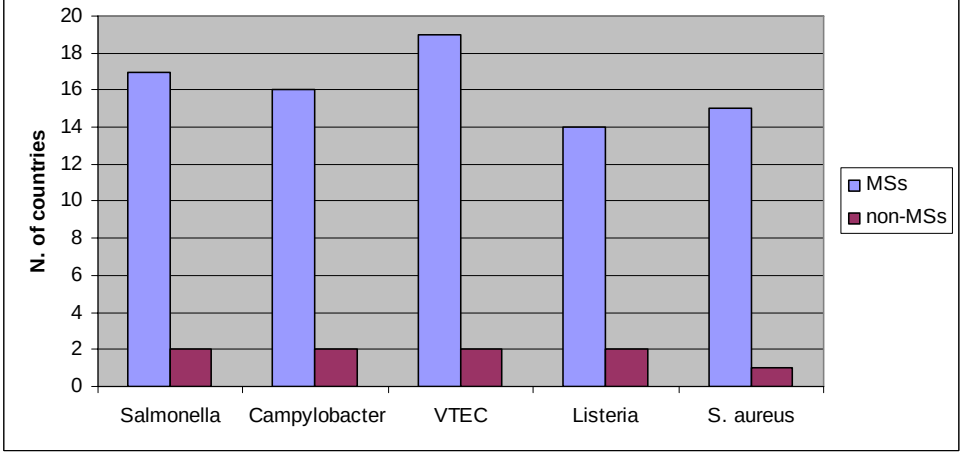
Figure 16. MSs and other reporting countries in which at least 1 food/veterinary laboratory performs molecular typing



The NRL of each pathogen is involved in molecular typing of isolates from animals, food or feed in several MSs and other reporting countries (MSs: 19 for VTEC, 17 for *Salmonella*; 16 for *Campylobacter*; 15 for *Staphylococcus aureus* and 14 for *Listeria monocytogenes*; non-MSs: 1 for *Staphylococcus aureus* and 2 for the other pathogens) (Figure 17).

There seems to be a slight discrepancy between the number of NRLs and the number of food/veterinary laboratories performing molecular typing, i.e. it could be supposed that most NRLs would be regarded as food/veterinary laboratories in MSs. This may be due to the fact that there was some missing information on the number of food/veterinary laboratories performing the typing and also in some MSs NRLs could be human health laboratories.

Figure 17. MSs and other reporting countries in which the NRL performs molecular typing



As regards the purchase of molecular typing analyses of isolates from food, feed or animals from laboratories of another MS/country, only four MSs buy the analyses abroad.

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7. References

Ayling, R.D., Woodward, M.J., Evans, S. and Newell, D.G., 1996. Restriction fragment length polymorphism of polymerase chain reaction products applied to the differentiation of poultry Campylobacters for epidemiological investigations. *Res. Vet. Sci*. 60, 168–172.

Beranek, A., Mikula, C., Rabold, P., Arnhold, D., Berghold, C., Lederer, I., Allerberger, F. and Kornschober, C., 2009. Multiple-locus variable-number tandem repeat analysis for subtyping of *Salmonella enterica* subsp. *Enterica* serovar Enteritidis. *Int. J. Med. Microbiol*. 299 (1), 43-51.

Best, E.L., Lindstedt, B.A., Cook, A., Clifton Hadley, F.A., Threlfall, E.J. and Liebana, E., 2007. Multiple-locus variable-number tandem repeat analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium: comparison of isolates from pigs, poultry and cases of human gastroenteritis. J. *Appl. Microbiol*. 103, 565-572.

Birnboim, H.C. and Doly, J., 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*. 7. (6), 1513-1523.

Campynet. <http://campynet.vetinst.dk/PFGE.html>

Champion, O.L., Best, E.L. and Frost, J.A., 2002. Comparison of Pulsed-Field Gel Electrophoresis and Amplified Fragment Length Polymorphism Techniques for Investigating Outbreaks of Enteritis Due to Campylobacters. *J. Clin. Microbiol*. 40 (6), 2263–2265.

Cho, S., Whittam, T.S., Boxrud, D.J., Bartkus, J.M. and Mahdi S.A., 2008. Allele distribution and genetic diversity of VNTR loci in Salmonella enterica serotype Enteritidis isolates from different sources. *BMC Microbiol*. 8,146.

De Neeling, A.J., van den Broek, M.J.M., Spalburg, E.C., van Santen-Verheuvel, M.G., Dam-Deisz, W.D.C., Boshuizen, H.C. , van de Giessen, A.W., van Duijkeren, E., Huijsdens, X.W., 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* 122, 366-372.

Dingle, K.E., Colles, F.M., Wareing, D.R., Ure, R., Fox, A.J. and Bolton, F.E., 2001. Multilocus sequence typing system for *Campylobacter jejuni*. *J. Clin. Microbiol*. 39, 14-23.

Dingle, K.E., Colles, F.M., Ure, R., Wagenaar, J., Duim, B., Bolton, F.J., Fox, A.J., Wareing, D.R. and Maiden, M.C., 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiological investigations. *Emerg. Infect. Dis*. 8, 949–55.

Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003, p. 31).

Duim, B., Wassenaar, T.M., Rigter, A. and Wagenaar, J., 1999. High-Resolution Genotyping of *Campylobacter* Strains Isolated from Poultry and Humans with Amplified Fragment Length Polymorphism Fingerprinting. *Appl. Environ. Microbiol*. 65 (6), 2369–2375.

Enright, M.C., Day, N.P.J., Davies, C.E., Peacock, S.J. and Spratt, B.J., 2000. Multilocus sequence typing for characterisation of methicillin-resistant and methicicllin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol*. 38, 1008-1015.

Harmony. <http://www.harmony-microbe.net/new_pfge_protocol.pdf>

Hopkins, K.L., Maguire, C., Best, E., Liebana, E. and Threlfall, E.J., 2007. Stability of Multiple-Locus Variable-Number Tandem Repeats in *Salmonella enterica* Serovar Typhimurium. *J. Clin. Microbiol.* 45 (9), 3058–3061.

Huijsdens, X.W., van Dijke, B.J, Spalburg, E., van Santen-Verheuvel, M.G., Heck, M.E., Pluister, G.N., Voss, A., Wannet, W.J. and de Neeling, AJ., 2006. Community-acquired MRSA and pig-farmimg. *Ann. Clin. Microbiol. Antimicrob*. 5, 26.

Hyytia-Trees, E., Gerner-Smidt, P., and Ribot, E., 2006. Use of Multiple-locus Variable-number Tandem Repeat Analysis (MLVA) to Further Characterize Shiga Toxin-producing *Escherichia coli* (STEC) O157 Clusters Detected by PFGE in PulseNet USA. In: Abstracts of the 6th International Symposium on Shiga Toxin (Verocytotoxin) –Producing *Escherichia coli* Infections. October 29-Nov 1, 2006, Melbourne, Australia. S. 142.

Kawamori, F., Hiroi, M., Harada, T., Ohata, K., Sugiyama, K., Masuda, T., and Ohashi, N., 2008. Molecular typing of Japanese *Escherichia coli* O157:H7 isolates from clinical specimens by multilocus variable-number tandem repeat analysis and PFGE. J. Med. Microbiol. 57, 58-63.

Lindstedt, B.A., Heir, E., Gjernes, E. and Kapperud, G., 2003. DNA fingerprinting of *Salmonella enterica* subsp. *enterica* serovar Typhimurium with emphasis on phage type DT104 based on variable number of tandem repeat loci. *J. Clin. Microbiol*. 41 (4), 1469-1479.

Lindstedt, B.A., Vardund, T., Aas, L. and Kapperud, G., 2004. Multiple-locus variable-number tandem repeat analysis of *Salmonella enterica* subsp. *Enterica* serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. *J. Microbiol. Methods*. 59 (2), 163-172.

Lindstedt, B.A., Torpdahl, M., Nielsen, E.M., Vardund, T., Aas, L. and Kapperud, G., 2007. Harmonization of the multiple-locus variable-number tandem repeat analysis method between Denmark and Norway for typing *Salmonella* Typhimurium isolates and closer examination of the VNTR loci. *J. Appl. Microbiol*. 102, 728-735.

Løvseth, A., Loncarevic, S. and Berdal, K.G., 2004. Modified Multiplex PCR Method for Detection of Pyrogenic Exotoxin Genes in Staphylococcal Isolates. *J. Clin. Microbiol*. 42 (8), 3869–3872.

Malorny, B., Junker, E. and Helmuth, R., 2008. Multi-locus variable-number tandem repeat analysis for outbreak studies of *Salmonella enterica* serotype Enteritidis. *BMC Microbiol*. 8, 84.

McDougal, L.K., Steward, C.D., Killgore, G.E, Chaitram, J.M., McAllister, S.K and Tenover, F.C., 2003. Pulsed-Field Gel Electrophoresis typing of oxacillin-resistant *Staphylococcus*

aureus isolates from the United States: establishing a national database. *J. Clin. Microbiol.* 41 (11), 5113–5120.

Meinersmann, R.J., Helsel, L.O., Fields, P.I. and Hiett, K.L., 1997. Discrimination of *Campylobacter jejuni* Isolates by *fla* Gene Sequencing. *J. Clin. Microbiol*. 35 (11), 2810-2814.

Melles, D.C., Schouls, L., François, P., Herzig, S., Verbrugh, H.A., van Belkum, A. and Schrenzel, J., 2009. High-throughput typing of *Staphylococcus aureus* by amplified fragment length polymorphism (AFLP) or multi-locus variable number of tandem repeat analysis (MLVA) reveals consistent strain relatedness. *Eur. J. Clin. Microbiol. Infect. Dis.* 28, 39–45.

Michaud, S., Menard, S., Gaudreau, C. and Arbeit R.D., 2001. Comparison of SmaI-defined genotypes of *Campylobacter jejuni* examined by KpnI: a population-based study. *J. Med. Microbiol.* 50, 1075-1081.

Miller, W.G., On, S.L.W., Wang, G., Fontanoz, S., Lastovica, A.J. and Mandrelli, R.E., 2005. Extended Multilocus Sequence Typing System for *Campylobacter coli, C. lari, C. upsaliensis*, and *C. helveticus. J. Clin. Microbiol*. 43 (5), 2315-2329.

Mulvey, M.R., Chui, L., Isamil, J., Louie, L., Murphy, C., Chang, N. and Alfa, M., 2001. Development of a Canadian standardized protocol for subtyping methicillin-resistant *Staphylococcus aureus*: using Pulsed Field Gel Electrophoresis. *J. Clin. Microbiol*. 39, 3481-3485.

Murchan, S., Kaufmann, M.E., Deplano, A., de Ryck, R., Struelens, M. and Zinn, C.E., 2003. Harmonisation of pulsed-filed gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application fro tracing the spread of related strains. *J. Clin. Microbiol*. 41, 1574-1585.

Nachamkin, I., Bohachick, K., Patton, C.M., 1993. Flagellin Gene Typing of *Campylobacter jejuni* by Restriction Fragment Length Polymorphism Analysis. *J. Clin. Microbiol.* 31 (6), 1531-1536.

Noller, A.C., McEllistrem, M.C., Pacheco, A.G.F., Boxrud, D.J. and Harrison, L.H., 2003. Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic *Escherichia coli* O157:H7 isolates. *J. Clin. Microbiol*. 41, 5389-5397.

On, S.L., Nielsen, E.M., Engberg, J. and Madsen, M., 1998. Validity of SamI defined genotypes of *Campylobacter jejuni* examined by SalI, KpnI and BamHI polimorphism: evidence of identical clones infecting humans, poultry and cattle. *Epidemiol. Infect.* 120, 231-237.

Peters, T.M., Berghold, C., Brown, D., Coia, J., Dionisi, A.M., Echeita, A., Fisher, I.S., Gatto, A.J., Gill, N., Green, J., Gerner-Smidt, P., Heck, M., Lederer, I., Lukinmaa, S., Luzzi, I., Maguire, C., Prager, R., Usera, M., Siitonen, A., Threlfall, E.J., Torpdahl, M., Tschäpe, H., Wannet, W. and Zwaluw, W.K., 2007. Relationship of pulsed-field profiles with key phage types of *Salmonella enterica* serotype Enteritidis in Europe: results of an international multi-centre study. *Epidemiol. Infect*. 135, 1274-1281.

Pulsenet Europe. <http://www.pulsenet-europe.org/docs.htm>

Pulsenet USA. <http://www.cdc.gov/PULSENET/protocols.htm>

Ribot, M., Fitzgerald, C., Kubota, K., Swaminathan, B., and Barrett, T. J., 2001. Rapid Pulsed-Field Gel Electrophoresis Protocol for Subtyping of *Campylobacter jejuni. J. Clin. Microbiol.* 39(5), 1889–1894.

Rivoal, K., Ragimbeau, C., Salvat, G., Colin, P. and Ermel, G., 2005. Genomic Diversity of *Campylobacter coli* and *Campylobacter jejuni* Isolates Recovered from Free-Range Broiler Farms and Comparison with Isolates of Various Origins. *Appl. Environ. Microbiol*. 71 (10), 6216–6227.

Ruppitsch, W., Indra, A., Stoeger, A., Mayer, B., Stadlbauer, S. and Wewalka, G., 2006. Classifying spa types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol*. 44, 2442-2448.

Sabat, A., Krzyszton-Russjan, J., Strzalka, W., Filipek, R., Kosowska, K., Hryniewicz, W., Travis, J. and Potemp J., 2003. New method for typing *Staphylococcus aureus* strains: Multiple-Locus Variable Number of Tandem Repeat Analysis of polimorphism and genetic relationship of clinical isolates. *J. Clin. Microbiol*. 41, 1801-1804.

Santesteban, E., Gibson, J. and Owen, R.J., 1996. Flagellin gene profiling of *Campylobacter jejuni* heat-stable serotype 1 and 4 complex. *Res. Microbiol*. 147, 641–649.

SEQnet. <www.SEQnet.org>

Shimizu, A., Kawano, J., Yamamoto, C., Kakutani, O., Anzai, T. and Kamada, M., 1997. Genetic analysis of equine methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis. *J. Vet. Med. Sci*. 59, 935–7.

Shopsin, B., Gomez, M., Montgomery, S.O., Smith, D.H., Waddington, M., Dodge, D.A., Bost, D.A., Riehman, M., Naidich, S. and Kreiswirth, B.N., 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J. Clin. Microbiol*. 37, 3556-3563

Staphylococcus aureus mlst. <http://saureus.mlst.net/>

Vali, L., Hamouda, A., Pearce, M.A., Knight, H.I., Evans, J. and Amyes, S.G.B., 1997. Detection of genetic diversity by pulsed-field gel electrophoresis among *Escherichia coli* O157 isolated from bovine faecal samples by immunomagnetic separation technique. *Lett. Appl. Microbiol.* 44, 19-23.

van Bergen, M.A., Dingle, K.E., Maiden, M.C., Newell, D.G., van der Graaf-Van Bloois, L., van Putten, J.P. and Wagenaar, J.A., 2005. Clonal nature of *Campylobacter fetus* as defined by multilocus sequence typing. *J. Clin. Microbiol*. 43 (12), 5888-5898.

Willshaw, G.A., Smith, H.R., Cheasty, T., Wall, P.G. and Rowe, B., 1997. Vero Cytoxin-Producing *Escherichia coli* O157 Outbreaks in England and Wales, 1995: Phenotypic Methods and Genotypic Subtyping. *Emerging Infect. Dis*. 3, 561-564.

Zhang, K., McClure, J. A., Elsayed, S., Louie, T. and Conly, J. M., 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol*. 43, 5026-5033.

ANNEX I

Table SA1. Molecular typing frequency of *Salmonella* isolates in countries by source of isolates

	Frequency of molecular typing		
	Animals	**Food**	**Feed**
MSs			
AT	Occasionally	Occasionally	Occasionally
BE	Occasionally	Occasionally	No
CY	Occasionally	Occasionally	No
DE	Occasionally	Occasionally	Occasionally
DK	Routine basis	Routine basis	No
ES	Occasionally	Occasionally	Occasionally
FI	Routine basis	No	Occasionally
FR	Occasionally	Occasionally	Occasionally
HU	No	Occasionally	No
IE	Occasionally	Occasionally	No
IT	Occasionally	Occasionally	Occasionally
LT	Occasionally	Occasionally	Occasionally
LU	Routine basis	Routine basis	Routine basis
NL	Occasionally	Occasionally	Occasionally
PL	Occasionally	Occasionally	Occasionally
RO	Occasionally	No	No
SE	Occasionally	Occasionally	Occasionally
SI	Occasionally	Occasionally	Occasionally
SK	Occasionally	Occasionally	Occasionally
UK	Occasionally	Occasionally	Occasionally
Non-MSs			
CH	Occasionally	Occasionally	Occasionally
NO	Occasionally	Occasionally	Occasionally
TR	Occasionally	Occasionally	No

Table SA2. Sampling context of *Salmonella* isolates for country

	Sampling related to official controls	Sampling related to outbreak investigations	Sampling related to research	Other sources
MSs				
AT	Yes	Yes	Yes	
BE	Yes	Yes	Yes	
CY	Yes	No	No	
DE	Yes	Yes	Yes	Zoo animals
DK	Yes	Yes	Yes	
ES	Yes	Yes	Yes	
FI	Yes	Yes	No	
FR	Yes	Yes	Yes	
HU	No	Yes	Yes	
IE	Yes	Yes	Yes	Samples from private laboratories
IT	No	Yes	Yes	
LT	Yes	Yes	No	
LU	Yes	Yes	Yes	
NL	Yes	Yes	Yes	
PL	Yes	No	Yes	
RO	No	No	Yes	
SE	Yes	Yes	Yes	
SI	Yes	Yes	Yes	
SK	Yes	Yes	Yes	
UK	Yes	Yes	Yes	
Non-MSs				
CH	No	Yes	Yes	
NO	Yes	Yes	Yes	
TR	Yes	Yes	Yes	

Table SA3. PFGE protocols for molecular typing of *Salmonella* isolates

	Standardised protocol	Details of standardised protocol	PulseNet Europe	Bionumerics
MSs				
AT	No		Yes	No
BE	No		Yes	Yes
CY	No		Yes	Yes
DE	No		Yes	Yes
DK	Yes		Yes	Yes
ES	No		Yes	Yes
FI	No		Yes	No
FR	Yes		Yes	Yes
HU	No		Yes	No
IE	No		Yes	Yes
IT	No		Yes	Yes
LU	No		Yes	Yes
NL	No		Yes	Yes
PL	Yes		Yes	Yes
RO	No		Yes	Yes
SE	No		Yes	Yes
SI	Yes	Biorad	Yes	Yes
SK	No		Yes	No
UK	Yes	Salm-gene protocol (Peters *et al*., 2007)	Yes	Yes
Non-MSs				
CH	Yes		Yes	Yes
NO	No		Yes	Yes
TR	Yes		No	Yes

Table SA4. MLVA protocols for molecular typing of *Salmonella* isolates

Country	MLVA protocols
AT	Beranek *et al*., 2009 - Lindstedt *et al*., 2004
BE	Lindstedt *et al*., 2004
DE	Lindstedt *et al*., 2004 - Malorny *et al*., 2008
DK	Lindstedt *et al*., 2004
FI	Lindstedt *et al*., 2004
IE	
LU	Lindstedt *et al*., 2004
NL	Lindstedt *et al*., 2004
SE	Lindstedt *et al*., 2004 - Lindstedt *et al*., 2003 - Lindstedt *et al*., 2007
SK	
UK	Linstedt *et al*., 2004 - Best *et al*., 2007 - Nordic standard method for *S*. Typhimurium which is a combination of Lindstedt *et al*., 2003; Lindstedt *et al*., 2004; plus Hopkins *et al.* 2007 - Trial method for *S*. Enteritidis based on Cho *et al*., 2008

Table SA5. Other methods for molecular typing of *Salmonella* isolates

Country	Other methods
DE	DNA microarray analysis, ribotyping, IS200 typing, PCR typing, plasmid profiling
ES	MLST
FR	Molecular serotyping, Diversilab
IE	Automated ribotyping (Qualicon)
IT	Ribotyping
PL	Plasmid profile analysis (Birnboim and Doly, 1989)
UK	Plasmid profile analysis, MLST, ribotyping, resistant/virulence array

Table CA1. Molecular typing frequency of *Campylobacter* isolates in countries by source of isolates

	Frequency of molecular typing		
	Animals	**Food**	**Feed**
MSs			
AT	Occasionally	Occasionally	No
BE	No	Occasionally	No
CZ	Routine basis	No	No
DE	Occasionally	Occasionally	No
DK	Occasionally	Occasionally	No
ES	Occasionally	Occasionally	Occasionally
FI	Occasionally	Occasionally	No
FR	Routine basis	Routine basis	No
HU	Occasionally	Occasionally	Occasionally
IE	Occasionally	Occasionally	No
IT	Routine basis	Routine basis	Routine basis
LT	Occasionally	Occasionally	Occasionally
LU	Routine basis	Routine basis	No
LV	Occasionally	Occasionally	No
NL	Occasionally	Occasionally	Occasionally
PL	Occasionally	Occasionally	No
SE	Occasionally	Occasionally	No
SI	Occasionally	Occasionally	No
UK	Occasionally	Routine basis	No
Non MSs			
CH	Routine basis	Occasionally	Occasionally
NO	Occasionally	Occasionally	No
TR	Occasionally	Occasionally	No

Table CA2. Sampling context of *Campylobacter* isolates for country

	Sampling related to official controls	Sampling related to outbreak investigations	Sampling related to research	Other sources
MSs				
AT	No	Yes	Yes	
BE	No	No	Yes	
CZ	Yes	No	No	
DE	Yes	Yes	Yes	
DK	Yes	Yes	Yes	
ES	Yes	Yes	Yes	
FI	Yes	Yes	Yes	
FR	Yes	Yes	Yes	
HU	No	Yes	Yes	
IE	Yes	Yes	Yes	
IT	Yes	Yes	Yes	
LT	Yes	No	No	
LU	Yes	No	Yes	
LV	Yes	No	Yes	
NL	Yes	Yes	Yes	
PL	No	Yes	Yes	
SE	Yes	No	Yes	
SI	Yes	Yes	Yes	
UK	Yes	Yes	Yes	On request from private companies, medical and veterinary laboratories
Non-MSs				
CH	Yes	Yes	Yes	
NO	Yes	Yes	Yes	
TR	No	Yes	Yes	

Table CA3. PFGE protocols for molecular typing of *Campylobacter* isolates

Country	Standardised protocol	Details of standardised protocol	Other protocols	Details of other protocols	Bionumerics
MSs					
AT	No		Yes	Modification of Ribot *et al*., 2001	Yes
BE	Yes	PulseNet USA	No		Yes
ES	Yes		No		Yes
FI	Yes	PulseNet USA	No		Yes
FR	Yes	Campynet protocol	Yes	Rivoal *et al.*, 2005	Yes
HU	Yes	Campynet protocol	Yes	Modification of Michaud *et al*., 2001 - Ribot *et al*., 2001	Unknown
IT	Yes	PulseNet USA	No		Yes
NL	No		Yes	On *et al.*, 1998	Yes
PL	Yes	PulseNet USA	No		No
SE	Yes	Campynet protocol	No		Yes
SI	Yes	PulseNet USA	Yes	Biorad	No
UK	Yes	Campynet protocol - PulseNet USA	No		Yes
Non-MSs					
CH	Yes		No		Yes

Table CA4. MLST protocols for molecular typing of *Campylobacter* isolates

	MLST protocols
MSs	
DE	Dingle *et al.,* 2001
DK	Dingle *et al.*, 2001
ES	
IE	Dingle *et al.,* 2001
LU	
NL	van Bergen *et al*., 2005
SE	
UK	Dingle *et al*., 2001 - Oxford method based on a combination of Dingle *et al.*, 2001 and Miller *et al.*, 2005 - Fla SVR as per Meinersmann *et al.*, 1997 and Dingle *et al*., 2002
Non-MSs	
CH	

Table CA5. Other methods for molecular typing of *Campylobacter* isolates

	Other methods
MSs	
BE	AFLP
CZ	PCR
DE	FlaA-SVR-sequencing; AFLP (Duim *et al*., 1999)
ES	RFLP of gene flaA
FR	PCR-RFLP
HU	RepPCR
IE	Automated ribotyping (Qualicon); FlaA-SVR typing
IT	AFLP (Champion *et al*., 2002)
LV	Cycle Sequencing of 16S rDNA
NL	PCR_RFLP (Nachamkin *et al*., 1993)
SE	FlaA typing
UK	Sequence based typing of *fla* short variable region (*fla*-SVR) (Santesteban *et al.*, 1996); occasionally fla typing (Ayling *et al.*, 1996)
Non-MSs	
CH	Fla typing
NO	AFLP

Table VT1. Molecular typing frequency of VTEC isolates in countries by source of isolates

	Frequency of molecular typing		
	Animals	**Food**	**Feed**
MSs			
AT	Occasionally	Routine basis	No
BE	Occasionally	Occasionally	Occasionally
CZ	Occasionally	Occasionally	No
DE	Routine basis	Routine basis	No
DK	Occasionally	Occasionally	No
ES	Occasionally	Occasionally	Occasionally
FI	Occasionally	Occasionally	No
FR	Routine basis	Routine basis	No
HU	No	Occasionally	No
IE	Occasionally	Occasionally	No
IT	Routine basis	Routine basis	No
LT	Occasionally	Occasionally	Occasionally
LU	Occasionally	No	No
LV	No	Routine basis	No
NL	Routine basis	Routine basis	Occasionally
PL	Occasionally	Occasionally	No
SE	Occasionally	Occasionally	No
SI	Occasionally	Occasionally	No
SK	No	Occasionally	No
UK	Occasionally	Occasionally	No
Non-MSs			
CH	Occasionally	Occasionally	Occasionally
NO	Occasionally	Occasionally	Occasionally

Table VT2. Sampling context of VTEC isolates for country

	Sampling related to official controls	Sampling related to outbreak investigations	Sampling related to research	Other sources
MSs				
AT	Yes	Yes	No	
BE	No	Yes	Yes	
CZ	No	No	Yes	
DE	Yes	Yes	Yes	
DK	Yes	Yes	Yes	
ES	No	Yes	Yes	
FI	Yes	Yes	Yes	
FR	No	Yes	Yes	Food industry controls
HU	Yes	No	No	
IE	Yes	Yes	Yes	Private laboratories
IT	Yes	Yes	Yes	
LT	Yes	No	No	
LU	Yes	No	Yes	
LV	Yes	No	No	
NL	Yes	Yes	Yes	
PL	No	Yes	Yes	
SE	Yes	Yes	Yes	
SI	Yes	Yes	Yes	Proficiency testing CRL for *E. coli*
SK	Yes	Yes	No	
UK	No	Yes	Yes	
Non-MSs				
CH	No	Yes	Yes	
NO	Yes	Yes	Yes	

Table VT3. PFGE protocols for molecular typing of VTEC isolates

	Standardised protocol	Details of standardised protocol	PulseNet protocol	Bionumerics
MSs				
AT	Yes	Internal procedure	No	Yes
BE	Yes		Yes	Yes
CZ	Yes	Vali *et al*., 1997	No	No
DE	Yes		Yes	Yes
DK	Yes		Yes	Yes
ES	No		Yes	Yes
FI	No		Yes	Yes
FR	No		Yes	No
IE	No		Yes	Yes
IT	No		Yes	Yes
LU	Unknown		Unknown	Unknown
NL	No		Yes	Yes
PL	No		Yes	No
SE	No		Yes	Yes
SI	Yes	Biorad	Yes	Yes
SK	No		Yes	No
UK	Yes	Willshaw *et al.*, 1997	Yes	Yes
Non-MSs				
CH	Yes		Yes	Yes
NO	No		Yes	Yes

Table VT4. MLVA protocols for molecular typing of VTEC isolates

	MLVA protocols
MSs	
CZ	Kawamori *et al*., 2008
DE	
ES	Noller *et al*., 2003
IE	Noller *et al*., 2003
NL	
SE	A modified PulseNet protocol
UK	PulseNet USA (based on Hyytia-Trees *et al*., 2006)
Non-MSs	
NO	Lindstedt *et al*., 2004

Table VT5. Other methods for molecular typing of VTEC isolates

	Other methods
MSs	
DE	MLST, virulotyping
FR	Rep PCR
HU	PCR based characterisation of the strain (serotype, eae gene, type of toxin)
IE	Automated ribotyping (Qualicon)
LU	Typing of shiga-toxins
LV	Conventional PCR for detection of verocytotoxins producing genes: vtx1, vtx2, eaeA
PL	RAPD-PCR, ERIC-PCR
UK	PCR to detect VT1, VT2 and intimin genes
Non-MSs	
CH	MLST, microarrays for virulence and serotyping

Table LI1. Molecular typing frequency of *Listeria monocytogenes* isolates in countries by source of isolates

	Frequency of molecular typing		
	Animals	**Food**	**Feed**
MSs			
AT	Occasionally	Routine basis	Occasionally
BE	No	Occasionally	No
CZ	Occasionally	Routine basis	Occasionally
DE	Occasionally	Occasionally	No
DK	Occasionally	Occasionally	No
FI	Occasionally	Routine basis	Occasionally
FR	Routine basis	Routine basis	Routine basis
IE	Occasionally	Occasionally	No
IT	Occasionally	Occasionally	Occasionally
LU	No	Routine basis	Routine basis
NL	Occasionally	Routine basis	Occasionally
SE	Occasionally	Occasionally	No
SI	Occasionally	Occasionally	No
SK	No	Occasionally	No
UK	No	Routine basis	No
Non-MSs			
CH	No	Occasionally	No
NO	Occasionally	Occasionally	Occasionally
TR	Occasionally	No	No

Table LI2. Sampling context of *Listeria monocytogenes* isolates for country

	Sampling related to official controls	Sampling related to outbreak investigations	Sampling related to research	Other sources
MSs				
AT	Yes	Yes	No	
BE	No	Yes	No	
CZ	Yes	No	No	HACCP verification by producers
DE	Yes	Yes	Yes	
DK	Yes	Yes	No	
FI	Yes	Yes	Yes	
FR	Yes	Yes	Yes	
IE	Yes	Yes	Yes	
IT	Yes	Yes	Yes	
LU	Yes	No	Yes	
NL	Yes	Yes	Yes	
SE	Yes	No	Yes	
SI	Yes	Yes	Yes	
SK	Yes	Yes	No	
UK	Yes	Yes	No	
Non-MSs				
CH	No	No	Yes	For the food industry
NO	No	Yes	Yes	
TR	No	Yes	Yes	

Table LI3. PFGE protocols for molecular typing of *Listeria monocytogenes* isolates

	Standardised method	Details of standardised method	PulseNet Europe	Bionumerics
MSs				
AT	No		Yes	Yes
BE	No		Yes	Yes
CZ	Unknown		Unknown	Unknown
DE	No		Yes	Yes
DK	Yes		Yes	Yes
FI	No		Yes	Yes
FR	No		Yes	Yes
IE	No		Yes	Yes
IT	No		Yes	Yes
LU	Unknown		Unknown	Unknown
NL	No		Yes	Yes
SE	Yes		Yes	Yes
SI	No		Yes	Yes
SK	No		Yes	No
Non-MSs				
CH	No		Yes	Yes
NO	No		Yes	Yes

Table LI4. Other methods for molecular typing of *Listeria monocytogenes* isolates

	Other methods
MSs	
CZ	PCR
FR	Molecular serotyping, Diversilab
IE	Automated ribotyping (Qualicon)
SE	Ribotyping
UK	Fluorescent AFLP
Non-MSs	
CH	REP-PCR, ERIC-PCR
TR	RT-PCR

Table ST1. Molecular typing frequency of *Staphylococcus aureus* isolates in countries by source of isolates

	Frequency of molecular typing		
	Animals	**Food**	**Feed**
MSs			
AT	Occasionally	Occasionally	Occasionally
BE	Routine basis	Occasionally	No
CZ	Occasionally	No	No
DE	Occasionally	Occasionally	No
DK	Occasionally	No	No
ES	Routine basis	No	No
FI	Routine basis	No	No
FR	Occasionally	Occasionally	No
HU	Occasionally	Occasionally	No
IE	Occasionally	Occasionally	No
IT	Occasionally	Occasionally	No
LT	Occasionally	Occasionally	Occasionally
LU	Routine basis	No	No
LV	Occasionally	No	No
NL	Occasionally	Occasionally	Occasionally
SE	Occasionally	Occasionally	No
SI	Occasionally	Occasionally	No
SK	Occasionally	Occasionally	No
UK	Occasionally	No	No
Non-MSs			
CH	Occasionally	Occasionally	No
NO	Occasionally	Occasionally	Occasionally
TR	Occasionally	No	No

Table ST2. Sampling context of *Staphylococcus aureus* isolates for country

	Sampling related to official controls	Sampling related to outbreak investigations	Sampling related to research	Other sources
MSs				
AT	Yes	Yes	Yes	
BE	Yes	Yes	Yes	
CZ	Yes	No	No	
DE	Yes	No	Yes	
DK	Yes	Yes	Yes	
ES	Yes	No	No	Ring trial
FI	Yes	Yes	Yes	Yes
FR	Yes	Yes	Yes	
HU	Yes	No	No	
IE	Yes	Yes	Yes	
IT	Yes	Yes	Yes	
LT	Yes	No	No	
LU	No	Yes	Yes	
LV	Yes	No	No	
NL	Yes	Yes	Yes	
SE	No	No	Yes	
SI	No	Yes	Yes	
SK	No	No	Yes	
UK	Yes	No	No	Veterinary diagnostic submission from mastitis
Non-MSs				
CH	No	No	Yes	
NO	No	No	Yes	Diagnostic samples
TR	No	Yes	Yes	

Table ST3. PFGE protocols for molecular typing of *Staphylococcus aureus* isolates

	Standardised protocol	Details of standardised protocol	PulseNet protocol	Bionumerics
MSs				
AT	Yes	Murchan *et al.*, 2003	No	No
BE	Yes		No	Yes
DE	Yes	Murchan *et al.*, 2003	No	No
DK	Yes	Murchan *et al*., 2003	No	Yes
FI	Yes	Murchan *et al.*, 2003	No	Yes
FR	Yes		No	Yes
IT	Yes		No	No
LU	Yes	Harmony protocol	No	Yes
NL	No		Yes*	Yes
SE	Yes	Shimizu *et al.*, 1997 - Harmony protocol	No	No
SI	Unknown		Unknown	Yes
SK	Yes	Mulvey *et al*., 2001 - Harmony protocol	No	No
UK	No		Yes*	Yes
Non-MSs				
CH	No		Yes*	No
NO	No		Yes**	Yes

* Using the PulseNet USA protocol

** Using the protocol described by McDougal *et al*., 2003

Table ST4. MLST protocols for molecular typing of *Staphylococcus aureus* isolates

	MLST protocols
MSs	
AT	Enright *et al.,* 2000
BE	
DE	Enright *et al.,* 2000
DK	Enright *et al.,* 2000
ES	CRL-AR
FI	
HU	Enright *et al*., 2000 - *S. aureus* MLST database
LU	
NL	Huijdens *et al.*, 2006
UK	

Table ST5. Spa typing protocols for molecular typing of *Staphylococcus aureus* isolates

	Spa typing protocol
MSs	
AT	Ruppitsch *et al.*, 2006
BE	
DE	Shopsin *et al.*, 1999
DK	www.SEQnet.org
ES	CRL-AR
FI	
FR	
HU	Shopsin *et al.*, 1999
IT	
LT	
LU	
NL	De Neeling *et al.*, 2007
UK	

Table ST6. Other methods for molecular typing of *Staphylococcus aureus* isolates

	Other methods
MSs	
BE	SCC*mec* typing
CZ	PCR
DE	SCC*mec* (Zhang *et al.*, 2005)
ES	SCC*mec* - Real Time PCR
FR	Genes encoding enterotoxins PCR detection
LV	Conventional PCR for detection of *mecA* gene
UK	Identibac microarray
Non-MSs	
NO	Multiplex PCR for enterotoxin genes (Løvseth *et al.*, 2004)

ANNEX II

List of general abbreviations

AFLP	Amplified Fragment Length Polymorphism
CRL	Community Reference Laboratory
CRL-AR	Community Reference Laboratory for Antimicrobial Resistance
MLST	Multi Locus Sequence Typing
MLVA	Multi Locus Variable-Number Tandem Repeat Analyses
MS	Member State
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RFLP	Restriction Fragment Length Polymorphism
SCC*mec*	Staphylococcal Cassette Chromosome *mec*

Countries

AT	Austria
BE	Belgium
BG	Bulgaria
CH	Switzerland
CZ	Czech Republic
CY	Cyprus
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
DE	Germany
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
LT	Lithuania
LU	Luxembourg
LV	Latvia
MK	Former Yugoslav Republic of Macedonia
MT	Malta
NL	Netherlands
NO	Norway
PL	Poland
PT	Portugal
RO	Romania
SK	Slovakia
SI	Slovenia
SE	Sweden
TR	Turkey
UK	United Kingdom