#### Technical University of Denmark



# The 19th EURL-AR Proficiency Test Salmonella, Campylobacter and genotypic characterisation 2015

Karlsmose Pedersen, Susanne; Cavaco, Lina; Hendriksen, Rene S.; Aarestrup, Frank Møller

Publication date: 2016

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

Karlsmose Pédersen, S., Cavaco, L., Hendriksen, R. S., & Aarestrup, F. M. (2016). The 19th EURL-AR Proficiency Test Salmonella, Campylobacter and genotypic characterisation 2015. Søborg: National Food Institute, Technical University of Denmark.

#### DTU Library

Technical Information Center of Denmark

#### General rights

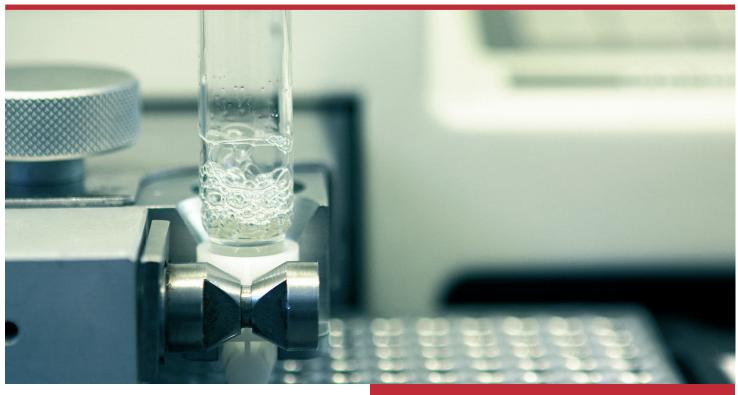
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



# The 19th EURL-AR Proficiency Test Salmonella, Campylobacter and genotypic characterisation 2015



Susanne Karlsmose Lina M. Cavaco Rene S. Hendriksen Frank M. Aarestrup







**DTU Food**National Food Institute

# The 19th EURL-AR Proficiency Test *Salmonella*, *Campylobacter* and genotypic characterisation 2015

1. edition, June 2016

Copyright: National Food Institute, Technical University of Denmark

Photo: Mikkel Adsbøl ISBN: 978-87-93109-86-5

The report is available at www.food.dtu.dk

National Food Institute Technical University of Denmark Mørkhøj Bygade 19 2860 Søborg





# Index

1.	Introduction		4
2.	Materials and Meth	nods	5
	2.1 Participants in	EQAS 2015	5
	2.2 Strains		6
	2.3 Antimicrobials.		6
	2.4 Distribution		7
	2.5 Procedure		7
3.	Results		9
	3.1 Data omitted fr	om the report	9
	3.2 Methods		9
	3.3 Deviations, over	erall	9
	3.4 Deviations by I	aboratory	11
	3.5 Deviations by r	reference strains	13
	3.6 Genotypic char	racterisation	14
4.	Discussion		14
	4.1 Salmonella tria	ıl	15
	4.2 Campylobacte	<i>r</i> trial	15
	4.3 Genotypic char	racterisation	16
5.	Conclusions		16
6.	References		17
	Appendix 1	Pre-notification	
	Appendix 2	Participant list	
	Appendix 3a	Reference values (MIC) – Salmonella	
	Appendix 3b	Reference values (MIC) – Campylobacter	
	Appendix 4a	Welcome letter	
	Appendix 4b	Protocol, text	
	Appendix 4c	Protocol, test forms	
	Appendix 4d	Instructions for opening and reviving lyophilised cultures	
	Appendix 4e	Subculture and maintenance of Quality Control strains	
	Appendix 5	Quality Control ranges for ATCC reference strains	
	Appendix 6a	Reference strain results – E. coli ATCC 25922	
	Appendix 6b	Reference strain results – C. jejuni ATCC 33560	
	Appendix 7a	Expected and obtained results – Salmonella	
	Appendix 7b	Expected and obtained results – Campylobacter	
	Appendix 8a	Deviations, Salmonella	
	Appendix 8b	Deviations, Campylobacter	
	Appendix 9	Genotypic characterisation (optional)	





## 1. Introduction

This report describes and summarises results from the nineteenth proficiency test trial conducted by the National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). proficiency test focuses on antimicrobial susceptibility testing (AST) of Salmonella and Campylobacter and is the ninth External Quality Assurance System (EQAS) conducted for these microorganisms (the first was EQAS 2006). In addition, the proficiency test includes categorization of the relevant Salmonella strains as presumptive AmpC-, ESBL-, or carbapenemase producing organisms, and identification of the Campylobacter species as either C. jejuni or C. coli,

In addition, for the seventh time, an optional element was included, consisting of genotypic characterization by PCR/sequencing of antimicrobial resistance genes. This optional component included characterization of genes related to production of AmpC, ESBL- and carbapenemases in the *Salmonella* test strains.

This EQAS aims to: i) monitor the quality of AST results produced by National Reference Laboratories (NRL-AR), ii) identify laboratories which may need assistance to improve their performance in AST, and iii) determine possible topics for further research or collaboration.

In reading this report, the following important considerations should be taken into account:

1) Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determinations for all test strains in two different occasions at the Technical University of Denmark, National Food Institute (DTU Food). These results were then verified by the United States Food and Drug Administration (FDA), Centre for Veterinary Medicine. Finally, a fourth MIC determination was performed at DTU Food after preparation of the agar stab culture for shipment to participants to confirm

that the vials contained the correct strains with the expected MIC values.

- 2) Evaluation is based on interpretations of AST values determined by the participants. This is in agreement with the method used by Member States (MS) to report AST data to the European Food Safety Authority (EFSA), and complies with the main objective of this EQAS, i.e. to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of Salmonella and Campylobacter reported to EFSA by different laboratories, as stated in the protocol.
- 3) The EURL-AR network agreed on setting the accepted deviation level for laboratory performance on AST to 5%. For the optional genotypic characterisation, no specific acceptance level has been set.

Evaluation of a result as "deviating from the expected interpretation" should be carefully analyzed in а self-evaluation procedure performed by the participant including also considerations related to any corrective actions introduced in the laboratory. Note, that since methods used for MIC determination have limitations, it is not considered a mistake to obtain a one-fold dilution difference in the MIC of a specific antimicrobial when testing the same strains. If, however, the expected MIC is close to the breakpoint value for categorizing the strain as susceptible or resistant, a one-fold dilution difference - which is acceptable - may result in two different interpretations, i.e. the same strain can be categorized as susceptible and resistant. This result will be evaluated as correct in one case but incorrect when the evaluation is based on interpretation of MIC values. This report is based on evaluation of AST interpretations, therefore some participants may find their results classified as incorrect even though the actual MIC they reported is only a one-fold dilution different from the





expected MIC. In these cases, the participants should be confident about the good quality of their performance of AST by MIC. In the organization of the EQAS, we try to avoid these situations by choosing test strains with MIC values distant from the breakpoints for resistance, which is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AR network unanimously established in 2008 that if there are less than 75% correct results for a specific strain/antimicrobial combination, the reasons for this situation must be further examined and, on selected occasions explained in details case by case, these results may subsequently be omitted from the evaluation report.

This report is approved in its final version by a technical advisory group composed by

competent representatives from all NRL-ARs. This group meets annually at the EURL-AR workshop.

All conclusions presented in this report are publically available. Participating laboratories are identified by codes and each code is known only by the corresponding laboratory. The full codes is confidential list of laboratory information known only by relevant representatives of the EURL-AR and the EU Commission.

The EURL-AR is accredited by DANAK as provider of proficiency testing (accreditation no. 516); working with zoonotic pathogens and indicator organisms as bacterial isolates (identification, serotyping and antimicrobial susceptibility testing).

#### 2. Materials and Methods

#### 2.1 Participants in EQAS 2015

A pre-notification (App. 1) to announce the EURL-AR EQAS on AST of Salmonella and Campylobacter was distributed on the 23<sup>th</sup> June 2015 by e-mail to the 43 NRLs in the EURL-ARnetwork including all EU countries and Iceland, Norway, Serbia, Switzerland and Turkey. All EU MS as well as Iceland, Norway, and Switzerland were represented as participants for both Salmonella and Campylobacter. In addition to the AST of Salmonella and Campylobacter, an optional genotypic characterization by PCR/sequencing of antimicrobial resistance aenes the AmpC-, ESBLand carbapenemase-producing Salmonella test strains was offered.

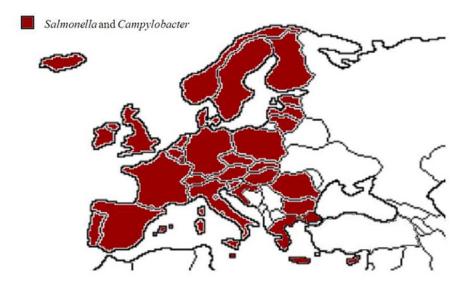
Appendix 2 shows that 29 of the 34 participating NRLs were appointed by the individual Member States' Competent Authority. Five additional laboratories were included; one from each of the following countries: Iceland, the Netherlands, Norway, Spain, and

Switzerland. These were invited to take part in the EQAS 2015 on the basis of their participation in previous EQAS iterations and/or affiliation to the EU network. These laboratories were charged a fee for their participation in the EQAS, whereas the NRLs from EU Member States participated free of charge.

Figure 1 illustrates that of the 31 participating countries, all tested both *Salmonella* and *Campylobacter*. Nine laboratories participated in the optional genotypic characterisation of the ESC-producing *Salmonella* test strains (not illustrated in Figure 1; see Appendix 2).

The results from the NRLs designated by the MS are presented and evaluated in this report in addition to national reference laboratories in affiliated non-MS; i.e. results from 31 countries consisting of 31 laboratories submitting Salmonella results and 31 laboratories submitting Campylobacter results. Results from the two laboratories not designated by the MS but enrolled on equal terms as these are not further presented or evaluated in this report.





**Figure 1:** Participating countries that performed antimicrobial susceptibility testing of *Salmonella* and *Campylobacter*.

#### 2.2 Strains

Eight Salmonella strains and eight Campylobacter strains were selected for this trial among isolates from the strain collection at DTU Food on the basis of antimicrobial resistance profiles and MIC values. For quality assurance purposes, one strain per bacterial species has been included in all EQAS iterations performed to date, representing an internal control.

Prior to distribution of the strains, AST was performed the Salmonella and Campylobacter strains at DTU Food and the verified by US Food Administration (FDA). When MIC-values were not in agreement but varied +/- one MIC-step, the value obtained by DTU Food was selected as the reference value. The obtained MIC values served as reference for the test strains (App. 3a and 3b). Results from the following antimicrobials were not verified by FDA: cefepime, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, colistin, ertapenem, imipenem, meropenem, temocillin, tigecycline and trimethoprim for Salmonella and furthermore, streptomycin for

#### Campylobacter.

Reference strains *Escherichia coli* CCM 3954 (ATCC 25922) and *Campylobacter jejuni* CCM 6214 (ATCC 33560) were provided to new participating laboratories with instructions to store and maintain them for quality assurance purposes and future EQAS trials.

#### 2.3 Antimicrobials

The antimicrobials tested in this EQAS are listed in the protocol (App. 4b).

The antimicrobials tested correspond to the panel of antimicrobials listed in Decision 2013/652/EU.

Guidelines for performing AST were set according to the Clinical and Laboratory Standards Institute (CLSI) document; M7-A10 (2015) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Tenth Edition": M100-S25 (2015)"Performance Standards for Antimicrobial Susceptibility Testing" (Twenty-Fifth Informational Supplement) and document VET01-A4 (2013) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial



**DTU Food** National Food Institute

Isolated From Animals" (Approved Standard – Fourth Edition).

MIC results were interpreted by using the interpretative criteria listed in Decision 2013/652/EU. Where values were not available, list interpretative criteria the of supplemented with CLSI-interpretative criteria as described and indicated in the protocol (App. 4). No interpretative criteria were available to determine the interpretation of MIC-values from testing of azithromycin, cefepime and temocillin. Results of ESC detection tests were interpreted according to the at deadline most recent EFSA recommendations (EFSA Journal 10(6):2742).

The selection of antimicrobials used in the trial ampicillin Salmonella were: (AMP), azithromycin (AZI), cefepime (FEP), cefotaxime (FOT), cefotaxime/clavulanic acid (FOT/CI), cefoxitin (FOX), ceftazidime (TAZ),ceftazidime/clavulanic acid (TAZ/CI), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), ertapenem (ERT), gentamicin (GEN), imipenem (IMI), meropenem (MER), nalidixic acid (NAL), sulfonamides (sulfamethoxazole) (SMX), tetracycline (TET), tigecycline (TGC), temocillin (TRM) trimethoprim (TMP).

Minimum Inhibitory Concentration (MIC) determination of the *Salmonella* test strains was performed using the Sensititre system from Trek Diagnostic Systems Ltd, UK. For ESC confirmatory test, the analysis included MIC determination by microbroth dilution.

For Campylobacter the following antimicrobials were included: ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), streptomycin (STR), and tetracycline (TET). MIC determination for the Campylobacter testing, was performed using the Sensititre systems from Trek Diagnostic Systems Ltd, UK, according quidelines from the CLSI document M45-A2 (2010)"Methods Antimicrobial Dilution and Disk Susceptibility

Testing of Infrequently Isolated or Fastidious Bacteria" (Approved Guideline – Second Edition) and VET01-S2 (2013) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals" (Second Informational Supplement). Participants of the *Campylobacter* EQAS were additionally requested to identify the species of the *Campylobacter* spp. as either *C. jejuni* or *C. coli.* 

#### 2.4 Distribution

On the 14<sup>th</sup> October 2015, bacterial strains in agar stab cultures (*Salmonella* spp.) or charcoal swabs in transport media (Stuarts) (*Campylobacter* spp.) together with a welcome letter (App. 4a) were dispatched in double pack containers (class UN 6.2) to the participating laboratories. The shipment (UN3373, biological substances category B) was sent according to International Air Transport Association (IATA) regulations.

#### 2.5 Procedure

Protocols and all relevant information were uploaded on the EURL-AR website (<a href="http://www.eurl-ar.eu">http://www.eurl-ar.eu</a>), thereby EQAS participants could access necessary information at any time.

Participants were instructed to subculture charcoal swabs immediately, store the agar stabs at 4°C (dark) and the freeze-dried strains cool and dark until performance of AST. Information related to the handling of the test strains and reference strains (App. 4b, c, d, e) was made available. Participants receiving an ATCC reference strain were requested to save and maintain this strain for future proficiency tests.

The participants were instructed to apply the interpretative criteria listed in the protocol (App. 4). Instructions for interpretation of AST results allowed for categorization of results as resistant or susceptible. Categorisations as 'intermediate' were not accepted.



The EURL-AR is aware that there are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data using epidemiological cutoff values, bacteria should be reported as 'wildtype' or 'non-wild-type' (Schwarz et al., 2010). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistant, even in cases where we are referring to wild-type and non-wild-type strains.

As regards the method for performing the antimicrobial susceptibility testing, the protocol referred to Decision 2013/652/EU and instructed participants to perform a dilution method, i.e. microbroth dilution or agar dilution. Results obtained by methods not complying with the description in Decision 2013/652/EU were not included in the present analysis.

A mandatory part of the proficiency test was to detect ESC-producing strains and interpret results according to the most recent EFSA recommendations (EFSA Journal 2012; 10(6):2742) as described in the protocol.

Results from QC reference strains would consist of MIC values for the reference strains *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560). The results were evaluated towards the quality control ranges according to the relevant guidelines; i.e. the CLSI documents VET01-S2 (2013) or M100-S25 (2015) (App. 5).

For the optional genotypic characterisation of the ESC-producing *Salmonella* test strains, participating laboratories were requested to report the genes conferring resistance to extended-spectrum beta lactam antimicrobials. The organizers, however, decided to include none-ESC TEM-genes resulting in *bla*<sub>TEM-1</sub> registered as an expected gene, also. The

genes listed in the table in the protocol (App. 4b) were included in the test. Identification of additional genes not listed in the protocol was not evaluated by the database. The results were evaluated based on the actual genes and variants identified.

The participating laboratories were encouraged to use their own laboratory's method(s) for the genotypic characterisation. The expected results for this component of the EQAS were obtained by whole-genome-sequencing and subsequent analysis using the ResFinder 2.1 platform available at http://cge.cbs.dtu.dk/services/ResFinder/. The positive identification of genes was not verified elsewhere.

All participating laboratories were invited to enter the obtained results into an electronic record sheet at the EURL-AR web-based database through a secured individual login and password. The record sheet contained space for reporting the results obtained for the QC reference strains. Alternatively, it was offered the possibility to fill-in a record sheet (provided with the protocol) and to send it to the EURL-AR by fax, mail or email.

In addition, participants were encouraged to complete an evaluation form available at the EURL-AR database with the aim to improve future EQAS trials.

finally The database was closed and evaluations were made available to participants on the 9<sup>th</sup> December 2015. After this date, the participants were invited to login to retrieve an individual, database-generated report which contained an evaluation of the submitted results including possible deviations from the expected interpretations. Deviations in the interpretation as resistant or susceptible were categorised as 'incorrect', as were also deviations concerning confirmation of an isolate as extended spectrum beta-lactamase-(ESBL-), ampCcarbapenemase-producer.





#### 3. Results

The participants were asked to report results, i.e. MIC values and the categorisation as resistant or susceptible. Only the categorisation was evaluated, whereas the MIC values were used as supplementary information.

#### 3.1 Data omitted from the report

As mentioned in the introduction, the EURL-AR network established that data should be examined and possibly omitted from the general analysis if there are less than 75% correct results based on strain/antimicrobial combination (see Appendix 8 for an overview of correct/incorrect results). In the present EQAS this occurred in two cases which have been examined and consequently omitted from the S10.4/imipenem analysis: 1) (expected interpretation was 'resistant', however, 30% (9 laboratories) found the strain susceptible to imipenem. All but one of the deviating interpretations were based on MIC values two steps from the expected, one was three steps expected; 2) C10.4/tetracycline from the interpretation was 'susceptible', (expected however, 26% of participants found the strain 'resistant' to tetracycline. ΑII deviating interpretations were based on MIC values only one step from the expected. Both these combinations were subsequently omitted from further analysis.

#### 3.2 Methods

The agar dilution method and MIC determination were evaluated together as they are both quantitative methods giving results corresponding to the MIC of the bacterial strain tested.

In the *Salmonella* as well as the *Campylobacter* trial, 30 laboratories performed microbroth dilution and one performed agar dilution.

With the aim to conclude on the strain's presumptive ESBL, AmpC or carbapenemase

phenotype, two panels of antimicrobials were included in the testing of the *Salmonella* strains. The test strains found resistant to cefotaxime, ceftazidime or meropenem on the first panel were additionally tested on the second panel according to the protocol indications.

#### 3.3 Deviations, overall

The list of deviations is shown in Appendix 8a and 8b. Figure 2 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. The internal control strains mainly followed the trend in deviation level of the different EQAS trials (Figure 2). The deviation level in 2015 is acceptable for both the Salmonella and the Campylobacter trials. For the current EQAS, for both microorganisms, it appears that there has been a decrease in the level of deviations, to 0.7% Salmonella for and 1.6% Campylobacter in 2015 from levels at 2.4% and 4.0%, respectively in 2014.

#### 3.3.1 Salmonella trial

For the *Salmonella* strains, 99.3% of the AST's were interpreted correctly. The number of AST's performed and the percentage of correct results for the individual strains in the EQAS, are listed in Table 1. Variations of obtained correct results ranged from 98.5-99.8% between the *Salmonella* strains. Table 2 illustrates the percentage of correct AST per antimicrobial by bacterial species. The level of correct AST was at 97.8% (tigecycline) or above, for all the *Salmonella* test strains.

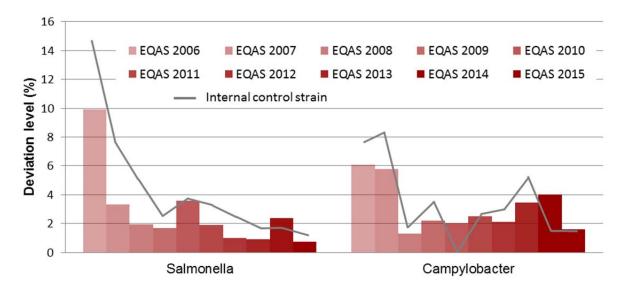
#### ESC-producing Salmonella test strains

Confirmation of beta-lactamase production is a mandatory component of this EQAS.

According to the protocol, which was based on the EFSA recommendations, the confirmatory test for ESC-production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone







**Figure 2:** A comparison between the EURL-AR EQAS's since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing performed by participating laboratories.

and in combination with a  $\beta$ -lactamase inhibitor. The MIC value for either antimicrobial agent (FOT or TAZ) tested in combination with clavulanic acid should be compared to the corresponding MIC when tested alone. Synergy is indicated if a three dilution steps difference is observed between the two MIC values (i.e. if the FOT:CTX/CI or TAZ:TAZ/CI ratio ≥8) (CLSI M100 2A: Table Enterobacteriaceae). Participants were instructed to test strains presenting resistance to cefotaxime (FOT), ceftazidime (TAZ or meropenem (MERO) on the second panel of antimicrobials.

The classification of the phenotypic results was based on the most recent EFSA recommendations (EFSA 2012), indicating:

- Presumptive ESBL-phenotype: strains with positive synergy test, susceptible to cefoxitin and resistant to cefepime
- Presumptive ESBL+pAmpC-phenotype: strains with positive or negative synergy test, resistant to cefoxitin and resistant to cefepime

- Presumptive pAmpC phenotype: strains with negative synergy test resistant to cefoxitin and susceptible to cefepime
- Presumptive carbapenemase phenotype: strain resistant to meropenem
- Unusual phenotype: any other combinations

In this EQAS, all laboratories uploaded results for the strains exhibiting resistance to the cephalosporins tested.

The strains S-10.3, S-10.7 and S-10.8 were ESBL-producers, and S-10.4 was a carbapenemase-producer. Note that when categorizing the presumptive phenotypes, the interpretation of the cefepime result had to be disregarded, as no interpretative criteria were available.

In total, the categorization as ESBL-, pAmpCor carbapenemase-producer was correct in almost all cases; i.e. out of 248 reported results, three were incorrect. The deviating results were presented by three different laboratories (#39, #56, and #60). For laboratory #39, the reported phenotypic results do not explain the selected presumptive phenotype for S-10.4 as 'unusual





**Table 1.** The number of AST performed and the percentage of correct results for each strain of *Salmonella* and *Campylobacter*.

EQAS	1     400     99.3       2     400     99.8       3     587     98.8       4     552     99.6       5     398     98.5       6     399     99.5		EQAS 2015 – Campylobacter							
Test strain	AST in total	% correct	Test strain	AST in total	% correct					
S-10.1	400	99.3	C-10.1 ( <i>C. jejuni</i> )	185	97.3					
S-10.2	400	99.8	C-10.2 (C. coli)	186	98.4					
S-10.3	587	98.8	C-10.3 ( <i>C. jejuni</i> )	186	97.3					
S-10.4	552	99.6	C-10.4 (C. coli)	155	100.0					
S-10.5	398	98.5	C-10.5 ( <i>C. jejuni</i> )	186	98.4					
S-10.6	399	99.5	C-10.6 ( <i>C. coli</i> )	185	98.4					
S-10.7	585	99.1	C-10.7 (C. coli)	186	97.8					
S-10.8	584	99.7	C-10.8 ( <i>C. jejuni</i> )	186	100.0					

**Table 2:** Percentage of correct antimicrobial susceptibility tests per antimicrobial by microorganism.

Antimicrobial	Salmonella	Campylobacter			
Ampicillin	99.2	-			
Cefotaxime	100.0	-			
Cefoxitin	99.2	-			
Ceftazidime	99.5	-			
Chloramphenicol	99.6	-			
Ciprofloxacin	98.8	99.6			
Colistin	98.0	-			
Ertapenem	100.0	-			
Erythromycin	-	99.6			
Gentamicin	99.6	97.6			
Imipenem	100.0	-			
Meropenem	100.0	-			
Nalidixic acid	99.6	98.0			
Streptomycin	-	97.2			
Sulphonamides	98.4	-			
Tetracycline	99.6	98.6			
Tigecycline	97.8	-			
Trimethoprim	99.2	-			

phenotype'. As follow-up, the laboratory reported that the cause for the incorrect categorization was based on the laboratory SOP not being strictly followed (the SOP clearly states that resistance to meropenem leads to 'presumptive carbapenemase phenotype'). For laboratory #56 and #60, however, the deviating results were caused by an incorrect result as resistant for cefoxitin (#56; S-10.7), and laboratory #60 commented that the positive

synergy test and susceptibility to cefoxitin for strain S-10.3 indicated presumptive ESBL. The laboratory stated that the cefepime MIC value is a little lower than they usually see for this profile, and together with the fact that the strain is ceftazidime sensitive, they concluded that the strain was 'unusual phenotype'.

#### 3.3.2 Campylobacter trial

For the *Campylobacter* strains, 98.4% of AST's were correctly tested. Table 1 presents that the variation in the obtained correct results ranged from 97.3-100% and Table 2 illustrates that the percentage of correct AST per antimicrobial was above 97.2% for the *Campylobacter* test strains with streptomycin exhibiting the lowest level.

The participants were requested to identify the *Campylobacter* species. All 31 laboratories delivered in total 248 results of which one identification was incorrect (laboratory #34). The incorrect species identification did not lead to incorrect AST results for this laboratory and strain.

#### 3.4 Deviations by laboratory

Figure 3 and 4 illustrate the percentage of deviations for each participating laboratory. The laboratories are ranked according to their performance determined by the percentage of deviating results in the antimicrobial susceptibility tests.





**Table 3**: Overview of ESBL-, pAmpC- and carbapenemase-producing *Salmonella* test strains and proportion of laboratories that obtained the expected result; number and percentages of laboratories which correctly detected and confirmed the ESBL-, pAmpC- and carbapenemase-producing *Salmonella* strains. Fields shaded in grey with numbers in *italics* indicate an unexpected result.

		Strain S-10.3	Strain S-10.4	Strain S-10.7	Strain S-10.8
ESC-gene	s harboured in the test strain	bla <sub>ТЕМ-1</sub> bla <sub>СТХ-М-9</sub>	bla <sub>TEM-1</sub> bla <sub>OXA-48</sub>	<i>bla</i> <sub>TEM-1</sub> <i>bla</i> <sub>SHV-12</sub> <i>bla</i> <sub>CTX-M-15</sub>	bla <sub>TEM-52</sub>
-	mpC- and carbapenemase-producing pected results	ESBL	carbapenemase	ESBL	ESBL
	Confirmed ESBL-producer	30/31 (97%)	-	30/31 (97%)	31/31 (100%)
	Confirmed ESBL + pAmpC-producer	-	-	1/31 (3%)	-
Obtained	Confirmed pAmpC-producer	-	-	-	-
results	Confirmed carbapenemase-producer	-	30/31 (97%)	-	-
	Confirmed unusual phenotype	1/31 (3%)	1/31 (3%)	-	-
	Not ESBL-, pAmpC- or carbapenemase-producing	-	-	-	-

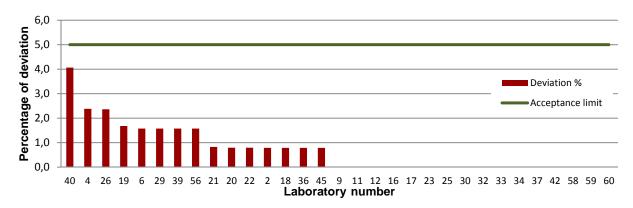


Figure 3: Individual participants' deviations in percent of their total number of Salmonella AST's.

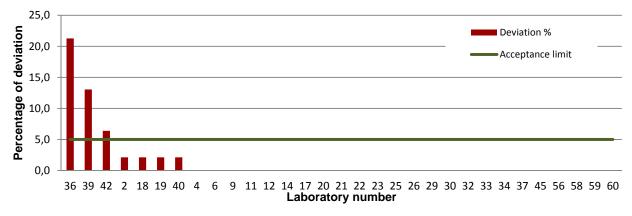


Figure 4: Individual participants' deviations in percent of their total number of Campylobacter AST's.



**Table 4** Obtained values for AST of *E. coli* ATCC 25922. AMP; ampicillin, FEP; cefepime FOT; cefotaxime, FOX; cefoxitin, TAZ; ceftazidime, CHL; chloramphenicol, CIP; ciprofloxacin, COL; colistin, ERT: ertapenem, GEN; gentamicin, IMI; imipenem, MER; meropenem, NAL; nalidixic acid, SMX; sulphonamides, TET; tetracycline, TGC; tigecycline, TMP; trimethoprim.

MIC det	ermination <i>E.</i>	coli ATCC	25922
	Proportion		values in MIC (min/max)
Antimicrobial	outside QC range	Below lower QC limit	Above upper QC limit
Panel 1, AMP	0/29 (0%)	-	-
Panel 1, FOT	0/27 (0%)	-	-
Panel 1, TAZ	0/28 (0%)	-	-
Panel 1, CHL	0/29 (0%)	-	-
Panel 1, CIP	1/29 (3%)	-	1 step
Panel 1, COL	0/29 (0%)	-	-
Panel 1, GEN	0/29 (0%)	-	-
Panel 1, MER	0/28 (0%)	-	-
Panel 1, NAL	0/29 (0%)	-	-
Panel 1, SMX	1/28 (4%)	-	1 step
Panel 1, TET	0/29 (0%)	-	-
Panel 1, TGC	0/28 (0%)	-	-
Panel 1, TMP	3/29 (10%)	1 step	-
Panel 2, FEP	1/24 (4%)	-	2 steps
Panel 2, FOT	1/24 (4%)	-	1 steps
Panel 2, FOX	0/25 (0%)	-	-
Panel 2, TAZ	0/25 (0%)	-	-
Panel 2, ERT	0/25 (0%)	-	-
Panel 2, IMI	0/25 (0%)	-	-
Panel 2, MER	0/25 (0%)	-	-

**Table 5** Obtained values for AST of *C. jejuni* ATCC 33560. CIP; ciprofloxacin, ERY; erythromycin, GEN; gentamicin, NAL; nalidixic acid, TET; tetracycline.

MIC de	termination C	. jejuni ATCO	33560				
		Obtained values in MIC					
	Proportion	steps (	(min/max)				
Antimicrobial	outside QC	Below					
	range	lower QC	Above upper				
		limit	QC limit				
CIP	1/29 (3%)	-	1 step				
ERY	0/29 (0%)	-	-				
GEN	3/28 (11%)	2 steps	-				
NAL	0/28 (0%)	-	-				
TET	2/28 (7%)	1 step	2 steps				

#### 3.4.1 Salmonella trial

All 31 participating laboratories obtained a result within the acceptance limit at 5% deviations for the *Salmonella* strains. The maximum percentage of deviations was at 4.1%, presenting a very good result across the EURL-AR network.

#### 3.4.2 Campylobacter trial

In the *Campylobacter* trial, most laboratories performed very well. Applying the 5% acceptance threshold, 28 of 31 participating laboratories performed acceptably, with 24 laboratories having no deviations (Figure 4).

Three laboratories present a deviation level above the 5% acceptance level (#36, #39, and #42). Of these, one laboratory with a deviation level at 21.3% (#36) was regarded as an outlier.

#### 3.5 Deviations by reference strains

In the following section, deviations are defined as results of antimicrobial susceptibility tests on the reference strain that are outside the quality control (QC) acceptance intervals (App. 5).

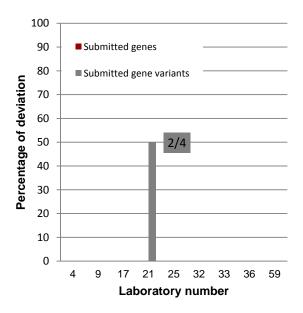
Obtained values from the participants' testing of the QC strains are listed in Appendix 6a and 6b, and in Table 4 and 5. For both the *Salmonella* and *Campylobacter* trial, 29 laboratories uploaded data from QC-testing on the relevant reference strain.

Appendix 6a indicates that of the 29 laboratories submitting AST-results for the reference strain *E. coli* ATCC 25922, six laboratories produced in all seven values outside the QC-limit. Table 4 illustrates the obtained results which are fully presented in Appendix 6a.

Table 5 presents the proportion of laboratories with results for the *C. jejuni* reference strain ATCC 33560 below or above the QC interval. six deviations were seen from five different laboratories.







**Figure 5:** Individual participants' deviations in percent of their total number of results from the genotypic characterization.

#### 3.6 Genotypic characterisation

For the optional genotypic characterisation of the ESC-producing Salmonella test strains, nine laboratories participated. In Appendix 9, information is collected on detected genes, genes which were tested but not detected, primers used, and references for the method used. One laboratory performed whole genome sequencing of the ESC-producing Salmonella whereas the remaining eight laboratories the use indicated of various types conventional PCR to identify the relevant genes.

Table 6 indicate the obtained results, both on gene and variant level. Moreover, Figure 5 indicates that two discordant results related to the gene variant were submitted by laboratory #21. CTX-M-15 belongs to the CTX M-1-group, however, it is not clear if this was the background for the deviation.

**Table 6:** Results from the participation of nine laboratories in the optional genotypic characterisation component of the EQAS

Test strain	Expected gene	Proportion of correct results (gene level)	Proportion of correct results (variant level)	Additional genes/variants identified
S-10.3	TEM-1	7/7 (100%)	5/5 (100%)	- CTX M-4
3-10.3	CTX-M-9	9/9 (100%)	7/8 (88%)	- C1X W-4
S-10.4	TEM-1	6/6 (100%)	4/4 (100%)	
3-10.4	OXA-48	9/9 (100%)	9/9 (100%)	_
	TEM-1	8/8 (100%)	6/6 (100%)	
S-10.7	SHV-12	8/8 (100%)	6/6 (100%)	CTX M-1
	CTX-M-15	9/9 (100%)	7/8 (88%)	_
S-10.8	TEM-52	9/9 (100%)	8/8 (100%)	

## 4. Discussion

It is important to consider that the number of EQAS participants differs from year to year, which implies that comparisons among different EQAS iterations should be interpreted with caution.

As also specified in the EU regulation 2013/652/EU, all participants in the present

EQAS performed AST by dilution methods, primarily as microbroth determination.

This 2015 proficiency test is the second possibility of testing *Salmonella* and *Campylobacter* strains with the panels designed to follow the requirements of Decision 2013/652/EU. This allows for the possibility that



**DTU Food** National Food Institute

the experience obtained since the introduction of the legislation and the focus it has created on AST in the laboratories has had an impact on the generally satisfactory results obtained at the present EQAS.

#### 4.1 Salmonella trial

Overall, the percentage of correct antimicrobial susceptibility test results of *Salmonella* was 99.3%. All (n=31) participants obtained satisfactory results according to the level of acceptance (<5% deviation).

As indicated in Figure 2, the overall quality of the results in the 2015-EQAS would appear to have increased again to the level of the years 2012 and 2013, also, the measure when comparing results obtained from testing the internal control strain indicates a steady and very good quality of results.

As indicated by Figure 3, all laboratories exhibited very good results with deviation levels below 5%. Follow-up has therefore not been necessary based on these results, and none of the laboratories were defined as outliers.

For the *E. coli* reference strain, the obtained results were in general in agreement with the CLSI recommendations. Two laboratories (#4 and #59) did not submit values related to the quality control strain, but within the submitted results, trimethoprim appeared to have most results outside the QC-range, and for cefepime one result was two steps above the QC-range.

For the two laboratories #57 and #58 which had a deviation level for the AST results above the acceptance limit in EQAS 2014 with values of 7.9% and 9.4%, respectively, one (#58) has this year increased their performance and have no deviations this year, and the other (#57) did not participate in the 2015-iteration.

#### ESC-producing Salmonella test strains

The detection of ESC-producing microorganisms remains to be important and is a mandatory part of this EQAS.

Of the four *Salmonella* test strains relevant for this component of the EQAS (S-10.3, S-10.4, S-10.7, and S-10.8), three were ESBL-phenotypes and one was a carbapenemase phenotype. The testing and interpretation of results for these strains appeared not to cause major difficulties for any of the participating laboratories.

Of the 31 laboratories which tested *Salmonella*, three laboratories (#39, #56 and #60) each submitted one incorrect AmpC-, ESBL-, carbapenemase categorization (App. 8a). The deviations all had background in the laboratory handling of the strains; for one, the incorrect categorization was based on an incorrect result as resistant for cefoxitin (#56; S-10.7). Even if no acceptance limit has been defined for this component of the EQAS, the overall result that 98.8% of the obtained results were as expected appears satisfactory.

#### 4.2 Campylobacter trial

For the *Campylobacter* component of this year's EQAS, 31 laboratories submitted results leading to an overall percentage of correct AST results at 98.4%. The performance varied from no deviations up to 21.3% deviations, with 28 laboratories performing satisfactorily according to the established acceptance range.

It appears that there has been a decrease in the level of deviations for the overall AST result. Also, results obtained from testing the internal control strain indicate a steady and very good quality of results.

Three laboratories (#36, #39, and #42) obtained deviation levels above 5%, one of these was defined as an outlier (#36) with a deviation level at 21.3%. For none of these laboratories, the values obtained for the QC-strain did not indicate methodical issues to be the reason for the obtained deviations.

The EURL-AR have been in contact with all three laboratories presenting deviation levels above 5% to identify possible causes of this



unsatisfactory performance and to improve the quality of results. As for laboratory #36, the high level of deviations was caused by the switch of panels used and not switching to the corresponding result sheet for reading the plates. The laboratory transferred the obtained results to the correct result sheet and subsequently found that not all obtained results were as expected. As for laboratory #39, retesting of strain C-10.3 that caused five deviations was performed, leading this time to one deviation, only. The laboratory will continue when fresh stocks follow-up recommended panel for Campylobacter are received. At laboratory #42, the obtained MICs were as expected, but the deviations were caused by typos when submitting interpretation into the database.

All participating laboratories except two (#4 and #59) uploaded data from tests performed on the *C. jejuni* reference strain and the proportion of results within the QC intervals was 95.8%. Four of the six values outside the QC intervals were one step below or above the QC-limits, the remaining two were two dilution steps above or below the QC-limits. The laboratories obtaining these values should follow-up on these high/low values, and it is suggested that these values are monitored over time to ensure that the tests render a reliable result for the particular antimicrobial.

Laboratories #29, and #40 which were regarded

as outliers for the 2014 *Campylobacter* EQAS with deviation levels at 22.9% and 34.0%, respectively, both increased their performance extensively in the 2015-iteration and obtained deviation levels at 0.0%, and 2.1%, respectively.

#### 4.3 Genotypic characterisation

The focus on genotypic characterization of microorganisms is increasing in the EU and worldwide. In EU, communication has been ongoing to improve laboratory detection and confirmation of ESBL- and AmpC-producing *Enterobacteriaceae*.

Furthermore, the agenda now is focusing at the implementation of detection of carbapenemase resistant organisms and the importance of determining the identity of the genes responsible for the carbapenemase production by molecular methods.

The optional genotypic characterisation offered as a supplementary part of this EQAS should therefore be seen as an important possibility for the NRL-AR's to introduce this method in the laboratory and thereby be at the forefront when the method proposals are adopted. This year, nine laboratories participated in this optional EQAS component and even if no acceptance limit has been defined, the 98.3% correct results (N=119) appears to be a satisfactory results.

# 5. Conclusions

The goal of the EURL-AR EQAS is to have all participating NRLs performing antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* with a deviation level below 5%. This seems within reach for *Salmonella* as well as for *Campylobacter*.

Compared to the EQAS 2014, the performance of the NRL's in 2015 appears to have improved for *Salmonella* AST's (99.3% in 2015 and

97.6% in 2014) and is now again at the level from 2013 and before (Figure 2). Regarding Campylobacter AST's, the performance of the NRL's also appear to have improved from 2014 to 2015, with a change in deviation level from 4.0% (2014) to 1.6% (2015). For the Campylobacter AST, one laboratory (#36) was regarded as an outlier. Follow-up/re-testing internally at the NRL's with deviation levels above 5% has shown acceptable results for all





three laboratories (#36, #39, and #42).

The test covering the identification of the phenotype of *Salmonella* test strains producing beta-lactamases of the ESBL-, AmpC, and carbapenemase-type rendered acceptable results. This is a priority area within the EURL-AR activities, and it is encouraging to see acceptable results in identifying and categorizing these strains.

Nine NRLs participated in the EQAS

component consisting of genotypic testing of ESBL-, AmpC- and carbapenemase-producing *Enterobacteriaceae* presenting satisfactory results.

Finally, the EURL-AR is open to suggestions to improve future EQAS trials and invites the entire network to contribute with ideas for training courses and specific focus areas to expand the network's knowledge in antimicrobial resistance.

#### 6. References

EFSA. Technical specifications on the harmonised monitoring and reporting of antimicrobial Salmonella, resistance in Campylobacter and indicator Escherichia coli and Enterococcus spp. bacteria transmitted through food. EFSA Journal 2012;10(6):2742 [64 pp.].

**European Commission**, 2013/652/EU: Commission Implementing Decision of 12

November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria

**Schwarz** S, Silley P, Simjee S, Woodford N, van DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 65: 601-604



G00-06-001/01.12.2014

#### EURL-AR EQAS pre-notification

# EQAS 2015 FOR SALMONELLA, CAMPYLOBACTER AND OPTIONAL GENOTYPIC CHARACTERISATION

The EURL-AR announces the launch of another EQAS, thus providing the opportunity for proficiency testing which is considered an essential tool for the generation of reliable laboratory results of consistently good quality.

This EQAS consists of antimicrobial susceptibility testing of eight *Salmonella* isolates and eight *Campylobacter* isolates. Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214) will be distributed to new participants.

This EQAS is specifically for NRL's on antimicrobial resistance. Laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. You may contact the EQAS-Coordinator if you wish to inform of changes in relation to your level of participation in previous years. The EURL-AR will be able to cover the expenses for one parcel per EU Member State. Therefore, countries with more than one laboratory registered on the EURL-AR contact-list will be contacted directly to confirm which laboratory will be included for participation free of charge.

The invitation to participate in the proficiency test is extended to additional participants from official NRLs and participants from laboratories which are involved in the network but are not designated NRLs (cost for participation will be 100 EURO).

#### TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is "UN3373, Biological Substance Category B": Eight *Salmonella* strains, eight *Campylobacter* and for new participants also the QC strains mentioned above. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g. specific text that should be written on the proforma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

## TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

<u>Shipment of isolates and protocol</u>: The isolates will be shipped in October 2015. The protocol for this proficiency test will be available for download from the website (www.eurl-ar.eu).

<u>Submission of results</u>: Results must be submitted to the National Food Institute **no later than December 4<sup>th</sup> 2015** via the password-protected website.



Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

<u>EQAS</u> report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

<u>Next EQAS</u>: The next EURL-AR EQAS that we will send out to the EURL-AR network focuses on isolation of ESBL and ampC-producing *E.coli* from samples which is expected to be sent to participating laboratories around 1<sup>st</sup> November, 2015.

Please contact me if you have comments or questions regarding the EQAS

Sincerely,

Susanne Karlsmose Pedersen (suska@food.dtu.dk)

**EQAS-Coordinator** 

#### Participant list

Salmonella	Campylobacter	Genotypic characterisation	Institute	Country
Х	Х	-	Austrian Agency for Health and Food Safety	Austria
Х	X	Х	Institute of Public Health	Belgium
Х	Х	-	National Diagnostic and Research Veterinary Institute	Bulgaria
Х	Х	-	Croatian Veterinary Institut	Croatia
Х	Х	-	Veterinary Services	Cyprus
Х	Х	Х	State Veterinary Institute Praha	Czech Republic
Х	Х	-	Danish Veterinary and Food Administration, DVFA	Denmark
Х	Х	-	Estonian Veterinary and Food Laboratory	Estonia
Х	Х	-	Finnish Food Safety Authority EVIRA	Finland
Х	-	-	Agence nationale de sécurité sanitaire ANSES - Fougères LERMVD	France
-	Х	-	Agence nationale de sécurité sanitaire ANSES - Ploufragan - LERAP	France
Х	Х	Х	Federal Institute for Risk Assessment	Germany
Х	Х	-	Veterinary Laboratory of Chalkis	Greece
Х	Х	-	Central Agricultural Office Veterinary Diagnostic Directorate	Hungary
X	Х	-	University of Iceland	Iceland
Х	Х	-	Central Veterinary Research Laboratory	Ireland
Х	Х	Х	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
Х	Х	-	Institute of Food Safety, Animal Health and Enviroment "BIOR"	Latvia
Х	Х	-	National Food and Veterinary Risk Assessment Institute	Lithuania
Х	Х	Х	Laboratoire national de Santé	Luxembourg
Х	Х	-	Public Health Laboratory	Malta
Х	Х	Х	Central Veterinary Institute of Wageningen UR	Netherlands
Х	Х	-	Food and Consumer Product Safety Authority (VWA)	Netherlands
Х	Х	Х	Veterinærinstituttet	Norway
Х	Х	-	National Veterinary Research Institute	Poland
Х	Х	-	Laboratorio National de Investigacáo Veterinaria	Portugal
Х	Х	-	Institute for Diagnosis and Animal Health	Romania
Х	Х	-	State Veterinary and Food Institute (SVFI)	Slovakia
Х	Х	-	National Veterinary Institute	Slovenia
Х	Х	Х	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	Х	-	VISAVET Health Surveillance Center, Complutense University	Spain
Х	Х	Х	National Veterinary Institute, SVA	Sweden
X	X	-	Vetsuisse Faculty Bern, Institute of Veterinary Bacteriology	Switzerland
X	X	<u>-</u>	The Veterinary Laboratory Agency	United Kingdom

Designated NRL-AR by the compentent authority of the member state

Non-NRL-AR enrolled by the EURL-AR

Not a Member State of the EU

#### Reference values (MIC-value and interpretation) - Salmonella

	Ampicillir AMP		Azithrom AZI	Cefepime FEP	Cefotaxir FOT		Cefotaxime/clav F/C		Cefoxitin FOX		Ceftazidi TAZ				Chloramp CHL	henicol	Ciproflox CIP	acin	Colistin COL		Ertapene	m
EURL S-10.1	= 2	SUSC	= 8		<= 0.25	SUSC					<= 0.5	SUSC			<= 8	SUSC	= 0.06	SUSC	= 8	RESIST		
EURL S-10.2	> 64	RESIST	= 8		<= 0.25	SUSC					<= 0.5	SUSC			> 128	RESIST	= 0.03	SUSC	<= 1	SUSC		
EURL S-10.3	> 64	RESIST	= 8	= 2	= 8	RESIST	= 0.25/4	>=8	= 2	SUSC	= 1	SUSC	= 0.25/4	<8	<= 8	SUSC	= 0.5	RESIST	<= 1	SUSC	<= 0.015	SUSC
EURL S-10.4	> 64	RESIST	= 8	= 1	= 2	RESIST	= 2/4	<8	= 4	SUSC	<= 0.5	SUSC	= 0.5/4	<8	<= 8	SUSC	= 0.03	SUSC	<= 1	SUSC	= 2	RESIST
EURL S-10.5	> 64	RESIST	= 8		<= 0.25	SUSC					<= 0.5	SUSC			= 64	RESIST	= 0.03	SUSC	<= 1	SUSC		
EURL S-10.6	= 2	SUSC	= 8		<= 0.25	SUSC					<= 0.5	SUSC			<= 8	SUSC	= 0.03	SUSC	<= 1	SUSC		
EURL S-10.7	> 64	RESIST	> 64	> 32	> 64	RESIST	= 0.25/4	>=8	= 8	SUSC	> 128	RESIST	= 2/4	>=8	> 128	RESIST	= 0.25	RESIST	<= 1	SUSC	= 0.03	SUSC
EURL S-10.8	> 64	RESIST	= 4	= 2	= 8	RESIST	= 0.06/4	>=8	= 2	SUSC	= 16	RESIST	= 0.25/4	>=8	<= 8	SUSC	= 0.03	SUSC	= 2	SUSC	= 0.015	SUSC

	Gentamicin GEN				MEROPENEM MER		Nalidixic acid Sulfam NAL SMX		Sulfamethoxazole SMX		TEMOCII TRM		Tetracycl TETRA		TIGECYCLINE TGC		Trimethor		ESBL-category	Relevant genes
EURL S-10.1	<= 0.5	SUSC			= 0.06	SUSC	<= 4	SUSC	= 32	SUSC			<= 2	SUSC	= 0.5	SUSC	<= 0.25	SUSC	N/A	N/A
EURL S-10.2	= 1	SUSC			= 0.06	SUSC	<= 4	SUSC	> 1024	RESIST			= 64	RESIST	= 0.5	SUSC	<= 0.25	SUSC	N/A	N/A
EURL S-10.3	= 1	SUSC	= 0.5	SUSC	<= 0.03	SUSC	> 128	RESIST	= 32	SUSC	= 8		= 64	RESIST	= 0.5	SUSC	<= 0.25	SUSC	Presumptive ESBL-phenotype	TEM-1; CTX M-9
EURL S-10.4	<= 0.5	SUSC	= 4	RESIST	= 2	RESIST	<= 4	SUSC	= 32	SUSC	> 128		<= 2	SUSC	= 0.5	SUSC	<= 0.25		Presumptive carbapenemase phenotype	TEM-1; OXA-48
EURL S-10.5	<= 0.5	SUSC			= 0.06	SUSC	<= 4	SUSC	> 1024	RESIST			> 64	RESIST	= 2	RESIST	> 32	RESIST	N/A	N/A
EURL S-10.6	<= 0.5	SUSC			= 0.06	SUSC	<= 4	SUSC	= 64	SUSC			= 4	SUSC	= 0.5	SUSC	<= 0.25	SUSC	N/A	N/A
EURL S-10.7	> 32	RESIST	= 0.5	SUSC	= 0.06	SUSC	= 8	SUSC	> 1024	RESIST	= 32	= 32 > 64 RESIST		= 1	SUSC	> 32	RESIST	Presumptive ESBL-phenotype	TEM-1; CTX M- 15; SHV-12	
EURL S-10.8	= 1	SUSC	= 0.5	SUSC	= 0.06	SUSC	= 8	SUSC	= 32	SUSC	= 8	= 8 <= 2 SUSC		= 0.5	SUSC	= 0.5	SUSC	Presumptive ESBL-phenotype	TEM-52	

Resistant

# Reference values (MIC-value and interpretation) - Campylobacter

Species	Code	Ciprofloxad		Erythromyd ERY	cin	Gentamicir GEN		Nalidixic ad NAL	cid	Streptomyo STR	cin	Tetracyclin TET	е
C. jejuni	C. jejuni EURL C-10.1		RESIST	> 128	RESIST	= 0.25	SUSC	> 64	RESIST	= 1	SUSC	<= 0.5	SUSC
C. coli	EURL C-10.2	<= 0.12	SUSC	<= 1	SUSC	= 0.25	SUSC	= 4	SUSC	= 16	RESIST	<= 0.5	SUSC
C. jejuni	EURL C-10.3	<= 0.12	SUSC	<= 1	SUSC	= 0.25	SUSC	= 2	SUSC	= 1	SUSC	<= 0.5	SUSC
C. coli	EURL C-10.4	= 0.25	SUSC	> 128	RESIST	= 0.5	SUSC	= 8	SUSC	= 1	SUSC	= 2	SUSC
C. jejuni	EURL C-10.5	= 8	RESIST	<= 1	SUSC	= 0.25	SUSC	> 64	RESIST	= 1	SUSC	> 64	RESIST
C. coli	EURL C-10.6	> 16	RESIST	> 128	RESIST	= 0.5	SUSC	> 64	RESIST	= 2	SUSC	> 64	RESIST
C. coli EURL C-10.7		= 16	RESIST	<= 1	SUSC	= 0.5	SUSC	> 64	RESIST	= 4	SUSC	<= 0.5	SUSC
C. jejuni	jejuni EURL C-10.8 = 16 RESIST > 128 RESIST		> 16	RESIST	> 64	RESIST	> 16	RESIST	> 64	RESIST			

Resistant





Appendix 4a, page 1 of 1

G00-06-001/01.12.2014

#### **EURL-AR External Quality Assurance System 2015**

- Salmonella, Campylobacter and optional genotypic characterisation

Id: «Lab\_no\_» «Name» «Institute\_\_» «Country»

Kgs. Lyngby, October 2015

Dear «Name»,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2015. Upon arrival to your laboratory, the strains should be stored dark and at 4°C for stabs, and dark and cool for lyophilized strains. Charcoal swabs must be subcultured straight away.

On the EURL-AR-website (<u>www.eurl-ar.eu</u>) the following documents relevant for the EURL-AR EQAS are available:

- Protocol for Salmonella and Campylobacter including test forms
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Control Strains

We ask you to examine the eight *Salmonella* and the eight *Campylobacter* strains that we send to you by performing antimicrobial susceptibility testing. The ESBL-producing *Salmonella* strains should be characterised genotypically (optional) according to the description in the protocol. In the protocol you can find detailed description of the procedures to follow. Additionally, you can find a description of the procedure to enter your results into the interactive web database. For accessing the database, you need this username and password.

Your username: «Username»

Your password: «Password»

Please keep this document Your username and password will not appear in other documents

Results should be submitted to the database no later than **December 4**<sup>th</sup> **2015**.

Please acknowledge receipt of this parcel immediately upon arrival (to <a href="mailto:suska@food.dtu.dk">suska@food.dtu.dk</a>). Do not hesitate to contact us for further information.

Yours sincerely,

Susanne Karlsmose Pedersen **EQAS-Coordinator** 





# **PROTOCOL**

For antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and optional genotypic characterisation of AmpC-, ESBL- and carbapenemase-producing test strains

1	INTRODUCTION	1
2	OBJECTIVES	2
3	OUTLINE OF THE SALM/CAMP EQAS 2015	2
3.1	Shipping, receipt and storage of strains2	
3.2	QC reference strains	
3.3	Antimicrobial susceptibility testing3	
3.4	Optional genotypic characterisation 6	
4	REPORTING OF RESULTS AND EVALUATION	6
5	HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE	7

#### 1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *Salmonella* and *Campylobacter* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The Salm/Camp EQAS 2015 will include AST of eight *Salmonella* and *Campylobacter* strains and AST of reference strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214).

The above-mentioned reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The reference strains will not be included in the years to come. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual 'Subculture and Maintenance of QC Strains' available on the EURL-AR website (see www.eurl-ar.eu).







Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

#### 2 OBJECTIVES

This EQAS aims to support laboratories to assess and, if necessary, to improve the quality of results obtained by AST of pathogens of food- and animal-origin, with special regard to *Salmonella* and *Campylobacter*. Further objectives are to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *Salmonella* and *Campylobacter* reported to EFSA by different laboratories.

#### 3 OUTLINE OF THE SALM/CAMP EQAS 2015

#### 3.1 Shipping, receipt and storage of strains

In October 2015, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *Salmonella* and *Campylobacter* strains from the National Food Institute. This parcel will also contain reference strains, but only for participants who did not receive them previously. All strains belong to UN3373, Biological substance, category B. Extended spectrum beta-lactamase (ESBL)-producing strains as well as carbapenemase producing strains are included in the selected material and are part of the optional EQAS-item, consisting of characterization of genes conferring ESBL- or carbapenemase production.

The reference strains are shipped lyophilised, the *Campylobacter* test strains are shipped as a charcoal swabs and the *Salmonella* test strains are stab cultures. On arrival, the stab cultures and the charcoal swabs must be subcultured, and all cultures should be adequately stored until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

#### 3.2 QC reference strains

For a suggested procedure for reconstitution of the lyophilised, please refer to the document 'Instructions for opening and reviving lyophilised cultures' on the EURL-AR-website (see <a href="https://www.eurl-ar.eu">www.eurl-ar.eu</a>).

Note that, for the testing of the *E. coli* ATCC25922 reference strain, the two compounds, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole.







#### 3.4 Antimicrobial susceptibility testing

The strains should be tested for susceptibility to the antimicrobials listed in Tables 1, 2 and 3, using the method implemented in your laboratory for performing monitoring for EFSA and applying the interpretative criteria listed below.

Participants should perform minimum inhibitory concentration (MIC) determination using the methods stated in the EC regulation EC 652/2013. For interpretation of the results, use the cut-off values listed in Tables 1, 2 and 3 (except where indicated) represent the current epidemiological cut-off values developed by EUCAST (<a href="www.eucast.org">www.eucast.org</a>), and allow categorisation of bacterial isolates into two categories; resistant or susceptible. A categorisation as intermediate is not accepted.

As the current regulation and recommendations focus on MIC testing only, results obtained by disk diffusion cannot be submitted.

#### 3.4.1 Salmonella

The interpretative criteria that should be applied for categorizing the *Salmonella* test strain as resistant or susceptible are those listed in Tables 1 and 2.

Table 1: Antimicrobials recommended for AST of *Salmonella* spp. and interpretative criteria according to table 1 in EC regulation 652/2013

Antimicrobial	MIC (μg/mL) (R>)
Ampicillin (AMP)	8
Azithromycin (AZI)	Not available*
Cefotaxime (FOT)	0.5
Ceftazidime (TAZ)	2
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.06
Colistin (COL)	2
Gentamicin (GEN)	2
Meropenem (MERO)	0.125
Nalidixic acid (NAL)	16
Sulfonamides (SMX)	256**
Tetracycline (TET)	8
Tigecycline (TGC)	1***
Trimethoprim (TMP)	2

<sup>\*</sup> Participants are requested to upload the MIC value obtained without selecting an interpretation.



<sup>\*\*</sup> CLSI M100 Table 2A

<sup>\*\*\*</sup> Data from EUCAST is available for *S.* Enteritidis, *S.* Typhimurium, *S.* Typhi and *S.* Paratyphi (for the purpose of this proficiency test, the ECOFF at 1 is applied)





Table 2: Antimicrobials recommended for additional AST of *Salmonella* spp. resistant to cefotaxime, ceftazidime or meropenem and interpretative criteria according to table 4 in EC regulation 652/2013

Antimicrobial	MIC (μg/mL) (R>)		
Cefepime, FEP	Not available*		
Cefotaxime, FOT	0.5		
Cefotaxime + clavulanic acid (F/C)	Not applicable		
Cefoxitin, FOX	8		
Ceftazidime, TAZ	2		
Ceftazidime+ clavulanic acid (T/C)	Not applicable		
Ertapenem, ETP	0.06		
Imipenem, IMI	1		
Meropenem, MERO	0.125		
Temocillin, TRM	Not available*		

<sup>\*</sup> Participants are requested to upload the MIC value obtained without selecting an interpretation

#### Plasmid-mediated quinolone resistance

When performing antimicrobial susceptibility testing of the *Salmonella* test strains, the interpretative criteria listed in Table 1 should be able to detect plasmid mediated quinolone resistant test strains.

#### Extended-beta-lactam- and carbapenem resistance

Confirmatory tests for AmpC-, ESBL- and carbapenemase production are **mandatory** on all strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem and should be performed by testing the second panel of antimicrobials (Table 2 in this document corresponding to Table 4 in EC regulation 652/2013).

Confirmatory tests for AmpC-, ESBL- and carbapenemase production require the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a  $\beta$ -lactamase inhibitor (clavulanic acid). Synergy is defined either as a  $\geq 3$  twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (MIC FOT: FOT/Cl or TAZ: TAZ/Cl ratio  $\geq 8$ ) (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production. Resistance to cefepime gives further indication of ESBL production, but is not essential. Confirmatory test for carbapenemase production requires the testing of meropenem (MERO).

Detection of AmpC-type beta-lactamases can be performed by testing the bacterium for susceptibility to cefoxitin (FOX). Resistance to FOX could indicate the presence of an AmpC-type beta-lactamase that may be verified by PCR and sequencing.







The classification of the phenotypic results should be based on the most recent EFSA recommendations (EFSA 2012<sup>1</sup>) indicating the strains as:

- Presumptive ESBL: strains with positive synergy test, susceptible to cefoxitin and resistant to cefepime
- Presumptive ESBL+pAmpC: strains with positive or negative synergy test, resistant to cefoxitin and resistant to cefepime
- Presumptive pAmpC phenotype: strains with negative synergy test, resistant to cefoxitin and susceptible to cefepime
- Presumptive carbapenemase phenotype: strain resistant to meropenem
- Unusual phenotype: any other combinations

We recommend, however, that strains showing synergy with clavulanic acid for at least one of the third generation cephalosporins (cefotaxime or ceftazidime) should be considered ESBL-producing, independently of the cefepime result.

#### 3.4.2 Campylobacter

For AST of *Campylobacter*, MIC methods should be applied, i.e. broth or agar dilution methods using incubation at 36-37°C for 48 hours or 42°C for 24 hours.

Table 3: Antimicrobials recommended for AST of *Campylobacter jejuni* and *C. coli* and interpretative criteria according to table 1 in EC regulation 652/2013

Antimicrobial	C. jejuni	C. coli	
Antimiciobiai	$MIC (\mu g/mL) (R>)$	$MIC (\mu g/mL) (R>)$	
Ciprofloxacin (CIP)	0.5	0.5	
Erythromycin (ERY)	4	8	
Gentamicin (GEN)	2	2	
Nalidixic acid (NAL)	16	16	
Streptomycin (STR)	4	4	
Tetracycline (TET)	1	2	

#### Identification of Campylobacter species

Species identification of the *Campylobacter* test strains must be performed by the NRLs using inhouse methods or adopting the protocol available on the EURL-AR website under: <a href="http://eurl-ar.eu/233-protocols.htm">http://eurl-ar.eu/233-protocols.htm</a>.

<sup>&</sup>lt;sup>1</sup> European Food Safety Authority; Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA Journal 2012; 10(6):2742. [64 pp.] doi:10.2903/j.efsa.2012.2742. Available online: www.efsa.europa.eu/efsajournal







#### 3.5 Optional genotypic characterisation

For the optional genotypic characterisation of the AmpC-, ESBL- or carbepenemase producing *Salmonella* test strains, the requested results are the genes conferring AmpC-, ESBL- or carbepenemase -production harboured in the test strains. The genes included in the test are the following: ACC, ACT, CMY, CTX, DHA, FOX, GES, IMP, KPC, MOX, NDM, OXA, PER, SHV, TEM, VEB, and VIM. The database lists the relevant variants of the genes.

When uploading the results in the database, the identified genes will be evaluated against the expected results. The results will be evaluated on the detected gene (ACC-, ACT-, CMY-, etc.) as well as the variant identified.

The method used for the genotypic characterisation should be your laboratory's routine method. The expected results listed in the database are those obtained by the EURL-AR.

#### 4 REPORTING OF RESULTS AND EVALUATION

Test forms are available for recording your results before you enter them into the interactive web database.

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than December 4<sup>th</sup> 2015.** After the deadline when all participants have uploaded results, you will be able to login to the database once again, and to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

If you experience difficulties in entering your results, please contact us directly.

All results will be summarized in a report which will be publicly available. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the complete list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions will be public.

If you have questions, please do not hesitate to contact the EQAS Coordinator:

Susanne Karlsmose Pedersen National Food Institute, Technical University of Denmark Søltofts Plads, Building 221, DK-2800 Lyngby Denmark

Tel: +45 3588 6601 Fax: +45 3588 6341

E-mail: suska@food.dtu.dk







#### 5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read carefully this paragraph before entering the web page.

Remember that you need by your side the completed test forms.

Enter the EURL-AR EQAS 2015 start web page (http://eurl-ar.food.dtu.dk), write your username and password (lower-case) and press enter. Your username and password are indicated in the letter following your strains. Do not hesitate to contact us if you experience problems with the login.

You can browse back and forth by using the Home or back keys, but please remember to save your inputs before.

Click on either "Salmonella test results" or "Campylobacter test results" for input of test results.

Click on "Start of Data Entry - Methods"

In the next page, you navigate among fields with the Tab-key and the mouse.

Complete the fields related to the method used for antimicrobial susceptibility testing and the brand of MIC trays, etc.

When submitting *Campylobacter* results, fill in the incubation conditions applied for susceptibility testing of *Campylobacter* – 36°C/48h or 42°C/24h.

Click on "save and go to next page"

In the data entry pages, you enter the species (for *Campylobacter* only), the obtained MIC-value and the interpretation (R, resistant or S, susceptible) for each *Salmonella* and *Campylobacter* strain.

For Salmonella, remember to also report the results for the ESBL detection tests.

If you did not test for susceptibility to a given antimicrobial, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains, please enter MIC values in  $\mu g/ml$ . Remember to use the operator keys to show symbols like "equal to", etc.

Click on "save".

Review the input pages by browsing through them and make corrections if necessary. Remember to save a page if you make corrections. If you press home a page without saving changes, you will see an error screen. In this case, click on "save" to save your results, browse back to the page and then continue.

Please complete the evaluation form.

Before approving your input, please be sure that you have filled in all the relevant fields as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database.

If you have performed the optional genotypic characterisation:







Click on "Gene test" and follow the description in the database for upload of the results of the optional genotypic characterization. Approve your input. Be sure that you have filled in all the results before approval. The approval blocks your data entry in the interactive database, but allows you to see the submitted results.







Appendix 4c, page 1 of 8

# Salmonella, Campylobacter and genetic characterisation

# **TEST FORMS**

Name:
Name of laboratory:
Name of institute:
City:
Country:
E-mail:
'ax:

Comments:







Appendix 4c, page 2 of 8

### **TEST FORM**

Does your laboratory have an accreditation for pe	erforming Salmonella AST?	Yes	☐ No
Which method did you use for antimicrobial suscing Broth microdilution  Agar dilution	eptibility testing of Salmonella	in this EQ	AS:
Brand of microbroth plates/agar: Incubation conditions: °C/	h		

How many Salmonella isolates does your laboratory annually isolate:

How many *Salmonella* isolates does your laboratory annually test for antimicrobial susceptibility by a MIC method:

Which method was followed for the preparation of the inoculum (please describe)

- Which standard was followed (TREK, CLSI...)
- Which solvent was used for the preparation of the 0.5 McFarland solution (water, saline)
- Please describe in detail how you prepared the dilution of the inoculum (including the volume in final MH-dilution and intended dilution level; e.g. diluted 1:1000 by adding 10µl of 0.5 McFarland solution in 10ml MH broth, for an expected inoculum of 1\*10<sup>5</sup> CFU/ml)

Comments or additional information:







Appendix 4c, page 3 of 8

# **TEST FORM**

Which method was followed for the preparation of the inoculum (please describe)

- Which standard was followed (TREK, CLSI...)
- Which solvent was used for the preparation of the 0.5 McFarland solution (water, saline)
- Please describe in detail how you prepared the dilution of the inoculum (including the volume in final MH-dilution and intended dilution level; e.g. diluted 1:1000 by adding 10μl of 0.5 McFarland solution in 10ml MH broth, for an expected inoculum of 1\*10<sup>5</sup> CFU/ml)

Comments or additional information:







#### Appendix 4c, page 4 of 8

# **TEST FORM**

Strain	Antimicrobial	Results and interpretation		
		<b>≤</b>	MIC-value (μg/ml)	S/R
		>	,, ,	
Salmonella	Ampicillin, AMP			
EURL S. 10.X	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

All strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem (MERO) must be included for testing in the second panel as part of confirmatory tests for ESBL-, AmpC or carbapenemase production. See further description in the protocol section '3.3.1 *Salmonella*'.

Strain	Antimicrobial	Results and interpretation		
		<b>≤</b>	MIC-value (μg/ml)	S/R
		>		
Salmonella	Cefepime, FEP			
EURL S. 10. <b>X</b>	Cefotaxime, FOT			
	Cefotaxime + clavulanic acid (F/C)			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid (T/C) Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			
☐ Presumptive ESBL       ☐ Presumptive pAmpC         ☐ Presumptive ESBL+ pAmpC       ☐ Presumptive carbapenemase		☐ Unusual phenotype ☐ No ESBL, AmpC- or carl	bapenemase	

Comments (include optional genotype or other results):







Appendix 4c, page 5 of 8

## **TEST FORM**

Antimicrobial susceptibility testing of reference strain E. coli ATCC 25922

	Antimicrobial	MIC-value (μg/ml)
l <sup>st</sup> panel	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2 <sup>nd</sup> panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid (F/C)	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid (T/C)	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

<sup>\*</sup> for the testing of the *E. coli* ATCC25922 reference strain, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole (CLSI M100, Table 3).







Appendix 4c, page 6 of 8

## **TEST FORM**

Antimicrobial	Interpretation				
	MIC-value (μg/ml)	S/R			
Ciprofloxacin					
Erythromycin					
Gentamicin					
Nalidixic acid					
Streptomycin					
Tetracycline					
Ciprofloxacin					
Erythromycin					
Gentamicin					
Nalidixic acid					
Streptomycin					
Tetracycline					
Ciprofloxacin					
Erythromycin					
Gentamicin					
Nalidixic acid					
Streptomycin					
Tetracycline					
Ciprofloxacin					
Erythromycin					
Gentamicin					
Nalidixic acid					
Streptomycin					
Tetracycline					
	Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Gentamicin Nalidixic acid Streptomycin Tetracycline Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Gentamicin Erythromycin Gentamicin Nalidixic acid Streptomycin	Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline			





#### Appendix 4c, page 7 of 8

### **TEST FORM**

Susceptibility testing of Campylobacter jejuni reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (μg/ml)				
		36 °C/48 hours	42 °C/24 hours			
	Ciprofloxacin					
C. jejuni ATCC 33560	Erythromycin					
	Nalidixic acid					
	Tetracycline					

#### For Agar dilution:

Susceptibility testing of Campylobacter jejuni reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (μg/ml)
	Ciprofloxacin	
C. jejuni ATCC 33560	Erythromycin	
	Gentamicin	
	Nalidixic acid	
	Tetracycline	







Appendix 4c, page 8 of 8

# **TEST FORM – genotypic characterisation**

Genotypic characterisation of the test strains

Strain code:	Method used: If PCR-methods, additional information should be given below
Gene:	Published method , reference:
Gene.	In-house method
Found	Primer used 5'→3':
Tested, not found	Primer used 3'→5':
G	Published method , reference:
Gene:	☐ In-house method
Found	Primer used 5'→3':
Tested, not found	Primer used 3'→5':
C	Published method , reference:
Gene:	In-house method
Found	Primer used 5'→3':
Tested, not found	Primer used 3'→5':
G	Published method , reference:
Gene:	☐ In-house method
Found	Primer used 5'→3':
Tested, not found	Primer used 3'→5':
G	Published method , reference:
Gene:	☐ In-house method
Found	Primer used 5'→3':
Tested, not found	Primer used 3'→5':

Comments:







# INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz.

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

- a. Check the number of the culture on the label inside the ampoule
- b. Make a file cut on the ampoule near the middle of the plug (see Figure 1)
- c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
- d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
- e. Remove the pointed end of the ampoule into disinfectant
- f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
- g. Incubate the inoculated medium at appropriate conditions for several days
- h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

#### Notes:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see http://www.sci.muni.cz)
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

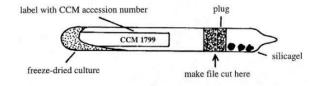


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz





# SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

#### 1.1 Purpose

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has published a guideline for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test results.

#### 1.2 References

M100-S24, January 2014 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A9, January 2012 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

#### 1.3 Definition of Terms

<u>Reference Culture</u>: A reference culture is a microorganism preparation that is acquired from a culture type collection.

<u>Reference Stock Culture</u>: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

<u>Working Stock Cultures</u>: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

<u>Subcultures (Passages)</u>: A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time

#### 1.4 Important Considerations

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC
- CLSI requires that QC be performed either on the same day or weekly (only after 30 day QC validation)
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides
- Periodically perform colony counts to check the inoculum preparation procedure





- Ideally, test values should be in the middle of the acceptable range
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

#### 1.5 Storage of Reference Strains

#### Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

#### Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

#### 1.6 Frequency of Testing

#### Weekly vs. daily testing

Weekly testing is possible if the lab can demonstrate satisfactory performance with daily testing as follows:

- Documentation showing reference strain results from 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more than 3 out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

#### **Corrective Actions**

If an MIC is outside the range in weekly testing, corrective action is required as follows:

- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

The problem is considered resolved only after the reference strain is tested for 5 consecutive days and each drug/organism result is within specification on each day.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

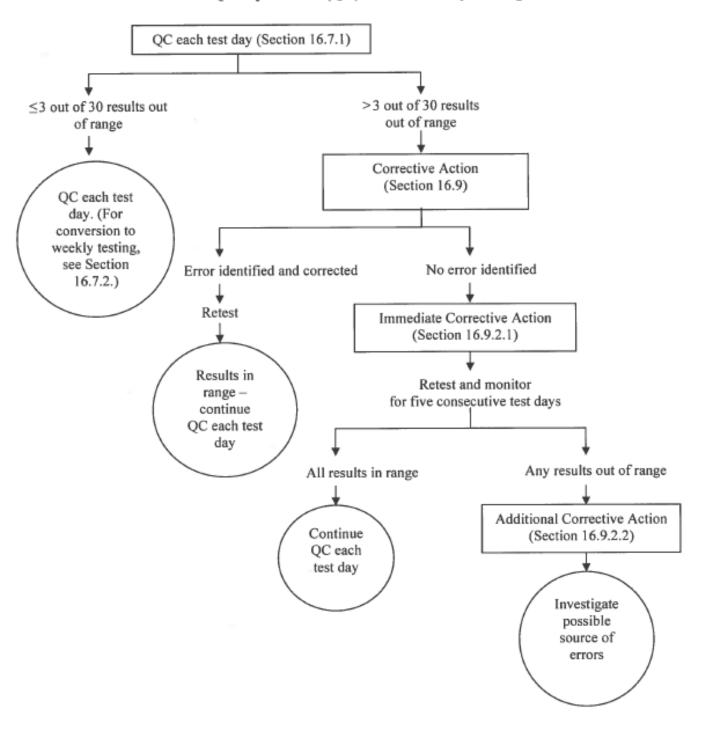


# European Union Reference Laboratory Antimicrobial Resistance

#### DAILY MIC QC CHART

#### Appendix A. Quality Control Protocol Flow Charts

#### Quality Control (QC) Protocol: Daily Testing



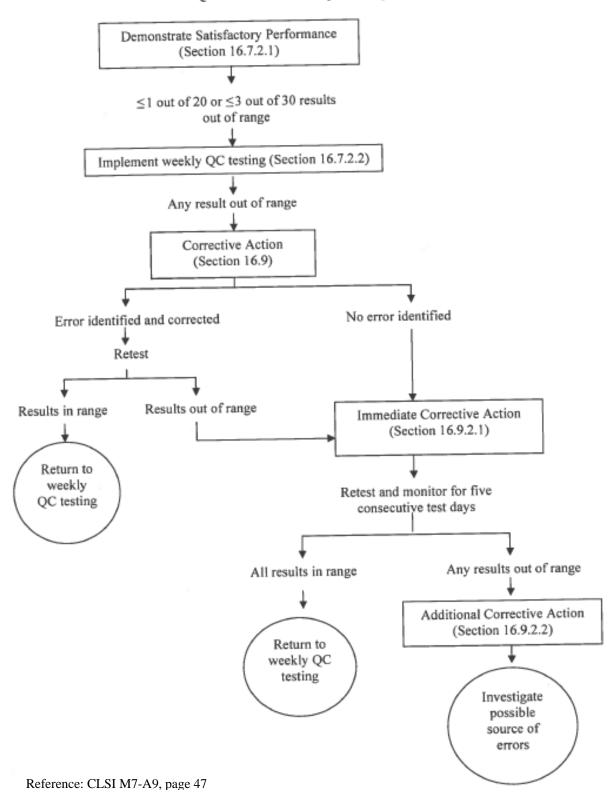
Reference: CLSI M7-A9, page 46



# European Union Reference Laboratory Antimicrobial Resistance

#### Appendix A. (Continued)

#### QC Protocol: Weekly Testing



#### Quality Control ranges for ATCC reference strains

E. coli ATCC 25922	
Antimicrobial	MIC
Ampicillin, AMP	2-8
Azithromycin, AZT	none
Cefepime, FEP	0.015-0.12
Cefotaxime, FOT	0.03-0.12
Cefotaxime + clavulanic acid, F/C	none
Cefoxitin, FOX	2-8
Ceftazidime, CAZ	0.06-0.5
Ceftazidime + clavulanic acid, T/C	none
Chloramphenicol, CHL	2-8
Ciprofloxacin, CIP	0.004-0.016
Colistin, COL	0.25-2
Ertapenem, ETP	0.004-0.016
Gentamicin, GEN	0.25-1
Imipenem, IMI	0.06-0.25
Meropenem, MERO	0.008-0.06
Nalidixic acid, NAL	1-4
Sulfisoxazole, FIS	8-32
Temocillin, TRM	none
Tetracycline, TET	0.5-2
Tigecycline, TGC	0.03-0.25
Trimethoprim, TMP	0.5-2

MIC ranges (μg/mL) are according to CLSI M100 S25 (range for ciprofloxacin and ertapenem extended to include 0.016).

Campylobacter jejuni ATCC 33560								
Antimicrobial	Microbroth (36-37°C/48h)	Microbroth (42°C/24h)	Agar dilution (36-37°C/48h)	Agar dilution (42°C/24h)				
Ciprofloxacin, CIP	0.06-0.25	0.03-0.12	0.12-1	0.06-0.5				
Erythromycin, ERY	0.5-2	0.25-2	1-8	1-4				
Gentamicin, GEN	0.5-2	0.25-2	0.5-2	0.5-4				
Nalidixic acid, NAL	4-16	4-16	None	None				
Tetracycline, TET	0.25-2	0.25-1	None	None				

MIC ranges (μg/mL) are according to CLSI (VET01-S2)

#### Test results from the reference strain E. coli ATCC 25922

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
2	1	Ampicillin	=	4	2	8	1	MIC
2	1	Azithromycin	=	4	-	-	-	MIC
2	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
2	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
2	1	Chloramphenicol	<=	8	2	8	1	MIC
2	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
2	1	Colistin	<=	1	0.25	2	1	MIC
2	1	Gentamicin	<=	0.5	0.25	1	1	MIC
2	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
2	1	Nalidixic acid	<=	4	1	4	1	MIC
2	1	Sulfamethoxazole	=	16	8	32	1	MIC
2	1	Tetracycline	<=	2	0.5	2	1	MIC
2	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
2	1	Trimethoprim	=	0.5	0.5	2	1	MIC
6	1	Ampicillin	=	4	2	8	1	MIC
6	1	Azithromycin	=	4	-	-		MIC
6	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
6	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
6	1	Chloramphenicol	<=	8	2	8	1	MIC
6	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
6	1	Colistin	<=	1	0.25	2	1	MIC
6	1	Gentamicin	<=	0.5	0.25	1	1	MIC
6	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
6	1	Nalidixic acid	<=	4	1	4	1	MIC
6	1	Sulfamethoxazole	=	32	8	32	1	MIC
6	1	Tetracycline	<=	2	0.5	2	1	MIC
6	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
6	1	Trimethoprim	=	1	0.5	2	1	MIC
6	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
6	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
6	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
6	2	Cefoxitin	=	4	2	8	1	MIC
6	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
6	2	Ceftazidime/clavulanic acid	<=	0.12	-	-	-	MIC
6	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
6	2	Imipenem	=	0.25	0.06	0.25	1	MIC
6	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
6	2	Temocillin	=	4	-	-	-	MIC
9	1	Ampicillin	=	4	2	8	1	MIC
9	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
9	1	Chloramphenicol	=	8	2	8	1	MIC
9	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
9	1	Colistin	<=	1	0.25	2	1	MIC
9	1	Gentamicin	<=	0.5	0.25	1	1	MIC
9	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
9	1	Nalidixic acid	<=	4	1	4	1	MIC
9	1	Sulfamethoxazole	=	16	8	32	1	MIC
9	1	Tetracycline	<=	2	0.5	2	1	MIC
9	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
9	1	Trimethoprim	=	1	0.5	2	1	MIC
9	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
9	2	Cefoxitin	=	4	2	8	1	MIC
9	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
9	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
9	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
9	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
<u> </u>		p=	· · · · · · · · · · · · · · · · · · ·				· · · · · · · · · · · · · · · · · · ·	

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
11	1	Ampicillin	' =	4	2	8	1	MIC
11	1	Azithromycin	=	8	-	-	-	MIC
11	1	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
11	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
11	1 1	Chloramphenicol	<=	8 0.015	0.004	8 0.016	<u>1</u> 1	MIC MIC
11	1	Ciprofloxacin Colistin	<= <=	1	0.004	2	1 1	MIC
11	1	Gentamicin	=	1	0.25	1	1	MIC
11	1	Meropenem	<=	0.03	0.008	0.06	<del>.</del> 1	MIC
11	1	Nalidixic acid	<=	4	1	4	1	MIC
11	1	Sulfamethoxazole	=	32	8	32	1	MIC
11	1	Tetracycline	<=	2	0.5	2	1	MIC
11	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
11	1	Trimethoprim	=	0.5	0.5	2	1	MIC
11 11	2	Cefepime	<=	0.06	0.015	0.125	<u>1</u> 1	MIC
11	2 2	Cefotaxime Cefotaxime/clavulanic acid	<= <=	0.25 0.12	0.03	0.125	1	MIC MIC
11	2	Cefoxitin	=	4	2	8	<del>-</del> 1	MIC
11	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
11	2	Ceftazidime/clavulanic acid	=	0.25	-	-	<u> </u>	MIC
11	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
11	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
11	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
11	2	Temocillin	=	32	-	-	-	MIC
12	1	Ampicillin	=	8	2	8	1	MIC
12	1	Azithromycin	=	4	-	- 0.405	- 4	MIC
12	1	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
12 12	1	Ceftazidime Chloramphenicol	<= <=	0.5 8	0.06	0.5 8	<u>1</u> 1	MIC MIC
12	1	Ciprofloxacin	<= <=	0.015	0.004	0.016	<u>'</u> 1	MIC
12	1	Colistin	<=	1	0.25	2	1	MIC
12	1	Gentamicin	=	1	0.25	1	1	MIC
12	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
12	1	Nalidixic acid	<=	4	1	4	1	MIC
12	1	Sulfamethoxazole	=	16	8	32	1	MIC
12	1	Tetracycline	<=	2	0.5	2	1	MIC
12	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
12 12	1 2	Trimethoprim Cefepime	=	0.5 0.06	0.5 0.015	2 0.125	<u>1</u> 1	MIC MIC
12	2	Cefotaxime	<= <=	0.06	0.015	0.125	1	MIC
12	2	Cefotaxime/clavulanic acid	<=	0.23	-	- 0.123	<u> </u>	MIC
12	2	Cefoxitin	=	4	2	8	1	MIC
12	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
12	2	Ceftazidime/clavulanic acid	=	0.25	-	-	-	MIC
12	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
12	2	Imipenem	<=	0.12	0.06	0.25	11	MIC
12	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
12	2	Temocillin	=	16	-	-	-	MIC
16 16	1 1	Ampicillin Azithromycin	=	4	2	8	1 -	MIC MIC
16	1	Cefotaxime	= <=	0.25	0.03	0.125	<u>-</u> 1	MIC
16	1	Ceftazidime	<=	0.25	0.06	0.123	1	MIC
16	1	Chloramphenicol	<=	8	2	8	<del>:</del> 1	MIC
16	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
16	1	Colistin	<=	1	0.25	2	1	MIC
16	1	Gentamicin	<=	0.5	0.25	1	1	MIC
16	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
16	1	Nalidixic acid	<=	4	1	4	11	MIC
16 16	1 1	Sulfamethoxazole	=	32 2	8	32 2	<u>1</u> 1	MIC
16	1	Tetracycline Tigecycline	<= <=	0.25	0.5 0.03	0.25	1 1	MIC MIC
16	1	Trimethoprim	=	0.25	0.03	2	<u>'</u> 1	MIC
16	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
16	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
16	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
16	2	Cefoxitin	=	4	2	8	1	MIC
16	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
16	2	Ceftazidime/clavulanic acid	=	0.25	-	-	-	MIC
16	2	Ertapenem	<=	0.015	0.004	0.016	11	MIC
16	2	Imipenem	<=	0.12	0.06	0.25	11	MIC
16	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
16		Temocillin	=	16	-	-	-	MIC

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
17	1	Ampicillin	=	8	2	8	1	MIC
17	1	Azithromycin	=	4	-	-	-	MIC
17	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
17	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
17	1	Chloramphenicol	<=	8	2	8	1	MIC
17 17	1	Ciprofloxacin	<=	0.01	0.004	0.016	<u>1</u> 1	MIC
17	1	Colistin Gentamicin	<=	1	0.25 0.25	2	<u> </u>	MIC MIC
17	1	Meropenem	= <=	0.03	0.25	0.06	<u>!</u> 1	MIC
17	1	Nalidixic acid	<=	4	1	4	1	MIC
17	1	Sulfamethoxazole	=	32	8	32	1	MIC
17	1	Tetracycline	<=	2	0.5	2	1	MIC
17	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
17	1	Trimethoprim	=	0.5	0.5	2	1	MIC
17	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
17	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
17	2	Cefotaxime/clavulanic acid	=	0.12	-	-	-	MIC
17	2	Cefoxitin	=	4	2	8	11	MIC
17	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
17	2	Ceftazidime/clavulanic acid	=	0.25	-	-	-	MIC
17	2	Ertapenem	<=	0.015	0.004	0.016	11	MIC
17	2	Imipenem	=	0.25	0.06	0.25	1	MIC
17 17	2	Meropenem Temocillin	<= =	0.03 16	0.008	0.06	1	MIC MIC
18	1	Ampicillin	=	2	2	8	1	MIC
18	1	Azithromycin	= <=	2	-	-	-	MIC
18	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
18	1	Ceftazidime	<=	0.5	0.06	0.5	<u>.</u> 1	MIC
18	1	Chloramphenicol	<=	8	2	8	1	MIC
18	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
18	1	Colistin	<=	1	0.25	2	1	MIC
18	1	Gentamicin	<=	0.5	0.25	1	1	MIC
18	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
18	1	Nalidixic acid	<=	4	1	4	1	MIC
18	1	Sulfamethoxazole	=	32	8	32	11	MIC
18	1	Tetracycline	<=	2	0.5	2	1	MIC
18	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
18 18	1	Trimethoprim	=	0.12	0.5	2 0.125	<u>1</u> 1	MIC
18	2	Cefepime Cefotaxime	=	0.12	0.015 0.03	0.125	1	MIC MIC
18	2	Cefoxitin	<=	4	2	8	1	MIC
18	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
18	2	Ertapenem	<=	0.015	0.004	0.016	<del>.</del>	MIC
18	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
18	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
18	2	Temocillin	=	8	-	-	-	MIC
19	1	Ampicillin	=	4	2	8	1	MIC
19	1	Azithromycin	=	4	-	-	-	MIC
19	1	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
19	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
19	1	Chloramphenicol	<=	8	2	8	1	MIC
19	1	Ciprofloxacin	<=	0.015	0.004	0.016	11	MIC
19	1	Contemisin	<=	1	0.25	2	1	MIC
19 19	1	Gentamicin Meropenem	<=	0.5 0.03	0.25 0.008	0.06	1	MIC MIC
19	1 1	Nalidixic acid	<= <=	4	1	4	<u>1</u> 1	MIC
19	1	Sulfamethoxazole	=	32	8	32	1	MIC
19	1	Tetracycline	= <=	2	0.5	2	1	MIC
19	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
19	1	Trimethoprim	=	5	0.5	2	0	MIC
19	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
19	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
19	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
19	2	Cefoxitin	=	4	2	8	1	MIC
19	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
19	2	Ceftazidime/clavulanic acid	<=	0.12	-	-	-	MIC
19	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
19	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
19	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
19	2	Temocillin	=	8	-	-	-	MIC

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
20	1	Ampicillin	=	4	2	8	1	MIC
20	1	Azithromycin	=	8	-	-	-	MIC
20	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
20	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
20	1	Chloramphenicol	<=	8	2	8	1	MIC
20	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
20	1	Colistin	<=	1	0.25	2	1	MIC
20	1	Gentamicin	<=	0.5	0.25	1	1	MIC MIC
20	1 1	Meropenem Nalidixic acid	<=	0.03	0.008	0.06	1	MIC
20	1	Sulfamethoxazole	=	32	8	32	1	MIC
20	1	Tetracycline	<=	2	0.5	2	1	MIC
20	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
20	1	Trimethoprim	=	0.5	0.5	2	1	MIC
20	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
20	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
20	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
20	2	Cefoxitin	=	2	2	8	1	MIC
20	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
20	2	Ceftazidime/clavulanic acid	=	0.25	-	-	-	MIC
20	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
20	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
20	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
20 21	2	Temocillin Ampicillin	=	8 4	2	- 0	<u>-</u> 1	MIC MIC
21	1 1	Azithromycin	=	4		8		MIC
21	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
21	1	Ceftazidime	<=	0.25	0.06	0.123	1	MIC
21	1	Chloramphenicol	<=	8	2	8	1	MIC
21	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
21	1	Colistin	<=	1	0.25	2	1	MIC
21	1	Gentamicin	<=	0.5	0.25	1	1	MIC
21	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
21	1	Nalidixic acid	<=	4	1	4	1	MIC
21	1	Sulfamethoxazole	=	32	8	32	1	MIC
21	1	Tetracycline	<=	2	0.5	2	1	MIC
21	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
21	1	Trimethoprim	=	0.5	0.5	2	1	MIC
21 21	2	Cefepime Cefotaxime	=	0.5 0.25	0.015	0.125	0	MIC MIC
21	2	Cerotaxime Cefotaxime/clavulanic acid	<=	0.25	0.03	0.125	<u>1</u>	MIC
21	2	Cefoxitin	<= =	8	2	8	1	MIC
21	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
21	2	Ceftazidime/clavulanic acid	=	0.25	-	-		MIC
21	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
21	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
21	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
21	2	Temocillin	=	16	-	-	•	MIC
22	1	Ampicillin	=	2	2	8	1	MIC
22	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
22	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
22	1	Chloramphenicol	<=	8	2	8	1	MIC
22	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
22	1	Colistin	<=	1	0.25	2	1	MIC
22	1	Gentamicin	<=	0.5	0.25	1	1	MIC MIC
22	1 1	Meropenem Nalidixic acid	<=	0.03	0.008	0.06 4	1 1	MIC
22	1	Tetracycline	<= <=	2	0.5	2	1	MIC
22	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
22	1	Trimethoprim	=	0.23	0.03	2	1	MIC
22	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
22	2	Cefoxitin	=	4	2	8	1	MIC
22	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
22	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
22	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
22	2							

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
23	1	Ampicillin	=	4	2	8	1	MIC
23	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
23	1	Ceftazidime	<b>&gt;=</b>	0.5	0.06	0.5	1	MIC
23	1	Chloramphenicol	<=	8	2	8	1	MIC
23	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
23	1	Colistin	<=	1	0.25	2	1	MIC
23	1	Gentamicin	=	1	0.25	1	1	MIC
23	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
23	1	Nalidixic acid	<=	4	1	4	1	MIC
23	1	Sulfamethoxazole	=	16	8	32	1	MIC
23	1	Tetracycline	<=	2	0.5	2	1	MIC
23	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
23	1	Trimethoprim	=	0.25	0.05	2	1	MIC
23	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
23	2	Cefotaxime		0.00	0.013	0.125	1	MIC
			<=	2	2		1	MIC
23	2	Cefoxitin	=			8		
23	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
23	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
23	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
23	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
25	1	Ampicillin	=	4	2	8	1	MIC
25	1	Azithromycin	=	4	-	-	-	MIC
25	1	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
25	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
25	1	Chloramphenicol	<=	8	2	8	1	MIC
25	1	Ciprofloxacin	=>	0.015	0.004	0.016	1	MIC
25	1	Colistin	<=	1	0.25	2	1	MIC
25	1	Gentamicin	<=	0.5	0.25	1	1	MIC
25	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
25	1	Nalidixic acid	<=	4	1	4	1	MIC
25	1	Sulfamethoxazole	<=	8	8	32	1	MIC
25	1	Tetracycline	<=	2	0.5	2	<del>:</del>	MIC
25	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
25	1	Trimethoprim	=	0.25	0.05	2	1	MIC
26	1	Ampicillin	=	4	2	8	1	MIC
26	1	Cefotaxime		0.25	0.03	0.125	1	MIC
26	1	Ceftazidime	<=	0.23	0.06	0.123	1	MIC
	_							
26	1	Chloramphenicol	<=	8	2	8	1	MIC
26	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
26	1	Colistin	<=	1	0.25	2	1	MIC
26	1	Gentamicin	=	1	0.25	1	1	MIC
26	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
26	1	Nalidixic acid	<=	4	1	4	11	MIC
26	1	Sulfamethoxazole	=	16	8	32	1	MIC
26	1	Tetracycline	<=	2	0.5	2	1	MIC
26	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
26	1	Trimethoprim	=	0.5	0.5	2	1	MIC
29	1	Ampicillin	=	8	2	8	1	MIC
29	1	Chloramphenicol	=	8	2	8	1	MIC
29	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
29	1	Colistin	=	1	0.25	2	1	MIC
29	1	Gentamicin	=	0.5	0.25	1	1	MIC
29	1	Nalidixic acid	<=	4	1	4	<del>:</del>	MIC
29	1	Sulfamethoxazole	=	16	8	32	1	MIC
29	1	Tetracycline	=	2	0.5	2	1	MIC
29	1	Trimethoprim		1	0.5	2	1	MIC
			=					
29	2	Cefepime	=	0.12	0.015	0.125	1	MIC
29	2	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
29	2	Ceftazidime	=	0.25	0.06	0.5	1	MIC
29	2	Ertapenem	=	0.015	0.004	0.016	1	MIC
29	2	Imipenem	=	0.12	0.06	0.25	11	MIC
29	2	Meropenem	=	0.03	0.008	0.06	1	MIC

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
30	1	Ampicillin		4	2	8	1	MIC
30	1	Azithromycin	=	4	-	-	-	MIC
30	1	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
30	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
30	1	Chloramphenicol	<=	8	2	8	1	MIC
30 30	1 1	Ciprofloxacin Colistin	<=	0.015 1	0.004 0.25	0.016	<u>1</u> 1	MIC MIC
30	1	Gentamicin	<= <=	0.5	0.25	1	1	MIC
30	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
30	1	Nalidixic acid	<=	4	1	4	1	MIC
30	1	Sulfamethoxazole	=	16	8	32	1	MIC
30	1	Tetracycline	<=	2	0.5	2	1	MIC
30	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
30	1	Trimethoprim	=	0.5	0.5	2	1	MIC
30	2	Cefepime	<=	0.06	0.015	0.125	11	MIC
30	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
30	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
30 30	2	Cefoxitin Ceftazidime	=	2 0.25	2	8 0.5	<u>1</u> 1	MIC MIC
30	2	Ceftazidime/clavulanic acid	<= <=	0.25	0.06	0.5	<u> </u>	MIC
30	2	Ertapenem	<=	0.12	0.004	0.016	1	MIC
30	2	Imipenem	<=	0.013	0.004	0.016	1	MIC
30	2	Meropenem	<=	0.12	0.008	0.25	1	MIC
30	2	Temocillin	=	8	-	-	<u> </u>	MIC
32	1	Ampicillin	=	4	2	8	1	MIC
32	1	Azithromycin	=	4	-	-	-	MIC
32	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
32	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
32	1	Chloramphenicol	<=	8	2	8	11	MIC
32	1	Ciprofloxacin	<=	0.15	0.004	0.016	1	MIC
32	1	Contaminin	<=	1	0.25	2	11	MIC
32 32	1 1	Gentamicin Meropenem	<=	0.5 0.03	0.25 0.008	1 0.06	<u>1</u> 1	MIC MIC
32	1	Nalidixic acid	<= <=	4	1	4	1	MIC
32	1	Sulfamethoxazole	=	16	8	32	1	MIC
32	1	Tetracycline	<=	2	0.5	2	1	MIC
32	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
32	1	Trimethoprim	=	0.5	0.5	2	1	MIC
32	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
32	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
32	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
32	2	Cefoxitin	=	4	2	8	1	MIC
32	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
32	2	Ceftazidime/clavulanic acid	<=	0.12	- 0.004	- 0.040	<u>-</u> 1	MIC
32	2	Ertapenem	<=	0.015	0.004	0.016		MIC
32 32	2	Imipenem Meropenem	= <=	0.25	0.06	0.25 0.06	<u>1</u> 1	MIC MIC
32	2	Temocillin	=	8	- 0.008	-	-	MIC
33	1	Ampicillin	=	4	2	8	1	MIC
33	1	Azithromycin	=	4	-	-	<del>-</del>	MIC
33	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
33	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
33	1	Chloramphenicol	<=	8	2	8	1	MIC
33	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
33	1	Colistin	<=	1	0.25	2	1	MIC
33	1	Gentamicin	<=	0.5	0.25	1	11	MIC
33	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
33	1	Nalidixic acid	<=	4	1	4	1	MIC
33	1	Sulfamethoxazole	=	32	8	32	11	MIC
33	1	Tetracycline Tigecycline	<=	2	0.5	2	1	MIC
33 33	1 1	Trimethoprim	<=	0.25 0.5	0.03	0.25 2	1 1	MIC MIC
33	2	Cefepime	= <=	0.5	0.015	0.125	1 1	MIC
33	2	Cefotaxime	<= <=	0.06	0.015	0.125	1	MIC
33	2	Cefotaxime/clavulanic acid	<=	0.25	-	-	<u> </u>	MIC
33	2	Cefoxitin	=	4	2	8	1	MIC
33	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
33	2	Ceftazidime/clavulanic acid	<=	0.12	-	-	<u> </u>	MIC
	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
33		a compare to the	+ · · · · · · · · · · · · · · · · · · ·					
33 33		Imipenem	=	0.25	0.06	0.25	1	MIC
	2	Imipenem Meropenem	= <=	0.25 0.03	0.06	0.25 0.06	1 1	MIC

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
34	1	Ampicillin	=	4	2	8	1	MIC
34	1	Azithromycin	=	4	-	-	-	MIC
34	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
34	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
34	1	Chloramphenicol	<=	8	2	8	1	MIC
34 34	1 1	Ciprofloxacin Colistin	<=	0.015 1	0.004	0.016	1	MIC MIC
34	1	Gentamicin	<= <=	0.5	0.25	1	1	MIC
34	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
34	1	Nalidixic acid	<=	4	1	4	1	MIC
34	1	Sulfamethoxazole	=	16	8	32	1	MIC
34	1	Tetracycline	<=	2	0.5	2	1	MIC
34	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
34	1	Trimethoprim	=	0.5	0.5	2	1	MIC
34	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
34	2 2	Cefotaxime Cefotaxime/clavulanic acid	<=	0.25 0.06	0.03	0.125	1 -	MIC MIC
34	2	Cefoxitin	<= =	4	2	8	1	MIC
34	2	Ceftazidime	= <=	0.25	0.06	0.5	1	MIC
34	2	Ceftazidime/clavulanic acid	<=	0.12	-	-	-	MIC
34	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
34	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
34	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
34	2	Temocillin	=	16	-	-	-	MIC
36	1	Ampicillin	=	4	2	8	1	MIC
36	1	Azithromycin	<=	2	-	- 0.405	-	MIC
36	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
36 36	1	Ceftazidime Chloramphenicol	<=	0.5 8	0.06	0.5 8	1	MIC MIC
36	1	Ciprofloxacin	<= =	0.03	0.004	0.016	0	MIC
36	1	Colistin	<=	1	0.004	2	1	MIC
36	1	Gentamicin	=	1	0.25	1	1	MIC
36	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
36	1	Nalidixic acid	<=	4	1	4	1	MIC
36	1	Sulfamethoxazole	<=	8	8	32	1	MIC
36	1	Tetracycline	<=	2	0.5	2	1	MIC
36	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
36 36	1 2	Trimethoprim Cefepime	<=	0.25 0.06	0.5 0.015	2 0.125	<b>0</b>	MIC MIC
36	2	Cefotaxime	<= <=	0.06	0.015	0.125	1	MIC
36	2	Cefotaxime/clavulanic acid	<=	0.25	-	-	-	MIC
36	2	Cefoxitin	=	4	2	8	1	MIC
36	2	Ceftazidime	<=	0.25	0.06	0.5	1	MIC
36	2	Ceftazidime/clavulanic acid	<=	0.12	-	-	-	MIC
36	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
36	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
36	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
36	2	Temocillin	=	16	-	-	-	MIC
37	1	Ampicillin	=	4	2	8	1	AGA
37 37	1 1	Azithromycin Cefotaxime	= <=	4 0.25	0.03	0.125	1	AGA AGA
37	1	Ceftazidime	<=	0.25	0.03	0.125	1	AGA
37	1	Chloramphenicol	<=	8	2	8	1	AGA
37	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	AGA
37	1	Colistin	<=	1	0.25	2	1	AGA
37	1	Gentamicin	<=	0.5	0.25	1	1	AGA
37	1	Meropenem	<=	0.03	0.008	0.06	1	AGA
37	1	Nalidixic acid	<=	4	1	4	1	AGA
37	1	Sulfamethoxazole	=	32	8	32	1	AGA
37	1	Tetracycline	<=	2	0.5	2	1	AGA
37 37	1	Tigecycline Trimethoprim	<=	0.25 1	0.03	0.25 2	1	AGA AGA
37	2	Cefepime	=	0.03	0.015	0.125	1	AGA
37	2	Cefotaxime	<=	0.03	0.013	0.125	1	AGA
37	2	Cefotaxime/clavulanic acid	<=	0.23	-	-	-	AGA
37	2	Cefoxitin	=	4	2	8	1	AGA
37	2	Ceftazidime	<=	0.25	0.06	0.5	1	AGA
37	2	Ceftazidime/clavulanic acid	<=	0.125	-	-	-	AGA
37	2	Ertapenem	<=	0.015	0.004	0.016	1	AGA
37	2	Imipenem	<=	0.125	0.06	0.25	1	AGA
37	2	Meropenem	<=	0.03	0.008	0.06	1	AGA
37	2	Temocillin	=	2				AGA

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit		Mark	Method
39	1	Ampicillin	=	4	2	8	11	MIC
39 39	1 1	Azithromycin Cefotaxime	= <=	<u>4</u> 0.25	0.03	0.125	<u>-</u> 1	MIC MIC
39	1	Ceftazidime	<=	0.25	0.03	0.125	1	MIC
39	1	Chloramphenicol	<=	8	2	8	1	MIC
39	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
39	1	Colistin	<=	1	0.25	2	1	MIC
39	1	Gentamicin	<=	0.5	0.25	1	1	MIC
39	1	Meropenem	<=	0.03	0.008	0.06	11	MIC
39	1	Nalidixic acid	<=	4	1	4	1	MIC
39 39	1	Sulfamethoxazole	=	16 2	8 0.5	32 2	<u>1</u> 1	MIC MIC
39	1	Tetracycline Tigecycline	<= <=	0.25	0.03	0.25	<u>1</u> 1	MIC
39	1	Trimethoprim	=	0.25	0.05	2	1	MIC
39	2	Cefepime	=	0.06	0.015	0.125	1	MIC
39	2	Cefotaxime	=	0.25	0.03	0.125	0	MIC
39	2	Cefoxitin	=	4	2	8	1	MIC
39	2	Ceftazidime	=	0.25	0.06	0.5	1	MIC
39	2	Ertapenem	=	0.015	0.004	0.016	1	MIC
39	2	Imipenem	=	0.25	0.06	0.25	1	MIC
39 39	2	Meropenem	=	0.03	0.008	0.06	<u>1</u>	MIC MIC
40	1	Temocillin Ampicillin	=	16 2	2	8	<u>-</u> 1	MIC
40	1	Cefotaxime	=	0.12	0.03	0.125	1	MIC
40	1	Ceftazidime	<=	0.5	0.06	0.5	<u>·</u> 1	MIC
40	1	Chloramphenicol	<=	8	2	8	1	MIC
40	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
40	1	Colistin	<=	1	0.25	2	1	MIC
40	1	Gentamicin	<=	0.5	0.25	1	11	MIC
40	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
40	1	Nalidixic acid	<=	4	1	4	1	MIC
40	1	Sulfamethoxazole Tetracycline	=	16 2	8 0.5	32 2	<u>1</u> 1	MIC MIC
40	1	Tigecycline	<= <=	0.25	0.03	0.25	1	MIC
40	1	Trimethoprim	=	0.25	0.5	2	1	MIC
40	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
40	2	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
40	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
40	2	Cefoxitin	=	2	2	8	11	MIC
40	2	Ceftazidime	=	0.5	0.06	0.5	1	MIC
40	2	Ceftazidime/clavulanic acid	<=	0.12	- 0.004	- 0.040	- 4	MIC
40	2	Ertapenem Imipenem	<= <=	0.015 0.12	0.004	0.016 0.25	<u>1</u> 1	MIC MIC
40	2	Meropenem	<=	0.12	0.008	0.23	1	MIC
42	1	Ampicillin	=	4	2	8	1	MIC
42	1	Azithromycin	=	8	-	-	-	MIC
42	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
42	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
42	1	Chloramphenicol	<=	8	2	8	1	MIC
42	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
42 42	1 1	Colistin Gentamicin	<=	1 0.5	0.25 0.25	1	1	MIC
42	1 1	Meropenem	<= <=	0.5	0.25	0.06	<u>1</u> 1	MIC MIC
42	1	Nalidixic acid	<= <=	4	1	4	<u> </u> 1	MIC
42	1	Sulfamethoxazole	=	64	8	32	0	MIC
42	1	Tetracycline	<=	2	0.5	2	1	MIC
42	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
42	1	Trimethoprim	=	1	0.5	2	1	MIC
42	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
42	2	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
42	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
42 42	2 2	Cefoxitin Ceftazidime	=	4 0.25	2 0.06	8 0.5	<u>1</u>	MIC MIC
42	2	Ceftazidime/clavulanic acid	<= <=	0.25	-	- 0.5	<u> </u>	MIC
42	2	Ertapenem	<=	0.125	0.004	0.016	1	MIC
42		Imipenem	=	0.25	0.06	0.25	1	MIC
	2	IIIIIDELIEIII	_					IVIIO
42	2	Meropenem	<=	0.03	0.008	0.06	1	MIC

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
45	1	Ampicillin	=	4	2	8	1	MIC
45	1	Azithromycin	=	4	-	-	-	MIC
45	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
45	1	Ceftazidime	<=	0.5	0.06	0.5	11	MIC
45 45	1 1	Chloramphenicol	<=	8 0.015	0.004	8 0.016	<u>1</u> 1	MIC MIC
45	1	Ciprofloxacin Colistin	<= <=	1	0.004	2	<u> </u>	MIC
45	1	Gentamicin	<=	0.5	0.25	1	1	MIC
45	1	Meropenem	<=	0.03	0.008	0.06	<del>.</del>	MIC
45	1	Nalidixic acid	<=	4	1	4	1	MIC
45	1	Sulfamethoxazole	=	32	8	32	1	MIC
45	1	Tetracycline	<=	2	0.5	2	1	MIC
45	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
45	1	Trimethoprim	=	1	0.5	2	11	MIC
45	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
45	2	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
45	2	Cefotaxime/clavulanic acid	<=	0.06	2	- 8	-	MIC
45 45	2 2	Cefoxitin Ceftazidime	= <=	4 0.25	0.06	0.5	<u>1</u> 1	MIC MIC
45	2	Ceftazidime/clavulanic acid	<=	0.23	0.00	0.5	<u>'</u>	MIC
45	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
45	2	Imipenem	<=	0.12	0.06	0.25	<u>·</u> 1	MIC
45	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
45	2	Temocillin	=	16	-	-		MIC
56	1	Ampicillin	=	4	2	8	1	MIC
56	1	Azithromycin	=	4	-	-	-	MIC
56	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
56	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
56	1	Chloramphenicol	<=	8	2	8	11	MIC MIC
56 56	1 1	Ciprofloxacin Colistin	<= <=	0.015 1	0.004 0.25	0.016 2	<u>1</u> 1	MIC
56	1 1	Gentamicin	<=	0.5	0.25	1	1	MIC
56	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
56	1	Nalidixic acid	<=	4	1	4	1	MIC
56	1	Sulfamethoxazole	=	16	8	32	1	MIC
56	1	Tetracycline	<=	2	0.5	2	1	MIC
56	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
56	1	Trimethoprim	=	1	0.5	2	1	MIC
56	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
56	2	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
56 56	2	Cefotaxime/clavulanic acid	<=	0.06	2	- 8	- 4	MIC MIC
56	2	Cefoxitin Ceftazidime	= <=	0.25	0.06	0.5	<u>1</u> 1	MIC
56	2	Ceftazidime/clavulanic acid	<=	0.23	-	-	<u>'</u>	MIC
56	2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
56	2	Imipenem	<=	0.12	0.06	0.25	1	MIC
56	2	Meropenem	<=	0.03	0.008	0.06	1	MIC
56	2	Temocillin	=	4	-	-	_	MIC
58	1	Ampicillin	=	8	2	8	1	MIC
58	1	Azithromycin	=	8	-	-	-	MIC
58	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
58	1	Ceftazidime	<=	0.5	0.06	0.5	11	MIC
58	1	Chloramphenicol	<=	8	2	8	1	MIC
58 58	1 1	Ciprofloxacin Colistin	<=	0.015 1	0.004 0.25	0.016	<u>1</u> 1	MIC MIC
58	1	Gentamicin	<=	0.5	0.25	1	<u> </u> 1	MIC
58	1	Meropenem	<=	0.03	0.23	0.06	1	MIC
58	1	Nalidixic acid	<=	4	1	4	<del>:</del> 1	MIC
58	1	Sulfamethoxazole	=	32	8	32	1	MIC
58	1	Tetracycline	<=	2	0.5	2	1	MIC
58	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
58	1	Trimethoprim	=	1	0.5	2	1	MIC
58	2	Cefepime	<=	0.06	0.015	0.125	1	MIC
58	2	Cefotaxime	<=	0.25	0.03	0.125	11	MIC
58	2	Cefotaxime/clavulanic acid	<=	0.06	-	-	-	MIC
58 58	2	Cefoxitin	=	4	2	8	11	MIC
n nx	2	Ceftazidime	<=	0.25 0.25	0.06	0.5	1	MIC MIC
		Coftazidimo/olavulania acid				_	_	I IVIIL,
58	2	Ceftazidime/clavulanic acid	=		0.004	0.016	1	
58 58	2 2	Ertapenem	<=	0.015	0.004	0.016	1	MIC
58	2		1		0.004 0.06 0.008	0.016 0.25 0.06	1 1 1	

Lab no.	Panel	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
60	1	Ampicillin	=	4	2	8	1	MIC
60	1	Azithromycin	=	4	-	-	-	MIC
60	1	Cefotaxime	<=	0.25	0.03	0.125	1	MIC
60	1	Ceftazidime	<=	0.5	0.06	0.5	1	MIC
60	1	Chloramphenicol	<=	8	2	8	1	MIC
60	1	Ciprofloxacin	<=	0.015	0.004	0.016	1	MIC
60	1	Colistin	<=	1	0.25	2	1	MIC
60	1	Gentamicin	<=	0.5	0.25	1	1	MIC
60	1	Meropenem	<=	0.03	0.008	0.06	1	MIC
60	1	Nalidixic acid	<=	4	1	4	1	MIC
60	1	Sulfamethoxazole	<=	8	8	32	1	MIC
60	1	Tetracycline	<=	2	0.5	2	1	MIC
60	1	Tigecycline	<=	0.25	0.03	0.25	1	MIC
60	1	Trimethoprim	<=	0.25	0.5	2	0	MIC

MIC: Microbroth dilution AGA: Agar dilution

### Test results from the reference strain *C. jejuni* ATCC 33560

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37°C/48h	42°C/24h
2	Ciprofloxacin	=	0.12	0.06	0.25	1	MIC	Х	
2	Erythromycin	=	1	0.5	2	1	MIC	Χ	
2	Gentamicin	=	0.25	0.5	2	0	MIC	Х	
2	Nalidixic acid	=	8	4	16	1	MIC	Χ	
2	Streptomycin	=	1	-	-	-	MIC	Х	
2	Tetracycline	=	2	0.25	2	1	MIC	Х	
6	Ciprofloxacin	<=	0.12	0.03	0.125	1	MIC		Χ
6	Erythromycin	<=	1	0.25	2	1	MIC		Χ
6	Gentamicin	=	1	0.25	2	1	MIC		X
6	Nalidixic acid	=	8	4	16	1	MIC		Χ
6	Streptomycin	=	4	-	-	_	MIC		Χ
6	Tetracycline	<=	0.5	0.25	1	1	MIC		Χ
9	Ciprofloxacin	=	0.25	0.06	0.25	1	MIC	Х	
9	Erythromycin	=	2	0.5	2	1	MIC	X	
9	Gentamicin	=	1	0.5	2	1	MIC	X	
9	Nalidixic acid	=	4	4	16	1	MIC	X	
9	Streptomycin	=	1	-	-	-	MIC	X	
9	Tetracycline	=	1	0.25	2	1	MIC	X	
11	Ciprofloxacin	=	0.25	0.25	0.25	1	MIC	X	
11	Erythromycin	<=	1	0.5	2	1	MIC	X	
11	Gentamicin	=	1	0.5	2	1	MIC	X	
11	Nalidixic acid	=	8	4	16	1	MIC	X	
11	Streptomycin	=	4	7	- 10	-	MIC	X	
11	Tetracycline		1	0.25	2	1	MIC	X	
12	Ciprofloxacin	=	0.25	0.25	0.25	1	MIC	X	
12		=		0.06	2	1	MIC	X	
	Erythromycin	<=	1 2		2			X	
12 12	Gentamicin	=		0.5 4		1	MIC MIC	X	
	Nalidixic acid	=	8	-	16	1			
12	Streptomycin	=	4		2	-	MIC	X	
12	Tetracycline	<=	0.5	0.25		1	MIC	X	V
14	Ciprofloxacin	<=	0.125	0.03	0.125	1	MIC		X
14	Erythromycin	<=	1	0.25	2	1	MIC		X
14	Gentamicin	=	1	0.25	2	1	MIC		X
14	Nalidixic acid	=	8	4	16	1	MIC		X
14	Streptomycin	=	4	-	-	-	MIC		X
14	Tetracycline	=	1	0.25	1	1	MIC		Х
17	Ciprofloxacin	=	0.25	0.06	0.25	1	MIC	X	
17	Erythromycin	<=	1	0.5	2	1	MIC	X	
17	Gentamicin	=	2	0.5	2	1	MIC	X	
17	Nalidixic acid	=	8	4	16	1	MIC	X	
17	Streptomycin	=	8	-	-	-	MIC	X	
17	Tetracycline	<=	0.5	0.25	2	1	MIC	Х	
18	Ciprofloxacin	<=	0.12	0.03	0.125	1	MIC		X
18	Erythromycin	<=	1	0.25	2	1	MIC		X
18	Gentamicin	=	1	0.25	2	1	MIC		X
18	Nalidixic acid	=	8	4	16	1	MIC		Χ
18	Tetracycline	=	1	0.25	1	1	MIC		Χ
19	Ciprofloxacin	<=	0.12	0.06	0.25	1	MIC	X	
19	Erythromycin	<=	1	0.5	2	1	MIC	Х	
19	Gentamicin	=	1	0.5	2	1	MIC	Χ	
19	Nalidixic acid	=	8	4	16	1	MIC	X	
19	Streptomycin	=	4	-	-	-	MIC	X	
19	Tetracycline	=	2	0.25	2	1	MIC	Χ	

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37°C/48h	42°C/24h
20	Ciprofloxacin	=	0.25	0.03	0.125	0	MIC		Χ
20	Erythromycin	<=	1	0.25	2	1	MIC		Χ
20	Gentamicin	=	1	0.25	2	1	MIC		Χ
20	Nalidixic acid	=	8	4	16	1	MIC		Χ
20	Streptomycin	=	2	-	-	-	MIC		Χ
20	Tetracycline	=	4	0.25	1	0	MIC		Χ
21	Ciprofloxacin	<=	0.12	0.03	0.125	1	MIC		Х
21	Erythromycin	<=	1	0.25	2	1	MIC		Χ
21	Gentamicin	=	0.5	0.25	2	1	MIC		Χ
21	Nalidixic acid	=	4	4	16	1	MIC		Χ
21	Streptomycin	=	2	-	-	-	MIC		Χ
21	Tetracycline	<=	0.5	0.25	1	1	MIC		Χ
22	Ciprofloxacin	<=	0.06	0.03	0.125	1	MIC		Χ
22	Erythromycin	<=	1	0.25	2	1	MIC		X
22	Nalidixic acid	=	4	4	16	1	MIC		X
22	Tetracycline	=	1	0.25	1	1	MIC		X
23	Ciprofloxacin	=	0.12	0.03	0.125	1	MIC		X
23	Erythromycin	<=	0.12	0.05	2	1	MIC		X
23	Gentamicin	=	1	0.25	2	1	MIC		X
23	Nalidixic acid	=	4	4	16	1	MIC		X
23	Tetracycline	=	0.5	0.25	1	1	MIC		X
25	Ciprofloxacin	=	0.25	0.25	0.25	1	MIC	Χ	
25	Erythromycin		1	0.00	2	1	MIC	X	
25	Gentamicin	<=	0.25	0.5	2	0	MIC	X	
25	Nalidixic acid	=		4	16	1	MIC	X	
		=	8 1	4	10	- 1		X	
25	Streptomycin	=	2	0.05	- 0	-	MIC	X	
25	Tetracycline	=		0.25	2	1	MIC MIC	X	
26	Ciprofloxacin	<=	0.12	0.06	0.25	1			
26	Erythromycin	<=	1	0.5	2	1	MIC	X	
26	Gentamicin	=	0.5	0.5	2	1	MIC	X	
26	Nalidixic acid	=	4	4	16	1	MIC		
26	Streptomycin	=	2	- 0.05	-	-	MIC	X	
26	Tetracycline	<=	0.5	0.25	2	1	MIC	Х	
29	Ciprofloxacin	=	0.06	0.03	0.125	1	MIC		X
29	Erythromycin	=	1	0.25	2	1	MIC		X
29	Gentamicin	=	2	0.25	2	1	MIC		X
29	Nalidixic acid	=	4	4	16	1	MIC		X
29	Tetracycline	=	0.25	0.25	1	1	MIC	.,	Χ
30	Ciprofloxacin	=	0.25	0.06	0.25	1	MIC	X	
30	Erythromycin	<=	1	0.5	2	1	MIC	X	
30	Gentamicin	=	1	0.5	2	1	MIC	X	
30	Nalidixic acid	=	8	4	16	1	MIC	X	
30	Streptomycin	=	2	-	-	-	MIC	X	
30	Tetracycline	=	2	0.25	2	1	MIC	X	
32	Ciprofloxacin	<=	0.12	0.06	0.25	1	MIC	Х	
32	Erythromycin	<=	1	0.5	2	1	MIC	X	
32	Gentamicin	=	0.5	0.5	2	1	MIC	Х	
32	Nalidixic acid	=	4	4	16	1	MIC	X	
32	Streptomycin	=	4	-	-	-	MIC	X	
32	Tetracycline	=	1	0.25	2	1	MIC	X	
33	Ciprofloxacin	=	0.25	0.06	0.25	1	MIC	X	
33	Erythromycin	<=	1	0.5	2	1	MIC	X	
33	Gentamicin	=	0.5	0.5	2	1	MIC	Х	
33	Nalidixic acid	=	8	4	16	1	MIC	X	
33	Streptomycin	=	2		-	-	MIC	X	
33	Tetracycline	<=	0.5	0.25	2	1	MIC	Х	

34	Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42°C/24h
34   Enthromycin										0,
34   Nalidixic acid   =   8							1			
Nalidixica acid										
34   Steptomycin										
Tetracycline		Streptomycin	=	0.5	-	-	-			
36			=		0.25	2	1			
36	36		<=	0.06		0.125	1	MIC		Х
36   Gentamicin	36	Erythromycin	<=			2	1	MIC		
36   Nalidixic acid   =   16   4   16   1   MIC   X   X   36   Streptomycin   =   2   -   -   -   MIC   X   X   36   Tetracycline   <=   0.12   0.25   1   0   MIC   X   X   X   37   Ciprofloxacin   =   0.25   0.12   1   1   AGA   X   X   37   Etythomycin   =   2   1   8   1   AGA   X   X   37   Streptomycin   =   2   0.5   2   1   AGA   X   X   37   Nalidixic acid   =   8   -   -   -   AGA   X   X   X   X   X   X   X   X   X			=				1			
36   Streptomycin   =   2   -   -   MIC   X     36   Tetracycline   <=   0.12   0.25   1   0   MIC   X     37   Ciprofloxacin   =   0.25   0.12   1   1   AGA   X     37   Erythromycin   =   2   1   8   1   AGA   X     37   Streptomycin   =   2   0.5   2   1   AGA   X     37   Nalidxic acid   =   8   -   -   -   AGA   X     37   Streptomycin   =   4   -   -   -   AGA   X     37   Streptomycin   =   4   -   -   -   AGA   X     38   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC   X     39   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC   X     39   Gentamicin   =   0.5   0.5   2   1   MIC   X     39   Gentamicin   =   0.5   0.5   2   1   MIC   X     39   Tetracycline   =   1   0.5   2   1   MIC   X     40   Ciprofloxacin   =   0.12   0.03   0.125   1   MIC   X     40   Ciprofloxacin   =   0.12   0.03   0.125   1   MIC   X     40   Ciprofloxacin   =   0.25   0.25   2   1   MIC   X     40   Ciprofloxacin   =   0.25   0.25   2   1   MIC   X     40   Ciprofloxacin   =   0.25   0.25   2   1   MIC   X     40   Ciprofloxacin   =   0.25   0.25   2   1   MIC   X     40   Ciprofloxacin   =   0.25   0.25   2   1   MIC   X     40   Tetracycline   =   0.25   0.25   1   MIC   X     40   Tetracycline   =   0.25   0.25   1   MIC   X     41   Tetracycline   =   0.25   0.06   0.25   1   MIC   X     42   Ciprofloxacin   =   0.25   0.06   0.25   1   MIC   X     42   Ciprofloxacin   =   0.25   0.06   0.25   1   MIC   X     42   Streptomycin   =   1   -   -   MIC   X     43   Tetracycline   =   0.12   0.5   2   0   MIC   X     44   Streptomycin   =   1   -   -   MIC   X     45   Ciprofloxacin   =   0.12   0.5   2   1   MIC   X     45   Ciprofloxacin   =   0.12   0.5   2   1   MIC   X     45   Ciprofloxacin   =   0.12   0.5   2   1   MIC   X     45   Ciprofloxacin   =   1   0.5   2   1   MIC   X     45   Ciprofloxacin   =   1   0.5   2   1   MIC   X     45   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC   X     45   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC   X     46   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC	36		=	16		16	1			
36   Tetracycline			=		-	-	-			Χ
37   Ciprofloxacin	36		<=	0.12	0.25	1	0	MIC		
37   Erythromycin	37		=	0.25	0.12	1	1	AGA	Х	
37   Gentamicin   =   2   0.5   2   1   AGA   X   37   Nalidixic acid   =   8   -   -   -   AGA   X   37   Tetracycline   =   1   -   -   -   -   AGA   X   39   Ciprofloxacin   =   0.12   0.06   0.25   1   MIC   X   39   Erythromycin   =   1   0.5   2   1   MIC   X   39   Erythromycin   =   1   0.5   2   1   MIC   X   39   Gentamicin   =   0.5   0.5   2   1   MIC   X   39   Nalidixic acid   =   8   4   16   1   MIC   X   39   Tetracycline   =   1   0.25   2   1   MIC   X   X   39   Tetracycline   =   1   0.25   2   1   MIC   X   X   X   X   X   X   X   X   X	37		=	2	1	8	1			
37   Streptomycin	37		=	2	0.5	2	1		Х	
37   Streptomycin	37		=	8	-	-	-	AGA	Х	
37   Tetracycline		Streptomycin	=		-	-	-			
39   Ciprofloxacin			=		-	-	-			
39   Erythromycin			=		0.06	0.25	1			
39   Gentamicin		•	=				1			
39   Nalidixic acid			=	0.5			1			
39   Tetracycline			=				1			
40	39	Tetracycline	=	1	0.25		1	MIC	Х	
40   Gentamicin   =   0.25   0.25   2   1   MIC   X   X   X   X   X   X   X   X   X	40		<=	0.12	0.03	0.125	1	MIC		Χ
40   Gentamicin   =   0.25   0.25   2   1   MIC   X   X   Nalidixic acid   =   4   4   16   1   MIC   X   X   X   X   X   X   X   X   X	40	Erythromycin	<=	1	0.25	2	1	MIC		Х
40   Tetracycline   <=   0.5   0.25   1   1   MIC   X     42   Ciprofloxacin   =   0.25   0.06   0.25   1   MIC   X     42   Erythromycin   <=   1   0.5   2   1   MIC   X     42   Gentamicin   <=   0.12   0.5   2   0   MIC   X     42   Nalidixic acid   =   8   4   16   1   MIC   X     42   Streptomycin   =   1   -   -   -   MIC   X     42   Streptomycin   =   1   -   -   -   MIC   X     42   Tetracycline   =   2   0.25   2   1   MIC   X     45   Erythromycin   <=   1   0.5   2   1   MIC   X     45   Erythromycin   <=   1   0.5   2   1   MIC   X     45   Gentamicin   =   1   0.5   2   1   MIC   X     45   Streptomycin   =   4   -   -   -   MIC   X     45   Streptomycin   =   4   -   -   -   MIC   X     45   Streptomycin   <=   4   -   -   -   MIC   X     45   Tetracycline   =   2   0.25   2   1   MIC   X     45   Tetracycline   =   2   0.25   2   1   MIC   X     45   Tetracycline   =   2   0.25   2   1   MIC   X     45   Tetracycline   =   4   -   -   -   MIC   X     56   Ciprofloxacin   <=   0.12   0.06   0.25   1   MIC   X     56   Centamicin   =   1   0.5   2   1   MIC   X     56   Streptomycin   =   4   -   -   -   MIC   X     56   Streptomycin   =   4   -   -   -   MIC   X     56   Tetracycline   =   1   0.25   2   1   MIC   X     58   Ciprofloxacin   <=   0.12   0.06   0.25   1   MIC   X     58   Streptomycin   <=   1   0.5   2   1   MIC   X     58   Gentamicin   =   0.5   0.5   2   1   MIC   X     58   Streptomycin   <=   1   0.5   2   1   MIC   X     58   Streptomycin   <=   1   0.5   2   1   MIC   X     58   Streptomycin   <=   1   0.5   2   1   MIC   X     58   Streptomycin   <=   1   0.5   2   1   MIC   X     58   Ciprofloxacin   =   0.5   0.5   2   1   MIC   X     59   Streptomycin   <=   1   0.5   2   1   MIC   X     50   Ciprofloxacin   =   0.5   0.25   2   1   MIC   X     50   Ciprofloxacin   =   0.5   0.25   1   MIC   X     50   Ciprofloxacin   =   0.5   0.25   2   1   MIC   X     50   Ciprofloxacin   =   0.5   0.25   2   1   MIC   X     50   Ciprofloxacin   =   0.5   0.25   0.06   0.25   1   M	40		=	0.25	0.25	2	1	MIC		Χ
42         Ciproffoxacin         =         0.25         0.06         0.25         1         MIC         X           42         Erythromycin         <=	40	Nalidixic acid	=	4	4	16	1	MIC		Х
42         Erythromycin         <=	40	Tetracycline	<=	0.5	0.25	1	1	MIC		Χ
42         Erythromycin         <=	42	Ciprofloxacin	=	0.25	0.06	0.25	1	MIC	Х	
42   Nalidixic acid   =   8	42	Erythromycin	<=	1	0.5	2	1	MIC	Х	
42         Streptomycin         =         1         -         -         -         MIC         X           42         Tetracycline         =         2         0.25         2         1         MIC         X           45         Ciprofloxacin         <=	42	Gentamicin	<=	0.12	0.5	2	0	MIC		
42         Tetracycline         =         2         0.25         2         1         MIC         X           45         Ciprofloxacin         <=	42	Nalidixic acid	=	8	4	16	1	MIC		
45         Ciprofloxacin         <=	42	Streptomycin	=		-	-	-	MIC		
45         Erythromycin         <=	42	Tetracycline	=	2	0.25	2	1	MIC	Х	
45         Gentamicin         =         1         0.5         2         1         MIC         X           45         Nalidixic acid         =         8         4         16         1         MIC         X           45         Streptomycin         =         4         -         -         -         MIC         X           45         Tetracycline         =         2         0.25         2         1         MIC         X           56         Ciprofloxacin         <=	45	Ciprofloxacin	<=	0.12	0.06	0.25	1	MIC	Х	
45         Nalidixic acid         =         8         4         16         1         MIC         X           45         Streptomycin         =         4         -         -         -         MIC         X           45         Tetracycline         =         2         0.25         2         1         MIC         X           56         Ciprofloxacin         <=	45		<=	1		2	1		Х	
45         Nalidixic acid         =         8         4         16         1         MIC         X           45         Streptomycin         =         4         -         -         -         MIC         X           45         Tetracycline         =         2         0.25         2         1         MIC         X           56         Ciprofloxacin         <=	45	Gentamicin	=	1	0.5	2	1	MIC	Х	
45         Tetracycline         =         2         0.25         2         1         MIC         X           56         Ciprofloxacin         <=	45	Nalidixic acid	=	8		16	1		Х	
56         Ciprofloxacin         <=			=		-	-	-			
56         Erythromycin         <=	45		=	2	0.25	2	1	MIC		
56         Gentamicin         =         1         0.5         2         1         MIC         X           56         Nalidixic acid         =         8         4         16         1         MIC         X           56         Streptomycin         =         4         -         -         -         MIC         X           56         Tetracycline         =         1         0.25         2         1         MIC         X           58         Ciprofloxacin         <=		Ciprofloxacin	<=	0.12			1		X	
56         Nalidixic acid         =         8         4         16         1         MIC         X           56         Streptomycin         =         4         -         -         -         MIC         X           56         Tetracycline         =         1         0.25         2         1         MIC         X           58         Ciprofloxacin         <=	56	Erythromycin	<=	1	0.5		1	MIC	Х	
56         Streptomycin         =         4         -         -         -         MIC         X           56         Tetracycline         =         1         0.25         2         1         MIC         X           58         Ciprofloxacin         <=	56		=	1	0.5	2	1	MIC	Х	
56         Streptomycin         =         4         -         -         -         MIC         X           56         Tetracycline         =         1         0.25         2         1         MIC         X           58         Ciprofloxacin         <=	56	Nalidixic acid	=	8	4	16	1	MIC		
56         Tetracycline         =         1         0.25         2         1         MIC         X           58         Ciprofloxacin         <=		Streptomycin	=				-			
58         Ciprofloxacin         <=	56		=	1	0.25	2	1	MIC		
58         Erythromycin         <=		Ciprofloxacin	<=	0.12		0.25	1	MIC	Х	
58         Gentamicin         =         0.5         0.5         2         1         MIC         X           58         Nalidixic acid         =         8         4         16         1         MIC         X           58         Streptomycin         =         2         -         -         -         MIC         X           58         Tetracycline         <=	58	Erythromycin	<=	1	0.5	2	1	MIC	Х	
58         Nalidixic acid         =         8         4         16         1         MIC         X           58         Streptomycin         =         2         -         -         -         MIC         X           58         Tetracycline         <=			=	0.5		2	1		Х	
58         Streptomycin         =         2         -         -         -         MIC         X           58         Tetracycline         <=			=			16	1			
58         Tetracycline         <=			=		-		-		Х	
60         Ciprofloxacin         =         0.25         0.06         0.25         1         MIC         X           60         Erythromycin         <=	58		<=	0.5	0.25	2	1	MIC		
60         Erythromycin         <=	60		=			0.25	1			
60         Gentamicin         =         1         0.5         2         1         MIC         X           60         Nalidixic acid         =         8         4         16         1         MIC         X           60         Streptomycin         =         4         -         -         -         MIC         X	60		<=	1			1	MIC		
60         Nalidixic acid         =         8         4         16         1         MIC         X           60         Streptomycin         =         4         -         -         -         MIC         X										
60 Streptomycin = 4 MIC X			=				1			
	60		=				-	MIC	Х	
60 Tetracycline = 1 0.25 2 1 MIC X					0.25	2	1		Х	

#### Salmonella - expected and obtained interpretation

Antimicrobial	Strain	Panel	Expected	% R	% S	No. correct	No. incorrect
Ampicillin AMP	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	R	100	0	31	0
	EURL S-10.3	Panel 1	R	100	0	31	0
	EURL S-10.4	Panel 1	R	97	3	31	1
	EURL S-10.5	Panel 1	R	100	0	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	97	3	31	1
	EURL S-10.8	Panel 1	R	100	0	31	0
Cefotaxime FOT	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	S	0	100	31	0
	EURL S-10.3	Panel 1	R	100	0	31	0
	EURL S-10.3	Panel 2	R	100	0	31	0
	EURL S-10.4	Panel 1	R	100	0	31	0
	EURL S-10.4	Panel 2	R	100	0	31	0
	EURL S-10.5	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.7	Panel 2	R	100	0	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.8	Panel 2	R	100	0	31	0
Cefoxitin FOX	EURL S-10.3	Panel 2	S	0	100	31	0
Celoxilin FOX	EURL S-10.3		S	0	100	31	0
		Panel 2	S	3	97		
	EURL S-10.7	Panel 2		_		31	1
0.6.11. 7.7	EURL S-10.8	Panel 2	S	0	100	31	0
Ceftazidime TAZ	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	S	0	100	31	0
	EURL S-10.3	Panel 1	S	3	97	31	1
	EURL S-10.3	Panel 2	S	3	97	31	1
	EURL S-10.4	Panel 1	S	0	100	30	0
	EURL S-10.4	Panel 2	S	0	100	31	0
	EURL S-10.5	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.7	Panel 2	R	100	0	31	0
	EURL S-10.8	Panel 1	R	100	0	31	0
	EURL S-10.8	Panel 2	R	100	0	31	0
Chloramphenicol CHL	EURL S-10.1	Panel 1	S	0	100	31	0
·	EURL S-10.2	Panel 1	R	100	0	31	0
	EURL S-10.3	Panel 1	S	0	100	31	0
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.5	Panel 1	R	97	3	31	1
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.8	Panel 1	S	0	100	31	0
Ciprofloxacin CIP	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	S	0	100	31	0
	EURL S-10.3	Panel 1	R	94	6	31	2
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	97	3	31	1
	EURL S-10.8	Panel 1	S	0	100	31	0

Antimicrobial	Strain	Panel	Expected	% R	% S	No. correct	No.
		Donal 1	R	90	10	31	incorrect
Colistin COL	EURL S-10.1 EURL S-10.2	Panel 1 Panel 1	S	0	100	30	3 0
	EURL S-10.2	Panel 1	S	3	97	31	1
	EURL S-10.3	Panel 1	S	0	100	31	0
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	S	3	97	31	1
	EURL S-10.7	Panel 1	S	0	100	31	0
Ertapenem ETP	EURL S-10.3	Panel 2	S	0	100	31	0
Litapeneni Lir	EURL S-10.4	Panel 2	R	100	0	31	0
	EURL S-10.7	Panel 2	S	0	100	31	0
	EURL S-10.8	Panel 2	S	0	100	31	0
Gentamicin GEN	EURL S-10.1	Panel 1	S	0	100	31	0
Gentamicin GLN	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.3	Panel 1	S	3	97	31	1
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.5	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	R	100	0	31	0
	EURL S-10.7	Panel 1	S	0	100	31	0
Imipenem IMI	EURL S-10.8	Panel 2	S	0	100	31	0
impenem ivii	EURL S-10.7	Panel 2	S	0	100	31	0
	EURL S-10.7	Panel 2	S	0	100	31	0
	EURL S-10.4*	Panel 2	R	70	30	21	9
Marananam MED	EURL S-10.4		S	0	100	31	0
Meropenem MER	EURL S-10.1	Panel 1 Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	S	0	100	31	0
		Panel 2	S	0	100	31	0
	EURL S-10.3	Panel 1	R	100	0	31	0
	EURL S-10.4		R	100	0	31	0
	EURL S-10.4	Panel 2	S	0	100	31	0
	EURL S-10.5	Panel 1	S	0	100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 2	S	0	100	31	0
	EURL S-10.8 EURL S-10.8	Panel 1 Panel 2	S	0	100	31	0
Notidivia acid NAI			S	0			
Nalidixic acid NAL	EURL S-10.1	Panel 1	S	0	100 100	31 31	0
	EURL S-10.2	Panel 1	R				0
	EURL S-10.3	Panel 1	S	100	0 97	31	0
	EURL S-10.4	Panel 1	S	3 0	_	31	1
	EURL S-10.5	Panel 1	S		100	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1			100	30	0
Culforn oth sussels ONAY	EURL S-10.8	Panel 1	S S	0	100	31	0
Sulfamethoxazole SMX	EURL S-10.1	Panel 1			100	30	0
	EURL S-10.2	Panel 1	R	100	0	31	0
	EURL S-10.3	Panel 1	S	3	97	31	1
	EURL S-10.4	Panel 1	S	0	100	29	0
	EURL S-10.5	Panel 1	R	100	0	31	0
	EURL S-10.6	Panel 1	S	7	93	30	2
	EURL S-10.7	Panel 1	R	100	0	31	0
T	EURL S-10.8	Panel 1	S	3	97	30	1
Tetracycline TET	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	R	97	3	31	1
	EURL S-10.3	Panel 1	R	100	0	31	0
	EURL S-10.4	Panel 1	S	0	100	30	0
	EURL S-10.5	Panel 1	R	100	0	31	0
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.8	Panel 1	S	0	100	31	0

Antimicrobial	Strain	Panel	Expected	% R	% S	No. correct	No. incorrect
Tigecycline TGC	EURL S-10.1	Panel 1	S	0	100	29	0
	EURL S-10.2	Panel 1	S	0	100	29	0
	EURL S-10.3	Panel 1	S	0	100	29	0
	EURL S-10.4	Panel 1	S	0	100	29	0
	EURL S-10.5	Panel 1	R	85	15	26	4
	EURL S-10.6	Panel 1	S	0	100	28	0
	EURL S-10.7	Panel 1	S	4	96	28	1
	EURL S-10.8	Panel 1	S	0	100	28	0
Trimethoprim TMP	EURL S-10.1	Panel 1	S	0	100	31	0
	EURL S-10.2	Panel 1	S	0	100	31	0
	EURL S-10.3	Panel 1	S	0	100	31	0
	EURL S-10.4	Panel 1	S	0	100	31	0
	EURL S-10.5	Panel 1	R	97	3	31	1
	EURL S-10.6	Panel 1	S	0	100	31	0
	EURL S-10.7	Panel 1	R	100	0	31	0
	EURL S-10.8	Panel 1	S	3	97	30	1

<sup>\*</sup>Strain/antimicrobial-combination excluded from the evaluation

Antimicrobial	Strain	Expected	% R	% S	No.	No.
Ciprofloxacin, CIP	EURL C-10.1	R	100	0	correct 31	incorrect 0
Cipiolioxaciii, Cii	EURL C-10.2	S	0	100	31	0
	EURL C-10.3	S	3	97	30	1
	EURL C-10.4	S	0	100	31	0
	EURL C-10.4	R	100	0	31	0
	EURL C-10.6	R	100	0	31	0
	EURL C-10.7	R	100	0	31	0
	EURL C-10.8	R	100	0	31	0
Erythromycin, ERY	EURL C-10.8	R	100	0	31	0
Liyamomyom, Erci	EURL C-10.2	S	0	100	31	0
	EURL C-10.3	S	3	97	30	1
	EURL C-10.4	R	100	0	31	0
	EURL C-10.5	S	0	100	31	0
	EURL C-10.6	R	100	0	31	0
	EURL C-10.7	S	0	100	31	0
	EURL C-10.8	R	100	0	31	0
Gentamicin, GEN	EURL C-10.1	S	6	94	29	2
Contamon, OLIV	EURL C-10.2	S	0	100	31	0
	EURL C-10.3	S	3	97	30	1
	EURL C-10.4	S	0	100	31	0
	EURL C-10.5	S	3	97	30	1
	EURL C-10.6	S	3	97	30	1
	EURL C-10.7	S	3	97	30	1
	EURL C-10.8	R	100	0	31	0
Nalidixic acid, NAL	EURL C-10.1	R	97	3	29	1
,	EURL C-10.2	S	3	97	30	1
	EURL C-10.3	S	0	100	31	0
	EURL C-10.4	S	0	100	31	0
	EURL C-10.5	R	97	3	30	1
	EURL C-10.6	R	97	3	30	1
	EURL C-10.7	R	97	3	30	1
	EURL C-10.8	R	100	0	31	0
Streptomycin, STR	EURL C-10.1	S	3	97	30	1
	EURL C-10.2	R	94	6	29	2
	EURL C-10.3	S	3	97	30	1
	EURL C-10.4	S	0	100	31	0
	EURL C-10.5	S	0	100	31	0
	EURL C-10.6	S	3	97	29	1
	EURL C-10.7	S	6	94	29	2
	EURL C-10.8	R	100	0	31	0
Tetracycline, TET	EURL C-10.1	S	3	97	30	1
	EURL C-10.2	S	0	100	31	0
	EURL C-10.3	S	3	97	30	1
	EURL C-10.4*	S	26	74	31	8
	EURL C-10.5	R	97	3	30	1
	EURL C-10.6	R	100	0	31	0
	EURL C-10.7	S	0	100	31	0
	EURL C-10.8	R	100	0	31	0

<sup>\*</sup>Strain/antimicrobial-combination excluded from the evaluation

#### Deviations - Salmonella

Lab no.	Strain	Panel	Antimicrobial	Obtained MIC value	Obtained interpretation	Expected MIC-value	Expected interpretation
2	EURL S-10.1	1	Colistin COL	8	S	8	R
4	EURL S-10.3	1	Ciprofloxacin CIP	= 0.5	S	= 0.5	R
4	EURL S-10.5	1	Tigecycline TGC	2	S	2	R
4	EURL S-10.5	1	Trimethoprim TMP	> 32	S	> 32	R
6	EURL S-10.4	1	Ampicillin AMP	> 64	S	> 64	R
6	EURL S-10.7	1	Ampicillin AMP	> 64	S	> 64	R
18	EURL S-10.6	1	Sulfamethoxazole SMX	> 1024	R	64	S
19	EURL S-10.8	1	Sulfamethoxazole SMX	1024	R	32	S
19	EURL S-10.8	1	Trimethoprim TMP	> 32	R	= 0.5	S
20	EURL S-10.3	1	Gentamicin GEN	<= 0.5	R	1	S
21	EURL S-10.4	1	Nalidixic acid NAL	128	R	<= 4	S
22	EURL S-10.3	1	Colistin COL	8	R	<= 1	S
26	EURL S-10.5	1	Tigecycline TGC	1	S	2	R
26	EURL S-10.7	1	Colistin COL	8	R	<= 1	S
26	EURL S-10.7	1	Tigecycline TGC	<= 0.25	R	1	S
29	EURL S-10.3	1	Ceftazidime TAZ	1	R	1	S
29	EURL S-10.3	2	Ceftazidime TAZ	1	R	1	S
36	EURL S-10.5	1	Tigecycline TGC	<= 0.25	S	2	R
39	EURL S-10.3	1	Ciprofloxacin CIP	= 0.5	S	= 0.5	R
39	EURL S-10.4		ESBL test conclusion Unusual phenotype		phenotype	Presumptive carbapenemase	
39	EURL S-10.5	1	Chloramphenicol CHL	64	S	64	R
40	EURL S-10.1	1	Colistin COL	2	S	8	R
40	EURL S-10.2	1	Tetracycline TET	<= 2	S	64	R
40	EURL S-10.3	1	Sulfamethoxazole SMX	> 1024	R	32	S
40	EURL S-10.5	1	Tigecycline TGC	1	S	2	R
40	EURL S-10.7	1	Ciprofloxacin CIP	= 0.06	S	= 0.25	R
45	EURL S-10.6	1	Sulfamethoxazole SMX	> 1024	R	64	S
56	EURL S-10.1	1	Colistin COL	2	S	8	R
56	EURL S-10.7	2	Cefoxitin FOX	16	R	8	S
56	EURL S-10.7		ESBL test conclusion	Presumptive ESBL + pAmpC		Presumptive ESBL	
60	EURL S-10.3		ESBL test conclusion	Unusual	phenotype	Presur	nptive ESBL

#### Deviations - Campylobacter

Lab no.	Strain	Antimicrobial	Obtained MIC value	Obtained interpretation	Expected MIC-value	Expected interpretation
2	EURL C-10.6	Streptomycin STR	16	R	2	S
18	EURL C-10.7	Streptomycin STR	8	R	4	S
19	EURL C-10.7	Streptomycin STR	8	R	4	S
36	EURL C-10.1	Gentamicin GEN	16	R	= 0.25	S
36	EURL C-10.1	Nalidixic acid NAL	4	S	> 64	R
36	EURL C-10.2	Nalidixic acid NAL	> 64	R	4	S
36	EURL C-10.2	Streptomycin STR	2	S	16	R
36	EURL C-10.5	Gentamicin GEN	> 16	R	= 0.25	S
36	EURL C-10.5	Nalidixic acid NAL	8	S	> 64	R
36	EURL C-10.6	Gentamicin GEN	> 16	R	= 0.5	S
36	EURL C-10.6	Nalidixic acid NAL	8	S	> 64	R
36	EURL C-10.7	Gentamicin GEN	16	R	= 0.5	S
36	EURL C-10.7	Nalidixic acid NAL	8	S	> 64	R
39	EURL C-10.2	Streptomycin STR	<= 0.5	S	16	R
39	EURL C-10.3	Ciprofloxacin CIP	> 8	R	<= 0.12	S
39	EURL C-10.3	Erythromycin ERY	> 64	R	<= 1	S
39	EURL C-10.3	Gentamicin GEN	> 64	R	= 0.25	S
39	EURL C-10.3	Streptomycin STR	8	R	1	S
39	EURL C-10.3	Tetracycline TET	> 16	R	<= 0.5	S
40	EURL C-10.5	Tetracycline TET	1	S	> 64	R
42	EURL C-10.1	Gentamicin GEN	= 0.5	R	= 0.25	S
42	EURL C-10.1	Streptomycin STR	1	R	1	S
42	EURL C-10.1	Tetracycline TET	<= 0.5	R	<= 0.5	S

#### Genotypic characterization (optional); obtained results

		Genetype	number	detected	Method	Reference	Primer 5 3	Primer 3 5
4	EURL-S10.3	CTX	M-9		PCR (published)	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.3	OXA	-	Х	PCR (published)	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.3	SHV	-	Х	PCR (published)	Arlet1997.FEMS ML. 152:163-7	-	-
4	EURL-S10.3	TEM	-1		PCR (published)	Olesen 2004. MDR. 10:334-340	-	-
4	EURL-S10.4	CTX	-	Х	PCR (published)	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.4	IMP	-	Х	PCR (published)	Poirel. 2011 Diag Micro Infect Dis.70(1):119-23.	-	-
4	EURL-S10.4	KPC	-	Х	PCR (published)	Poirel. 2011 Diag Micro Infect Dis.70(1):119-23.	-	-
4	EURL-S10.4	OXA	-48		PCR (published)	Poirel. 2011 Diag Micro Infect Dis.70(1):119-23.	-	-
4	EURL-S10.4	SHV	-	X	PCR (published)	Arlet1997.FEMS ML. 152:163-7	-	-
4	EURL-S10.4	TEM	-1		PCR (published)	Olesen 2004. MDR. 10:334-340	-	-
4	EURL-S10.4	VIM	-	Х	PCR (published)	Poirel. 2011 Diag Micro Infect Dis.70(1):119-23.	-	-
4	EURL-S10.7	CTX	M-15		PCR (published)	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.7	OXA	-	Х	PCR (published)	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.7	SHV	-12		PCR (published)	Arlet1997.FEMS ML. 152:163-7	-	-
4	EURL-S10.7	TEM	-1		PCR (published)	Olesen 2004. MDR. 10:334-340	-	-
4	EURL-S10.8	CTX	-	Х	-	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.8	OXA		Х	-	Hasman 2005. JAC 56:115-121	-	-
4	EURL-S10.8	SHV	-	Х	-	Arlet1997.FEMS ML. 152:163-7	-	-
4	EURL-S10.8	TEM	-52		-	Olesen 2004. MDR. 10:334-340	-	-
9	EURL-S10.3	CTX	M-9		PCR (published)	JAC 2010;65;490-495	-	-
9	EURL-S10.3	TEM	-1		PCR (published)	JAC 2010;65;490-495	-	-
9	EURL-S10.4	OXA	-48		PCR (published)	JAC 2010;65;490-495	-	=
9	EURL-S10.7	CTX	M-15		PCR (published)	JAC 2010;65;490-495	-	-
9	EURL-S10.7	SHV	-12		PCR (published)	JAC 2010;65;490-495	-	-
9	EURL-S10.7	TEM	-1		PCR (published)	JAC 2010;65;490-495	-	-
9	EURL-S10.8	TEM	-52		PCR (published)	JAC 2010;65;490-495	-	-
17	EURL-S10.3	CTX	M-9		PCR (published)	Paauw et al. (2006)	TGGTGACAAAGAGAGTGCAACG	ATCGCCGAAGGGCTGTGA
17	EURL-S10.3	TEM	-1		PCR (published)	Guerra et al. (2001)	TTGGGTGCACGAGTGGGT	GCTTCCCGGCAACAATTA
17	EURL-S10.4	OXA	-48		PCR (published)	Guerra et al. (2000)	AGCAGCGCCAGTGCATCA	GGAAACTTGGGGTCGAAT
17	EURL-S10.4	TEM	-1		PCR (published)	Guerra et al. (2001)	TTGGGTGCACGAGTGGGT	GCTTCCCGGCAACAATTA
17	EURL-S10.7	CTX	M-15		PCR (published)	Carrattoli et al 2008	CCCATGGTTAAAAAATCACTGC	CTTAGACGGCAAAAGCGCTG
17	EURL-S10.7	SHV	-12		PCR (published)	Weill et al., (2004)	TTATCTCCCTGTTAGCCACC	CCGAGCGAAATCAGCAAATC
17	EURL-S10.7	TEM	-1		PCR (published)	Guerra et al. (2001)	TTGGGTGCACGAGTGGGT	GCTTCCCGGCAACAATTA
17	EURL-S10.8	TEM	-52		PCR (published)	Guerra et al. (2001)	TTGGGTGCACGAGTGGGT	GCTTCCCGGCAACAATTA
21	EURL-S10.3	CTX	M-4		PCR (published)	doi: 10.1093/jac/dki412	attggaaagcgttcatcacc	caaagagagtgcaacggatg
21	EURL-S10.4	OXA	-48		PCR (published)	doi: 10.3201/eid1710.110655	CATCAAGTTCAACCCAACCG	GCGTGGTTAAGGATGAACAC
21	EURL-S10.7	CTX	M-1		PCR (published)	doi:10.1128/JCM.42.12.5715-5721.2004	agccgccgacgctaataca	gacgatgtcactggctgagc
21	EURL-S10.7	SHV	-		PCR (published)	doi:10.1016/j.diagmicrobio.2006.04.016	tctttccgatgccgccgccagtca	gccgggttattcttatttgt
21	EURL-S10.8	TEM	-52		PCR (in-house)	-	ttaccaatgcttaatca	atgagtattcaacatttccg
25	EURL-S10.3	CTX	M-9		PCR (in-house)	-	-	-
25	EURL-S10.3	TEM	-		PCR (in-house)	=	-	=
25	EURL-S10.4	OXA	-48		PCR (in-house)	-	-	-
25	EURL-S10.4	TEM	-		PCR (in-house)	-	-	-
25	EURL-S10.7	CTX	M-15		PCR (in-house)	-	-	-
25	EURL-S10.7	TEM	-		PCR (in-house)	-	-	-
25	EURL-S10.8	TEM	-52		PCR (in-house)	-	-	-
32	EURL-S10.3	ACC	-	Х	PCR (published)	(Hasman et al. 2005)	-	-
32	EURL-S10.3	ACT	-	Х	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.3	CMY	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.3	CTX	M-9		PCR (published)	PediatrInfectDisJ28:814-818	-	-

Labno	Strain	Genetype	Gene number	Not detected	Method	Reference	Primer 5 3	Primer 3 5
32	EURL-S10.3	DHA	-	X	PCR (published)	(Gonzalez-Sanz et al.2009)	-	-
32	EURL-S10.3	FOX	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.3	IMP		Х	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.3	KPC	•	Χ	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.3	MOX	•	Χ	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.3	NDM	-	X	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.3	OXA	-48	Х	PCR (published)	Voets et al 2011	-	-
32	EURL-S10.3	OXA	-10	X	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.3	OXA	-	X	PCR (published)	J. Antimic.Chemothe(2009) 64	-	-
32	EURL-S10.3	SHV	-	X	PCR (published)	FEMSMicrobiolLett1997152:1637	-	-
32	EURL-S10.3	TEM*	-	X	PCR (published)	AntimicrAgentsChemotherap2009	-	-
32	EURL-S10.3	VEB	-	X	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.3	VIM	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.4	ACC	-	X	PCR (published)	Hasman et al. 2005)	-	-
32	EURL-S10.4	ACT	ı	Х	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.4	CMY	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.4	CTX	ı	Х	PCR (published)	PediatrInfectDisJ28:814-818	-	-
32	EURL-S10.4	DHA	•	Χ	PCR (published)	(Gonzalez-Sanz et al.2009)	-	-
32	EURL-S10.4	FOX		Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.4	IMP	•	Χ	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.4	KPC	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.4	MOX	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.4	NDM	-	Х	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.4	OXA	-48		PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.4	OXA	-10	Х	PCR (published)	J. Antimic.Chemothe(2009) 64	-	-
32	EURL-S10.4	OXA	-10	Х	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.4	SHV	-	Х	PCR (published)	FEMSMicrobiolLett1997152:1637	-	-
32	EURL-S10.4	TEM	-	Х	PCR (published)	AntimicrAgentsChemotherap2009	-	-
32	EURL-S10.4	VEB	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.4	VIM	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.7	ACC	-	Х	PCR (published)	(Hasman et al. 2005)	-	-
32	EURL-S10.7	ACT	-	Х	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.7	CMY	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.7	CTX	M-15		PCR (published)	PediatrInfectDisJ28:814-818	-	-
32	EURL-S10.7	DHA	-	Х	PCR (published)	(Gonzalez-Sanz et al.2009)	-	-
32	EURL-S10.7	FOX	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.7	IMP	-	Х	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.7	KPC	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.7	MOX	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.7	NDM	-	Х	PCR (published)	L. Poirel et al 2011	-	-
32	EURL-S10.7	OXA	-48	Х	PCR (published)	Voets et al 2011	-	-
32	EURL-S10.7	OXA	-10	Х	PCR (published)	Voets et al 2011	-	-
32	EURL-S10.7	OXA	-	Х	PCR (published)	J. Antimic.Chemothe(2009) 64	-	-
32	EURL-S10.7	SHV	-12		PCR (published)	FEMSMicrobiolLett1997152:1637	-	-
32	EURL-S10.7	TEM	-1		PCR (published)	AntimicrAgentsChemotherap2009	-	-
32	EURL-S10.7	VEB	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.7	VIM	-	Х	PCR (published)	Dallanne et al 2010	-	-
32	EURL-S10.8	ACC	-	Х	PCR (published)	(Hasman et al. 2005)	-	-
32	EURL-S10.8	ACT	-	Х	PCR (published)	Voets et al. 2011	-	-
32	EURL-S10.8	CMY	-	Х	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.8	CTX	-	Х	PCR (published)	PediatrInfectDisJ28:814-818	-	-
32	EURL-S10.8	DHA	-	Х	PCR (published)	(Gonzalez-Sanz et al.2009)	-	-

Labno	Strain	Genetype	Gene	Not	Method	Reference	Primer 5 3	Primer 3 5
32	EURL-S10.8	FOX	number	detected X	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.8	IMP	-	X	PCR (published)	L. Poirel et al 2011		
32	EURL-S10.8	KPC	-	X	PCR (published)	Dallanne et al 2010	-	
32	EURL-S10.8	MOX	-	X	PCR (published)	A.AGE.CHEM2006.ClinMicr1999-37	-	-
32	EURL-S10.8	NDM	-	X	PCR (published)	L. Poirel et al 2011	<u> </u>	-
32	EURL-S10.8	OXA	-48	X	PCR (published)	Voets et al 2011	_	
32	EURL-S10.8	OXA	-10	X	PCR (published)	Voets et al 2011	-	
32	EURL-S10.8	OXA	-	X	PCR (published)	J. Antimic.Chemothe(2009) 64	<u> </u>	-
32	EURL-S10.8	SHV	-	X	PCR (published)	FEMSMicrobiolLett1997152:1637	<u> </u>	-
32	EURL-S10.8	TEM	-52		PCR (published)	AntimicrAgentsChemotherap2009	_	_
32	EURL-S10.8	VEB	-	Х	PCR (published)	Dallanne et al 2010	<u> </u>	-
32	EURL-S10.8	VIM	-	X	PCR (published)	Dallanne et al 2010	1_	-
33	EURL-S10.3	CTX			PCR (published)	Woodford.et al. (2006)	CAAAGAGARTGCAACGGATG	ATTGGAAAGCGTTCATCACC
33	EURL-S10.3	TEM	-		PCR (published)	Fang, et al. (2008)	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT
33	EURL-S10.4	OXA	-48		PCR (published)	Poirel et al.(2011)	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG
33	EURL-S10.4	TEM	-		PCR (published)	Fang,et al. (2008).	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT
33	EURL-S10.7	CTX	-		PCR (published)	Woodford,et al. (2006)	AAAAATCACTGCGYCAGTTC	AGCTTATTCATCGCCACGTT
33	EURL-S10.7	SHV	_		PCR (published)	Fang,et al. (2008).	CTTTATCGGCCCTCACTCAA	AGGTGCTCATCATGGGAAAG
33	EURL-S10.7	TEM			PCR (published)	Fang, et al. (2008)	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT
33	EURL-S10.8	TEM	-		PCR (published)	Fang,et al. (2008)	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT
36	EURL-S10.3	CTX	M-9		PCR (published)	Hasman et al. JAC. 2005 Jul;56(1):115-21	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG
36	EURL-S10.3	SHV	-	Х	PCR (published)	Briñas et al. AAC. 2002 Oct;46(10):3156-63	CACTCAAGGATGTATTGTG	TTAGCGTTGCCAGTGCTCG
36	EURL-S10.3	TEM	-1		PCR (published)	Briñas et al. AAC. 2002 Oct;46(10):3156-63	TTCTTGAAGACGAAAGGGC	ACGCTCAGTGGAACGAAAAC
36	EURL-S10.4	CTX	-	Х	PCR (published)	Hasman et al. JAC 2005 Jul;56(1):115-21	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG
36	EURL-S10.4	IMP		X	PCR (published)	Ellington JAC (2007)59(2):321-322	GGAATAGAGTGGCTTAAYTCTC	CCAAACYACTASGTTATCT
36	EURL-S10.4	NDM	_	X	PCR (published)	Mushtaq et. al. JAC (2011)66(9):2002-2005	GGGCAGTCGCTTCCAACGGT	GTAGTGCTCAGTGTCGGCAT
36	EURL-S10.4	OXA	-48		PCR (published)	Poirel et al. DiaMicInfDis. 2011 May;70(1):119-23	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG
36	EURL-S10.4	SHV	- 40	Х	PCR (published)	Briñas et al. AAC 2002 Oct;46(10):3156-63	CACTCAAGGATGTATTGTG	TTAGCGTTGCCAGTGCTCG
36	EURL-S10.4	TEM	-1		PCR (published)	Briñas et al. AAC 2002 Oct;46(10):3156-63	TTCTTGAAGACGAAAGGGC	ACGCTCAGTGGAACGAAAAC
36	EURL-S10.4	VIM	-	Х	PCR (published)	Ellington JAC (2007)59(2):321-322	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG
36	EURL-S10.7	CTX	M-15		PCR (published)	Hasman et al. JAC. 2005 Jul;56(1):115-21.	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG
36	EURL-S10.7	SHV	-12		PCR (published)	Briñas et al. AAC. 2003 0ct;46(10):3156-63.	CACTCAAGGATGTATTGTG	TTAGCGTTGCCAGTGCTCG
36	EURL-S10.7	TEM	-1		PCR (published)	Briñas et al. AAC. 2002 Oct;40(10):3156-63.	TTCTTGAAGACGAAAGGGC	ACGCTCAGTGGAACGAAAAC
36	EURL-S10.8	CTX	-	Х	PCR (published)	Hasman et al. JAC. 2005 Jul;56(1):115-21.	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG
36	EURL-S10.8	SHV	-	X	PCR (published)	Briñas et al. AAC. 2003 Oct;46(10):3156-63.	CACTCAAGGATGTATTGTG	TTAGCGTTGCCAGTGCTCG
36	EURL-S10.8	TEM	-52		PCR (published)	Briñas et al. AAC. 2002 Oct;40(10):3156-63.	TTCTTGAAGACGAAAGGGC	ACGCTCAGTGGAACGAAAAC
59	EURL-S10.3	CTX	M-9		Whole genome sequenced	- 2002 Oct, 40(10).3130*03.	-	-
59	EURL-S10.3	TEM	-1		Whole genome sequenced	_	-	-
59	EURL-S10.4	OXA	-48		Whole genome sequenced	<u> </u>		-
59	EURL-S10.4	TEM	-1		Whole genome sequenced	<u> </u>	-	1-
59	EURL-S10.4	CTX	M-15		Whole genome sequenced	_	-	-
59	EURL-S10.7	SHV	-12		Whole genome sequenced	<u> </u>	-	1-
59	EURL-S10.7	TEM	-1		Whole genome sequenced	_	-	-
59	EURL-S10.7	TEM	-52		Whole genome sequenced	1_		1_
Ja	LUINE-010.0	I LIVI	-32		writione genome sequenced	ĮF	Į <sup>Ę</sup>	<u> </u> =

Legend:

Fields shaded grey indicate that the gene was expected

Genes in bold and white font, were detected but not expected
\*TEM-1 does not confer ESBL-production and is as such not included as an expected result. TEM-1 was, however, present in S-10.3, S-10.4 and S-10.7

#### Genotypic characterization (optional); comments by participants

Labno	Strain	Comment
4	S-9.5	TEM-1 detected
4	S-9.5	TEM-1 detected
17	S-9.3	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. CTX-M9 primer is group specific, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.3	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. CTX-M9 primer is group specific, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.3	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. CTX-M9 primer is group specific, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.4	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer.  Validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.4	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. Validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.4	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. Validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.7	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. All used primers are specific for a respective group of genes, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.7	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. All used primers are specific for a respective group of genes, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.7	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. All used primers are specific for a respective group of genes, validation was done via sequencing TEM-1 was additionally found, it's just added for additional information, not as ESBL-gene
17	S-9.8	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. TEM primer is group specific, validation was done via sequencing
17	S-9.8	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. TEM primer is group specific, validation was done via sequencing
17	S-9.8	All primers were 5'->3'. The Primer in 3'->5' box is the reverse complement to the original primer. TEM primer is group specific, validation was done via sequencing
21	S-9.7	qnrB positive.
21	S-9.7	qnrB positive.
32	S-9.3	SPM Gene tested for/not detected (L. Poirel et al 2011) SPM Gene tested for/not detected (L. Poirel et al 2011)
32 32	S-9.3 S-9.3	SPM Gene tested for/not detected (L. Poirei et al 2011)  SPM Gene tested for/not detected (L. Poirei et al 2011)
32	S-9.3 S-9.4	SPM Gene tested for/not detected (L. Poirel et al 2011)  SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.4	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.4	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.7	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.7	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.7	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.8	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.8	SPM Gene tested for/not detected (L. Poirel et al 2011)
32	S-9.8	SPM Gene tested for/not detected (L. Poirel et al 2011)
59	S-9.3	genenumber for genetype TEM: TEM-1B
59	S-9.4	genenumber for genetype TEM: TEM-1D
59	S-9.7	genenumber for genetype TEM: TEM-1B
59	S-9.8	genenumber for genetype TEM: TEM-52B

National Food Institute Technical University of Denmark Mørkhøj Bygade 19 2860 Søborg

Tel. 35 88 70 00 Fax 35 88 70 01

www.food.dtu.dk

ISBN: 978-87-93109-86-5