The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017

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The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017
List of Abbreviations

AGISAR, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR, Antimicrobial Resistance
AST, Antimicrobial Susceptibility Testing
ATCC, American Type Culture Collection
CAZ, Ceftazidime
CDC, Centers for Disease Control and Prevention
COL, Colistin
CRO, Ceftriaxone
CTX, Cefotaxime
DTU Food, Technical University of Denmark, National Food Institute
EQAS, External Quality Assurance System
ESBL, Extended Spectrum Beta-Lactamase
GMI, Global Microbial Identifier
IP, Institute Pasteur
MERO, Meropenem
MIC, Minimum Inhibitory Concentration
SMX, Sulfamethoxazole
SXT, Sulfamethoxazole-trimethoprim (co-trimoxazole)
WHO, World Health Organization
WHO GFN, WHO Global Foodborne Infections Network
1. Introduction

Since 2000, 16 WHO External Quality Assurance System (EQAS) reports have been issued with this report being the 17th. The WHO Global Foodborne Infections Network (WHO GFN) and the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) focus on enhancing World Health Organization (WHO) Member States’ capacity to detect and respond to foodborne disease outbreaks and the emerging of antimicrobial resistance (AMR) in bacterial pathogens by conducting laboratory-based surveillance of *Salmonella* and other important foodborne pathogens. Thus, the WHO EQAS 2017 aligns with the 2015 WHO global action plan to target AMR worldwide, objective 2: Strengthen knowledge through surveillance and research, action 2, laboratory capacity.

Since its inception, the scope of the WHO EQAS has expanded to include additional foodborne pathogens than *Salmonella* such as *Shigella* and *Campylobacter*. *Salmonella*, *Campylobacter* and *Shigella* are among the most important foodborne pathogens worldwide and accounts for millions of cases of diarrheal disease and thousands of deaths per year impacting both developing and industrialized countries. Furthermore, the increased number of *Salmonella*, *Campylobacter* and *Shigella* isolates which are resistant to antimicrobials is of major concern since these bacterial isolates are associated with infections characterized by increased morbidity and mortality.

In the 2017 iteration of the WHO EQAS, a major change was applied as it focuses only on *Salmonella* serotyping and antimicrobial susceptibility testing (AST). This adjustment was made to balance the costs and focus efforts at continuing the development of the genomic proficiency test adopted by WHO and offered through the Global Microbial Identifier (GMI) ([http://www.globalmicrobialidentifier.org/workgroups/about-the-gmi-proficiency-tests](http://www.globalmicrobialidentifier.org/workgroups/about-the-gmi-proficiency-tests)).

The WHO EQAS is organized annually by DTU Food in collaboration with World Health Organization (WHO) in Geneva, Switzerland, Centers for Disease Control and Prevention (CDC) in Atlanta, USA, and Institute Pasteur (IP) in Paris, France.

Individual laboratory data are confidential and known only by the participating laboratory, the EQAS Organizer (DTU Food) and possibly the respective WHO GFN regional centre/WHO AGISAR country representative. All summary conclusions are public. The goal set by WHO GFN/AGISAR aims at having all national reference laboratories perform *Salmonella* serotyping with a maximum of one deviation out of eight strains tested (error rate of 13%) and performing AST of *Salmonella* with a maximum error rate of 10% (either less than 5% very major / major errors and less than 5% minor errors, or less than 10% minor errors). Minor deviations are defined as classification of an intermediate strain as susceptible, resistant or vice versa (*i.e.* I ↔ S or I ↔R). Major deviation is the classification of a susceptible strain as resistant (*i.e.* S → R). Very major deviation is the classification of a resistant strain as susceptible (*i.e.* R → S). In this report, the deviations of AST results are divided into two categories, *i.e.* critical deviations which include major and very major deviations, and total deviations which include also the minor deviations. In EQAS 2014, the regions were re-defined for all countries worldwide in relation to the analysis of data from the WHO GFN EQAS. This resulted in some reorganization of countries into new regions compared to previous years, why interpretation of regional-based results from 2014 and onwards
with results from before 2014 should be conducted with care. The countries belonging to each region is listed in Appendix 1.

Appendices 2-5 present additional background information in relation to the WHO EQAS 2017.

2. Summary

The summary report is divided into sections; the serotyping component, AMR as well as reporting resistance to Extended Spectrum Beta-Lactamases (ESBL) producing Salmonella. All results reported in the summary can be found in Appendix 1.

Participation

A total of 191 laboratories responded to the pre-notification and were enrolled in the WHO EQAS. When the deadline for submitting results was reached, 181 laboratories in 81 countries had uploaded data.

The following countries provided data for at least one of the EQAS components (Appendix 1): Argentina, Australia (3), Bahrain, Bangladesh, Barbados, Belgium, Belize, Bolivia, Brazil (2), Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada (11), Chile, China (15), Colombia (4), Costa Rica (2), Croatia, Cuba, Curaçao, Cyprus, Czech Republic (2), Denmark, Ecuador, Egypt, Gambia, Germany (2), Ghana, Greece (2), Guatemala (2), Honduras, India (4), Iran, Islamic rep. of (3), Ireland, Israel, Italy (14), Jamaica, Japan (2), Kenya (2), Korea, Rep of (2), Kosovo, Lao PDR, Luxembourg (2), Madagascar, Malaysia (5), Malta, Mauritius, Mexico (3), Morocco, Nepal (6), New Zealand, Nigeria (4), Panama (2), Paraguay, Peru, Philippines (2), Poland (3), Portugal, Serbia (2), Singapore (2), Slovakia, Slovenia, South Africa, Spain, Sri Lanka (2), Suriname, Sweden, Taiwan, Tanzania, United Republic of, Thailand (16), Trinidad and Tobago, Turkey, Ukraine, United Kingdom, United States of America (5), Uruguay, Venezuela (2), Viet Nam (2), Zambia, and Zimbabwe.

The level of participation in the WHO EQAS 2017 was the same as at the WHO EQAS 2016.

Salmonella EQAS components

The acceptance threshold for the EQAS Salmonella serotyping component was met by 77% (n = 111) of the 145 participating laboratories (Table 1). In addition, 88% (n = 127) of the laboratories tested all eight strains with a total of 90% (n = 1,014) of all tests being correct, representing results almost at the same level as in 2016 which was one of the best performances observed since the initiation of the EQAS (Table 2). The ability to correctly serotype the internal control strain increased in 2017 to the same level as in 2014, 98%, which is the best performance, recorded and only observed previously in 2011 and 2014. The increase in performance observed compared to
2016 was most likely due to a lower number of participating laboratories serotyping this specific strain. In 2017, the participation in testing the internal control strain decreased from 159 to 142, a level previously recorded over the years (Table 3). On a region-based categorization of participating laboratories, Africa and the Central Asia & Middle East both correctly serotyped between 63% and 66% of the test strains whereas the Caribbean, Southeast Asia, and Latin America, correctly serotyped between 81% and 89% of the test strains. The performance of correct serotyping in Europe, China, North America was between 94 and 99% but reached 100% correct serotyping of all eight strains in only Oceania. In 2017, Russia was again the only region not participating (Table 4). In all regions except for the Central Asia & Middle East region either a marked or consistent improvement was observed and in line with the other data presented.

In 2017, the main problem regarding the Salmonella serotyping appeared relatively to be associated with strain, WHO 2017 S-17.8 (Kentucky) whereas the deviations for the rest of the strains seems to be acceptable at a level of approximately 10% (n=5) and for the remaining two strains at 6% and 2%.

As indicated, WHO 2017 S-17.8 (Kentucky, I 8,20:i:z6), revealed a considerable level of deviation at 17.0% (Table 5). Of the 23 deviations, 14 were attributed to Tumodi (I 1,4,12:i:z6) which only differs from the somatic O antigen compared to Kentucky. It is surprising that the problem of the serotyping procedure seems very often to be associated with the somatic O antigen of relatively common antigens. The level of deviation is surprising since the serovars included the 2017 should not pose major difficulties. The somatic O antigens of all the test strains belong to the major serogroups such as O:4, O:3,10, O:7, O:8, and O:9, and the flagella antigens belong to well-known polyvalent antisera complex G and HMD.

Concerning the Salmonella AST component for the EQAS 2017, the performance slightly decreased compared to the EQAS of 2016, with deviations of 3% minor, 2% major, and 3% very major deviations. Thus, the percentages of critical deviation was 5% (Table 6). Deviations categorized by the tested antimicrobials revealed that ceftazidime (CAZ), ciprofloxacin (CIP), colistin (COL), ceftriaxone (CRO), cefotaxime (CTX), meropenem (MERO), sulfamethoxazole (SMX) and co-trimoxazole (SXT) caused most of the difficulties observed with the following level of total deviations: 22%, 18%, 6%, 7%, 8%, 6%, 7%, and 7%, respectively (Table 7). The deviations to CIP was mostly attributed to minor deviations and most likely due to the often observed hazy double zone when performing disk diffusion where the outer zone often incorrectly is measured. In this year’s iteration, participating laboratories appears to have been too strict measuring the zone diameter categorizing the susceptible strains intermediate. Similarly, the deviations observed to SMX and SXT are due to the bacteriostatic effect complicating reading when conducting both disk diffusion and minimum inhibitory concentration (MIC) determination where 20% of the lawn of growth for disk diffusion equal to 80% reduction of growth for MIC determination determines the read-value. This year, a resistant isolate caused most problems. For the disk diffusion results, it was not surprising to see deviations in relation to COL as disk diffusion is not recommended as a method for AST to colistin. This resulted in 10 participants incorrectly reporting one isolate susceptible despite it being resistant. For the four antimicrobials used to confirm ESBL and carbapenemase production, CAZ, CRO, CTX and MERO, all were responsible
for critical deviations with 17% of all tests incorrect for CAZ, which is a great concern (Table 7, Table 8). Assessing the data for the four antimicrobials, no clear patterns was observed, resistance reported as susceptible and visa versa (Table 8).

On a region-based categorization of participating laboratories, all regions performed poorly compared to 2016. A greater number of deviations was observed in developing regions, which partly could explain the results as well as the difficulties reporting the results for the third generation cephalosporins. The Caribbean region obtained the highest percentages of total deviations, 24.3% whereas a number of regions obtained a total deviations around 10%, i.e. Africa (12.8%), China (6.6%), Southeast Asia (8.1%), Latin America (8.9%), Europe (7.2%), and Central Asia & Middle East (11.1%). None of the regions obtained a performance of 100% correct AST results, however, North America and Oceania performed better than the other regions with 97.1% and 96.1% correct AST-results. Russia did not participate in the 2017 EQAS (Table 9).

For the 150 laboratories performing the Salmonella AST component (MIC (n = 41)/Disk diffusion (n = 74)), only 77% (115 laboratories) reported data for AST of the control strain E. coli ATCC 25922. As in previous years, this is a very alerting number as it is expected that all participating laboratories follow quality assurance procedures (Table 10). It is of extreme importance to once again emphasize that this component represents the true indicator for the laboratory as to the performance of AST. It is noteworthy that the WHO EQAS organizers provide the control strain E. coli ATCC 25922 free of charge to all new participants of the AST component, why there should not be any excuses not to test this strain.

**ESBL EQAS component**

The participants of the AST component are offered to detect and confirm ESBL production in the Salmonella strains. If participating in this component of the EQAS, all strains showing reduced susceptibility to cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ) ceftriaxone (CRO) and/or meropenem (MERO) should be tested for ESBL, AmpC and carbapenemase production.

For the EQAS 2017, four AmpC-, ESBL-, carbapenemase-producers were included represented by WHO 2017 S-17.1 Infantis (ESBL), WHO 2017 S-17.2 Havana (AmpC), WHO 2017 S-17.4 Rissen (ESBL), and WHO 2017 S-17.8 Kentucky (carbapenemase producers) (Table 11). The two ESBL producing strains harboured the bla\text{CTX-M14}a, and bla\text{CTX-M14} genes whereas the gene accounting for the AmpC phenotype till now curiously is unknown. The carbapenemase producer was conferred by bla\text{NDM-1} and bla\text{CMY-16}. The confirmatory tests (CAZ/C1:CAZ and CTX/C1:CTX) showed 87% (WHO 2017 S-17.1) and 90% (WHO 2017 S-17.4) of deviations in reporting correct ESBL results (based on phenotypic characteristics). For the WHO 2017 S-17.2 (AmpC) and WHO 2017 S-17.4 (carba), deviations of the confirmatory test resulted in 66% and 34%. In general, the level of correctly identified ESBL, AmpC and carbapenemase producing Salmonella is a great concern and it is suggested that the participating laboratories take steps to ensure that it is improved.
3. List of Appendices

Appendix 1: Figures and Tables
Appendix 2: Prenotification
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Appendix 4: WHO EQAS 2017 Protocol
Appendix 5a: Subculture and Maintenance of Quality Control Strains
Appendix 5b: Instructions for Opening and Reviving Lyophilized Cultures
Figure 1. Countries participating* in the WHO EQAS 2017

*marked in green
## List of Countries in the 10 Regions

### Africa
- Algeria
- Angola
- Benin
- Botswana
- Burkina Faso
- Burundi
- Cameroon
- Cape Verde
- Central African Republic
- Chad
- Comoros
- Congo (Brazzaville)
- Congo, Democratic Republic of the
- Cote d'Ivoire (Ivory Coast)
- Djibouti
- Egypt
- Equatorial Guinea
- Eritrea
- Ethiopia
- Gabon
- Ghana
- Guinea
- Guinea-Bissau
- Kenya
- Lesotho
- Liberia
- Libyan Arab Jamahiriya
- Madagascar
- Malawi
- Mali
- Mauritania
- Mauritius
- Mayotte
- Morroco
- Mozambique
- Namibia
- Niger
- Nigeria
- Reunion
- Rwanda
- Saint Helena
- Sao Tome and Principe
- Senegal
- Seychelles
- Sierra Leone
- Somalia
- South Africa
- South Sudan
- Sudan
- Swaziland
- Tanzania, United Republic of
- Togo
- Tunisia
- Uganda
- Western Sahara
- Zambia
- Zimbabwe

### Caribbean
- Anguilla
- Antigua and Barbuda
- Aruba
- Bahamas
- Barbados
- Bonaire, Saint Eustatius and Saba
- British Virgin Islands
- Cayman Islands
- Cuba
- Curaçao
- Dominica
- Dominican Republic
- Grenada
- Guadeloupe
- Haiti
- Jamaica
- Martinique
- Monserrat
- Puerto Rico
- Saint Lucia
- Saint Martin
- Saint Vincent and the Grenadines
- Saint-Barthélémy
- Sint Maarten
- St. Kitts and Nevis
- Trinidad and Tobago
- Turks and Caicos Islands
- Virgin Islands (US)

### Central Asia & Middle East
- Afganistan
- Armenia
- Azerbaijan
- Bahrain
- Bangladesh
- Bhutan
- Georgia
- Hong Kong
- India
- Indonesia
- Iran, Islamic rep. Of
- Iraq
- Israel
- Jordan
- Kazakhstan
- Kuwait
- Kyrgyzstan
- Lebanon
- Macao
- Maldives
- Mongolia
- Myanmar (ex-Burma)
- Nepal
- Oman
- Pakistan
- Palestine
- Qatar
- Saudi Arabia
- Syria
- Tajikistan
- Timor Leste (West)
- Turkmenistan
- United Arab Emirates
- Uzbekistan
- Yemen

### China
- China

### Europe
- Albania
- Andorra
- Austria
- Belarus
- Guernsey and Alderney
- Hungary
- Iceland
- Ireland
- Norway
- Poland
- Portugal
- Romania
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<td>Turkey</td>
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<td>Finland</td>
<td>Moldova</td>
<td>Ukraine</td>
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<td>Vatican City State (Holy See)</td>
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<td>Gibraltar</td>
<td>Netherlands</td>
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<td>Greece</td>
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**Latin America**

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**Oceania**

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<td>Korea, North</td>
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Table 1. Ability of EQAS participating laboratories to serotype the test *Salmonella* strains

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<td>7</td>
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<td>1</td>
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<td>0</td>
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<tr>
<td>In total</td>
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Table 2. EQAS participating laboratories’ performance of *Salmonella* serotyping

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<td><strong>Average</strong></td>
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Table 3. EQAS participating laboratories’ performance of internal quality control strain (WHO S-17.3, *Salmonella* Enteritidis) serotyping.

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Table 5. *Salmonella* serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2017

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<th>% DSG</th>
<th>No. of labs reporting ST</th>
<th>% DST</th>
<th>Deviating results (*)</th>
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*number of participants reporting the specified deviating result
Table 6. EQAS participating laboratories’ performance of antimicrobial susceptibility testing of *Salmonella* strains

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<th>% correct test results</th>
<th>% minor deviations (S ↔ I or I ↔ R)^</th>
<th>% major deviations (S → R)^</th>
<th>% very major deviations (R→ S)^</th>
<th>% critical deviations (R→ S &amp; S → R)^</th>
<th>% total deviations (S → R &amp; R → S &amp; S ↔ I or I ↔ R)^</th>
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*Data do not include one strain which may have lost resistance due to transport or storage stress

^S, susceptible; I, intermediate; R, resistant
Table 7. EQAS participants’ performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial

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Table 7 (continued). EQAS participants’ performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial.

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*For antimicrobial abbreviations: see List of Abbreviations page 1

*R→ S & S→ R (R, resistant; S, susceptible)

^S→R & R→S & S↔I or I↔R (I, intermediate)

* Data do not include one strain which may have lost resistance due to transport or storage stress

-, not determined
Table 8. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2017 *Salmonella* strains*

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<th>CRO</th>
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^For antimicrobial abbreviations: see List of Abbreviations page 1
*The result for the *Salmonella* strain WHO S-17.3 for chloramphenicol was omitted from evaluation (during the process of analyzing the WHO EQAS 2017 data, it was clear to the organizers that the database evaluation of the result related to the *Salmonella* strain WHO S-17.3 for chloramphenicol caused a large number of deviations. The expected result related to the testing of WHO S-17.3/chloramphenicol was 16/intermediate, only, due to the large number of deviations, the organizers decided not to evaluate the submitted results related to this strain/antimicrobial combination.)
Table 9. Region-based categorization of EQAS participants’ performance of Salmonella AST

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<th>Region</th>
<th>EQAS iteration</th>
<th>No. of labs</th>
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<th>% minor deviations (S ↔ I or I ↔ R)^</th>
<th>% major deviations (S → R)^</th>
<th>% very major deviations (R → S)^</th>
<th>% critical deviations (S → R &amp; R → S)^</th>
<th>% total deviations (S→R &amp; R→S &amp; S↔I or I→R)^</th>
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Table 9 (continued). Region-based categorization of EQAS participants’ performance of *Salmonella* antimicrobial susceptibility testing

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<th>EQAS iteration</th>
<th>No. of labs</th>
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<th>% minor deviations (S ↔ I or I ↔ R)$^\alpha$</th>
<th>% major deviations (S → R)$^\beta$</th>
<th>% very major deviations (R → S)$^\gamma$</th>
<th>% critical deviations (S → R &amp; R → S) or I ↔ R)$^\zeta$</th>
<th>% total deviations (S → R &amp; R → S) or I ↔ R)$^\zeta$</th>
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|               | 2002           | 7           | 91.7                 | 6.2                                      | 0.0                                 | 2.0                                 | 2.0                                         | 8.3                                         | |
|               | 2003           | 9           | 94.3                 | 2.5                                      | 1.2                                 | 2.0                                 | 3.2                                         | 5.7                                         | |
|               | 2004           | 11          | 97.1                 | 2.5                                      | 0.3                                 | 0.1                                 | 0.4                                         | 2.9                                         | |
|               | 2006           | 7           | 93.4                 | 4.6                                      | 0.9                                 | 1.1                                 | 2.0                                         | 6.6                                         | |
|               | 2007           | 1           | 98.9                 | 1.1                                      | 0.0                                 | 0.0                                 | 0.0                                         | 1.1                                         | |
|               | 2008           | 4           | 93.9                 | 3.8                                      | 0.0                                 | 2.3                                 | 2.3                                         | 6.1                                         | |
|               | 2009           | 4           | 95.9                 | 3.2                                      | 0.3                                 | 0.6                                 | 0.9                                         | 4.1                                         | |
|               | 2010           | 4           | 92.5                 | 4.6                                      | 0.6                                 | 2.3                                 | 2.9                                         | 7.5                                         | |
|               | 2011           | 4           | 93.8                 | 5.6                                      | 0.6                                 | 0.0                                 | 0.6                                         | 6.2                                         | |
|               | 2012           | 4           | 95.5                 | 3.1                                      | 0.6                                 | 0.9                                 | 1.4                                         | 4.5                                         | Australia, New Zealand |
|               | 2013           | 4           | 96.8                 | 2.9                                      | 0.0                                 | 0.3                                 | 0.3                                         | 3.2                                         | |
|               | 2014           | 5           | 97.4                 | 2.0                                      | 0.0                                 | 0.6                                 | 0.6                                         | 2.6                                         | |
|               | 2015           | 5           | 95.3                 | 3.8                                      | 0.5                                 | 0.5                                 | 1.0                                         | 4.8                                         | |
|               | 2016           | 3           | 98.1                 | 0.0                                      | 0.5                                 | 1.4                                 | 1.9                                         | 1.9                                         | |
|               | 2017           | 2           | 96.1                 | 2.6                                      | 0.0                                 | 1.3                                 | 1.3                                         | 3.9                                         | |

| Oceania | 2017           | 2           | 96.1                 | 2.6                                      | 0.0                                 | 1.3                                 | 1.3                                         | 3.9                                         | |

$^\alpha$ deviations due to change of test method, $^\beta$ deviations due to change of reference organism, $^\gamma$ deviations due to change of test methodology.
Table 9 (continued). Region-based categorization of EQAS participants’ performance of *Salmonella* antimicrobial susceptibility testing.

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<th>Region</th>
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<th>% minor deviations (S ↔ I or I ↔ R)*</th>
<th>% major deviations (S → R)*</th>
<th>% very major deviations (R → S)*</th>
<th>% critical deviations (S → R &amp; R → S &amp; S ↔ I or I ↔ R)*</th>
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</tr>
</tbody>
</table>

* S. susceptible; I. intermediate; R. resistant
Table 9 (continued). Region-based categorization of EQAS participants’ performance of *Salmonella* antimicrobial susceptibility testing.

<table>
<thead>
<tr>
<th>Region</th>
<th>EQAS iteration</th>
<th>No. of labs</th>
<th>% correct test result</th>
<th>% minor deviations (S ↔ I or I ↔ R)(^\wedge)</th>
<th>% major deviations (S → R)(^\wedge)</th>
<th>% very major deviations (R → S)(^\wedge)</th>
<th>% critical deviations (S → R &amp; R → S)(^\wedge)</th>
<th>% total deviations (S→R &amp; R→S &amp; S↔I or I↔R)(^\wedge)</th>
<th>Countries participating in the 2017 iteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southeast Asia</td>
<td>2001</td>
<td>16</td>
<td>88.1</td>
<td>7.7</td>
<td>2.3</td>
<td>1.9</td>
<td>4.2</td>
<td>11.9</td>
<td>Cambodia, Japan (2), Korea, Rep of (2), LAO PDR, Malaysia (5), Philippines (2), Singapore, Sri Lanka (2), Taiwan, Thailand (13), Viet Nam</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>18</td>
<td>89.0</td>
<td>8.1</td>
<td>1.4</td>
<td>1.6</td>
<td>3.0</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>17</td>
<td>87.4</td>
<td>5.2</td>
<td>4.7</td>
<td>2.7</td>
<td>7.4</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>16</td>
<td>92.8</td>
<td>4.4</td>
<td>2.3</td>
<td>0.5</td>
<td>2.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>15</td>
<td>90.0</td>
<td>8.1</td>
<td>1.2</td>
<td>0.8</td>
<td>2.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>20</td>
<td>93.9</td>
<td>4.0</td>
<td>1.4</td>
<td>0.7</td>
<td>2.1</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>19</td>
<td>90.5</td>
<td>4.7</td>
<td>2.2</td>
<td>2.6</td>
<td>4.8</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>27</td>
<td>91.8</td>
<td>4.1</td>
<td>3.0</td>
<td>1.2</td>
<td>4.2</td>
<td>8.3</td>
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</tr>
<tr>
<td></td>
<td>2010</td>
<td>25</td>
<td>92.8</td>
<td>3.8</td>
<td>1.5</td>
<td>1.9</td>
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<td>90.5</td>
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<td>2012</td>
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<td>91.7</td>
<td>3.9</td>
<td>3.5</td>
<td>0.9</td>
<td>4.4</td>
<td>8.3</td>
<td></td>
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<tr>
<td></td>
<td>2013</td>
<td>35</td>
<td>93.4</td>
<td>3.2</td>
<td>2.5</td>
<td>0.7</td>
<td>3.2</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>8</td>
<td>97.0</td>
<td>1.2</td>
<td>0.1</td>
<td>1.6</td>
<td>1.8</td>
<td>3.0</td>
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</tr>
<tr>
<td></td>
<td>2015</td>
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<td>89.9</td>
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<td>1.5</td>
<td>4.1</td>
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<tr>
<td></td>
<td>2016</td>
<td>30</td>
<td>93.5</td>
<td>2.2</td>
<td>3.5</td>
<td>0.8</td>
<td>4.3</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>31</td>
<td>91.9</td>
<td>2.9</td>
<td>2.1</td>
<td>3.2</td>
<td>5.2</td>
<td>8.1</td>
<td></td>
<td></td>
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</tbody>
</table>

\(^\wedge\)S. susceptible; I. intermediate; R. resistant
Table 10. EQAS participants’ performance of antimicrobial susceptibility testing of quality control strain *Escherichia coli* ATCC 25922

<table>
<thead>
<tr>
<th>Accepted interval</th>
<th>Method</th>
<th>Performance</th>
<th>AMP</th>
<th>CAZ</th>
<th>CHL</th>
<th>CIP</th>
<th>COL</th>
<th>CRO</th>
<th>CTX</th>
<th>FIS (SMX)</th>
<th>FOX</th>
<th>GEN</th>
<th>MER</th>
<th>NAL</th>
<th>STR</th>
<th>SXT</th>
<th>TET</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 (44)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>37</td>
<td>38</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>-</td>
<td>39</td>
<td>37</td>
<td>-</td>
<td>42</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2001 (107)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>97</td>
<td>97</td>
<td>97</td>
<td>-</td>
<td>-</td>
<td>53</td>
<td>-</td>
<td>99</td>
<td>74</td>
<td>81</td>
<td>97</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2002 (114)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>19</td>
<td>20</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>-</td>
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<td>12</td>
<td>14</td>
<td>22</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2003 (144)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>140</td>
<td>137</td>
<td>138</td>
<td>-</td>
<td>-</td>
<td>82</td>
<td>-</td>
<td>138</td>
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<td>105</td>
<td>137</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2004 (149)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>132</td>
<td>128</td>
<td>132</td>
<td>-</td>
<td>-</td>
<td>111</td>
<td>84</td>
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<td>126</td>
<td>110</td>
<td>129</td>
<td>87</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>2005 (159)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>10</td>
<td>13</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>16</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2006 (137)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>133</td>
<td>126</td>
<td>127</td>
<td>-</td>
<td>-</td>
<td>115</td>
<td>74</td>
<td>131</td>
<td>122</td>
<td>106</td>
<td>125</td>
<td>74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2007 (126)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>124</td>
<td>123</td>
<td>121</td>
<td>-</td>
<td>-</td>
<td>104</td>
<td>64</td>
<td>124</td>
<td>120</td>
<td>97</td>
<td>117</td>
<td>67</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2008 (147)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>147</td>
<td>135</td>
<td>144</td>
<td>-</td>
<td>-</td>
<td>124</td>
<td>71</td>
<td>145</td>
<td>136</td>
<td>101</td>
<td>129</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2009 (129)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>16</td>
<td>12</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>15</td>
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<td>14</td>
<td>9</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2010 (116)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>114</td>
<td>108</td>
<td>115</td>
<td>-</td>
<td>-</td>
<td>79</td>
<td>100</td>
<td>112</td>
<td>104</td>
<td>84</td>
<td>101</td>
<td>110</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2011 (112)</td>
<td>MIC &amp; Disk</td>
<td>%</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Equation 1 – Figure and Tables, page 18 of 20
Table 10 (continued). EQAS participants’ performance of antimicrobial susceptibility testing of quality control strain *Escherichia coli* ATCC 25922

| Method | Performance | AMP | CAZ | CHL | CIP | COL | CRO | CTX | FIS (SMX) | FOX | GEN | MER | NAL | STR | SXT | TET | TMP |
|--------|-------------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC (μg/ml) | 2-8 | 0.006-0.5 | 2-8 | 0.004-0.016 | 0.025-2 | 0.03-0.12 | 0.03-0.12 | 8-32 | 2-8 | 0.25-1 | 0.008-0.06 | 1-4 | 4-16<sup>3</sup> | ≤0.5/9.5 | 0.5-2 | 0.5-2 |

**EQAS Iteration (total no. of Participants)**

| Year | MIC & Disk | No.<sup>4</sup> | %<sup>4</sup> | MIC (37) | No.<sup>5</sup> | %<sup>5</sup> | Disk (98) | No.<sup>6</sup> | %<sup>6</sup> | MIC & Disk | No.<sup>7</sup> | %<sup>7</sup> | MIC (33) | No.<sup>8</sup> | %<sup>8</sup> | Disk (89) | No.<sup>9</sup> | %<sup>9</sup> | MIC & Disk | No.<sup>10</sup> | %<sup>10</sup> | MIC (28) | No.<sup>11</sup> | %<sup>11</sup> | Disk (87) | No.<sup>12</sup> | %<sup>12</sup> | MIC & Disk | No.<sup>13</sup> | %<sup>13</sup> | MIC (31) | No.<sup>14</sup> | %<sup>14</sup> | Disk (85) | No.<sup>15</sup> | %<sup>15</sup> | MIC & Disk | No.<sup>16</sup> | %<sup>16</sup> | MIC (30) | No.<sup>17</sup> | %<sup>17</sup> | Disk (76) | No.<sup>18</sup> | %<sup>18</sup> | MIC & Disk | No.<sup>19</sup> | %<sup>19</sup> | MIC (41) | No.<sup>20</sup> | %<sup>20</sup> | Disk (74) | No.<sup>21</sup> | %<sup>21</sup> |
|-------|-------------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2012  | 134 | 111 | 121 | 131 | - | 90 | 115 | 53 | - | 127 | - | 121 | 89 | 112 | 129 | 66 |
|        | 13 | 12 | 7 | 6 | - | 11 | 10 | 11 | - | 9 | - | 9 | 8 | 13 | 10 | 21 |
| 2013  | 37 | 26 | 31 | 35 | - | 23 | 28 | 19 | - | 35 | - | 31 | 26 | 23 | 35 | 22 |
|        | 3 | 4 | 0 | 3 | - | 0 | 4 | 5 | - | 3 | - | 3 | 8 | 0 | 0 | 9 |
| 2014  | 97 | 85 | 90 | 96 | - | 67 | 87 | 34 | - | 92 | - | 90 | 63 | 89 | 94 | 44 |
|        | 16 | 14 | 9 | 7 | - | 15 | 11 | 15 | - | 11 | - | 11 | 8 | 16 | 14 | 27 |
| 2015  | 117 | 100 | 112 | 119 | - | 82 | 104 | 44 | - | 113 | - | 113 | - | 101 | 114 | 59 |
|        | 12 | 7 | 5 | 7 | - | 4 | 8 | 10 | - | 6 | - | 11 | - | 8 | 8 | 11 |
|        | 6 | 4 | 4 | 13 | - | 5 | 11 | 18 | - | 9 | - | 11 | - | 5 | 6 | 5 |
| 2017  | 86 | 75 | 84 | 87 | - | 63 | 80 | 27 | - | 81 | - | 85 | - | 79 | 82 | 37 |
|        | 13 | 8 | 6 | 5 | - | 5 | 6 | 7 | - | 4 | - | 9 | - | 10 | 7 | 8 |

<sup>1</sup>For antimicrobial abbreviations: see List of Abbreviations page 1

<sup>2</sup>CLSI standard. Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 22nd Informational supplement. CLSI document M100-S22. 2012 Wayne. PA. USA

<sup>3</sup>FIS (sulfisoxazole) covers the group of SMX (sulfanamides)

<sup>4</sup>Quality control range developed by the manufacturer of Sensititre®; No.: number of laboratories performing the analysis; %: percentage of laboratories reporting erroneous results; -, not determined.
Table 11. Proportion of laboratories that obtained the expected result. Number (n/N) and percentages of laboratories which correctly detected and confirmed the ESBL-producing *Salmonella* strains.

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Expected interpretation</th>
<th>Confirmatory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 2017 S-17.1</td>
<td>Presumptive ESBL-phenotype</td>
<td>80/92 (87%)</td>
</tr>
<tr>
<td>WHO 2017 S-17.2</td>
<td>Presumptive AmpC-phenotype</td>
<td>26/77 (34%)</td>
</tr>
<tr>
<td>WHO 2017 S-17.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO 2017 S-17.4</td>
<td>Presumptive ESBL-phenotype</td>
<td>82/91 (90%)</td>
</tr>
<tr>
<td>WHO 2017 S-17.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO 2017 S-17.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO 2017 S-17.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO 2017 S-17.8</td>
<td>Presumptive carbapenemase-phenotype</td>
<td>62/94 (66%)</td>
</tr>
</tbody>
</table>
SIGN-UP FOR EQAS 2017

Greetings to the WHO Global Foodborne Infections Network (WHO GFN) Members:

WHO GFN strives to increase the quality of laboratory-based surveillance of *Salmonella* by encouraging national and regional reference laboratories that attended WHO GFN training courses to participate in the External Quality Assurance System (EQAS). We are pleased to announce the launch of the 2017 EQAS cycle.

**WHY PARTICIPATE IN EQAS?**

EQAS provides the opportunity for proficiency testing which is considered an important tool for the production of reliable laboratory results of consistently good quality.

**WHAT IS OFFERED IN EQAS?**

This year, WHO EQAS offers the following components:

- Serogrouping, serotyping and antimicrobial susceptibility testing of eight *Salmonella* isolates.

**WHO SHOULD PARTICIPATE IN EQAS 2017?**

All national and regional reference laboratories which perform analysis on *Salmonella* and are interested in participating in an external quality assurance program are invited to participate.

We expect that all national and regional reference laboratories that attended WHO GFN Training Courses will participate in the EQAS.

The WHO GFN Regional Centers in cooperation with the EQAS Coordinator will evaluate the list of laboratories that sign up for EQAS 2017. Laboratories which signed up and received bacterial isolates in year 2016 but did not submit any result should provide a consistent explanation for this if they want to participate in 2017.

**COST FOR PARTICIPATING IN EQAS**

There is no participation fee. Laboratories should, however, cover the expenses for parcel shipment if they can afford it. If FedEx has ‘Dangerous Goods-service’ in your country or if you have a DHL-account no, please provide your FedEx or DHL import account number (for import of UN3373 Biological Substance Category B) in the sign-up form or, alternatively, to the EQAS Coordinator (please find contact information below). We need this information at this stage to save time and resources. Participating laboratories are responsible for paying any expenses related to taxes or custom fees applied by their country.
HOW TO SIGN- UP FOR EQAS 2017

This link will open a sign-up webpage: http://eqas.food.dtu.dk/who/signup

In this webpage, you will be asked to provide the following information:
- Name of institute, department, laboratory, and contact person
- Complete mailing address for shipment of bacterial isolates (no post-office box number)
- Telephone and fax number, e-mail address
- FedEx or DHL import account number (if available)
- Approximate number of Salmonella isolates annually serogrouped/serotyped
- Approximate number of Salmonella isolates annually tested for antimicrobial susceptibility
- Availability of ATCC 25922 E. coli reference strain
- Components of EQAS 2017 you plan to participate in
- Level of reference function in your country

If you experience any problem in the sign-up webpage, please try again a few days later. If problems persist, please contact the EQAS Coordinator Susanne Karlsmose Pedersen: E-mail suska@food.dtu.dk.

TIMELINE FOR SHIPMENT OF ISOLATES AND AVAILABILITY OF PROTOCOLS

A number of different institutions will ship the bacterial isolates, and you will receive information concerning the institution shipping your parcel. The bacterial isolates will be shipped in October 2017.

In order to minimize delays, please send a valid import permit to the EQAS coordinator. Please apply for a permit to receive the following: “UN3373, Biological Substance Category B”: eight Salmonella strains, and (for new participants performing antimicrobial susceptibility testing on Salmonella) one Escherichia coli reference strain.

Protocols and all relevant information will be available for download from the website http://www.antimicrobialresistance.dk/233-169-215-eqas.htm.

DEADLINE FOR SUBMITTING RESULTS TO THE NATIONAL FOOD INSTITUTE

Results must be submitted to the National Food Institute (DTU Food) by 28th February 2018 through the password-protected website. An evaluation report will be generated upon submission of results. Full anonymity is ensured, and only DTU Food and the WHO GFN Regional Centre in your region will have access to your results.

Deadline for sign-up for the WHO GFN EQAS 2017 is August 4th, 2017
| WHO 2017 S-17.1 | Salmonella Infantis | I 6,7:r:1,5 | ESBL | >64 | RESIST | >64 | RESIST | synergy | 4 | SUSC | 16 | RESIST | synergy | >32 | RESIST | <=8 | SUSC | 0.25 | INTER |
| WHO 2017 S-17.2 | Salmonella Havana | I 13,23,f,g,- | AmpC | <=1 | SUSC | 1 | SUSC | no synergy | 32 | RESIST | 4 | RESIST | no synergy | 0.12 | SUSC | <=8 | SUSC | 0.06 | SUSC |
| WHO 2017 S-17.3 | Salmonella Enteritidis | I 9,12,g,m,- | - | 4 | SUSC | 0.5 | SUSC | 1 | SUSC | 0.25 | SUSC | 16 | INTER | 0.06 | SUSC |
| WHO 2017 S-17.4 | Salmonella Rissen | I 6,7,f,g,- | ESBL | >64 | RESIST | 32 | RESIST | synergy | 8 | SUSC | 2 | RESIST | synergy | 64 | RESIST | >128 | RESIST | 0.03 | SUSC |
| WHO 2017 S-17.5 | Salmonella Weltevreden | I 3,10,r,z6 | - | <=1 | SUSC | <=0.25 | SUSC | <=0.5 | SUSC | 0.03 | SUSC | 128 | RESIST | 0.03 | SUSC |
| WHO 2017 S-17.6 | Salmonella Schwarzengrund | I 4,12,d:1,7 | - | 2 | SUSC | <=0.25 | SUSC | <=0.5 | SUSC | 0.08 | SUSC | <=8 | SUSC | 0.03 | SUSC |
| WHO 2017 S-17.7 | Salmonella Typhimurium | I 4,5,12:i,1,2 | - | >64 | RESIST | 0.5 | SUSC | <=0.5 | SUSC | 0.12 | SUSC | 64 | RESIST | 0.5 | INTER |
| WHO 2017 S-17.8 | Salmonella Kentucky | I 8,20:i,z6 | Carbapenemase | >64 | RESIST | >64 | RESIST | no synergy | 64 | RESIST | >128 | RESIST | no synergy | >256 | RESIST | >128 | RESIST | 8 | RESIST |
PROTOCOL for
serotyping and antimicrobial susceptibility testing of Salmonella test strains

1 INTRODUCTION

In 2000, the Global Foodborne Infections Network (formerly known as WHO Global Salm-Surv) launched an External Quality Assurance System (EQAS). The EQAS is organized by the National Food Institute, Technical University of Denmark (DTU Food), in collaboration with partners and Regional Sites in WHO GFN.

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 for the subcontractor’s work.

The WHO EQAS 2017 includes serotyping and antimicrobial susceptibility testing of eight Salmonella strains and antimicrobial susceptibility testing of the Escherichia coli ATCC 25922 (CCM 3954) reference strain for quality control (QC).

The above-mentioned QC reference strain is included in the parcel only for new participants of the EQAS who did not receive it previously. The QC reference strain supplied is an original
CERTIFIED culture provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strain 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 WHO Collaborating Centre website (see www.antimicrobialresistance.dk).

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of serotyping and antimicrobial susceptibility testing of enteric human pathogens, especially Salmonella. A further objective is to assess and improve the comparability of surveillance data on Salmonella serotypes and antimicrobial susceptibility reported by different laboratories. Therefore, the laboratory work for this EQAS should be done by using the methods routinely used in your laboratory.

3 OUTLINE OF THE EQAS 2017

3.1 Shipping, receipt and storage of strains

In October 2017 around 200 laboratories located worldwide will receive a parcel containing eight Salmonella strains. An E. coli ATCC 25922 reference strain will be included for participants who signed up to perform antimicrobial susceptibility testing (AST) and did not receive it previously. All provided strains belong to UN3373, Biological substance category B. Extended Spectrum Beta Lactamase (ESBL)-, AmpC- or carbapenemase-producing strains could be included in the selected material.

Please confirm receipt of the parcel through the confirmation form enclosed in the shipment

The Salmonella strains are shipped as agar stab cultures whereas the reference strain is shipped lyophilised. On arrival, the agar stab culture must be stored in a dark place at 2°C to 8°C. If receiving a lyophilized reference culture, store in a dark, cool place. The agar stab cultures must be sub-cultured and prepared for storage in your strain collection (e.g. in a -80°C freezer). This set of cultures should serve as reference if discrepancies are detected during the testing (e.g. they can be used to detect errors such as mis-labelling or contamination).

3.2 Serotyping of Salmonella

The eight Salmonella strains should be serotyped by using the method routinely used in the laboratory. Also serogroup results will be evaluated, therefore, if you do not have all the necessary antisera for a serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and
Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

Please fill in information concerning the brand of antisera used for typing in the fields available in the database for entering results. In addition, we kindly ask you to report which antisera you think are required to complete the serotyping, if relevant.

### 3.3 Antimicrobial susceptibility testing of *Salmonella* strains and *Escherichia coli* ATCC 25922

The *Salmonella* strains as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in your laboratory.

For reconstitution of the *E. coli* reference strain, please see the document ‘Instructions for opening and reviving lyophilised cultures’ on the WHO Collaborating Centre website (see [www.antimicrobialresistance.dk](http://www.antimicrobialresistance.dk)).

Testing of gentamicin susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* should not be reported as susceptible to aminoglycosides.

The breakpoints used in this EQAS for interpreting MIC results are in accordance with CLSI values (Table 1). Consequently, interpretation of MIC results will lead to categorization of strains into three categories: resistant (R), intermediate (I) and susceptible (S). In the evaluation report you receive upon result submission, you can find that obtained interpretations in accordance with the expected interpretation will be defined as ‘correct’, whereas deviations from the expected interpretation will be defined as ‘minor’ (I ↔ S or I ↔ R), ‘major’ (S interpreted as R) or ‘very major’ (R interpreted as S).

Please report the breakpoints that you routinely use in your laboratory for interpretation of antimicrobial susceptibility test results in the fields available in the database (or in the test forms).
### Table 1. Interpretive breakpoint for *Salmonella* antimicrobial susceptibility testing

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Reference value, MIC (µg/mL)</th>
<th>Reference value, Disk diffusion (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Amoxicillin, AMP</td>
<td>≤8</td>
<td>16</td>
</tr>
<tr>
<td>Cefotaxime, CTX*</td>
<td>≤1</td>
<td>-</td>
</tr>
<tr>
<td>Cefoxitin, FOX</td>
<td>≤8</td>
<td>16</td>
</tr>
<tr>
<td>Ceftazidime, CAZ*</td>
<td>≤1</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone, CRO*</td>
<td>≤1</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol, CHL</td>
<td>≤8</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxacin, CIP</td>
<td>≤0.06</td>
<td>0.12-0.5</td>
</tr>
<tr>
<td>Colistin, COL***</td>
<td>≤2</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin, GEN</td>
<td>≤4</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem, MER*</td>
<td>≤0.12</td>
<td>-</td>
</tr>
<tr>
<td>Nalidixic acid, NAL</td>
<td>≤16</td>
<td>-</td>
</tr>
<tr>
<td>Sulfonamides, SMX</td>
<td>≤256</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline, TET</td>
<td>≤4</td>
<td>8</td>
</tr>
<tr>
<td>Trimethoprim, TMP</td>
<td>≤8</td>
<td>-</td>
</tr>
<tr>
<td>Trimethoprim + sulamethoxazole, TMP+SMX, SXT</td>
<td>≤2/38</td>
<td>-</td>
</tr>
</tbody>
</table>

Reference values used in this EQAS are according to CLSI (M100, 27th edition), with the following exceptions:
- For the cephalosporins and meropenem, the application of the interpretative criteria is intended to indicate if the microorganism is a presumptive ESBL- or carbapenemase-producer. Reference values for the cephalosporins are according to CLSI M100 Table 3A. These interpretative criteria are also applied for *Salmonella* test strains for interpretation of AST results in this EQAS. Reference values for meropenem are based on epidemiological cut off values from www.eucast.org.
- ** The publication by Cavaco LM and Aarestrup FM (J. Clin. Microbiol. 2009. Sep;47(9):2751-8) provides the background for these interpretative criteria in the WHO GFN EQAS.
- *** Reference values for colistin are based on CLSI M100 Table 2A-2. In the current EQAS these values should be applied for the interpretation of *Salmonella* AST results into the category as susceptible or resistant.
Concerning ciprofloxacin susceptibility tests, the applied breakpoints take into consideration mechanisms of resistance due to plasmid-mediated quinolone resistance genes (e.g. *qnr*-genes) and one-point-mutation in the gyrase gene.

**Important notes: beta-lactam resistance**

The following tests for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes are optional in relation to the current WHO GFN EQAS.

If choosing to participate in this component of the EQAS, all strains displaying reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ), and/or ceftriaxone (CRO) should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests. Reduced susceptibility to any of the above-mentioned antimicrobials indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype.

Confirmatory test for ESBL production requires the use of both cefotaxime (CTX) and ceftazidime (CAZ) alone, and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) by microbroth dilution methods or E-test; a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/Cl or CAZ : CAZ/Cl ratio ≥ 8) or ii) by disk diffusion; a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases can be performed by testing the bacterial culture for susceptibility to cefoxitin (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

Confirmatory test for carbapenemase production requires the testing of meropenem (MER). Reduced susceptibility to MER indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (available in The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015, EFSA Journal 2017;15(2):4694,212 pp (page 43).

The following summary of these recommendations indicate how the phenotypes should be categorized:
ESBL-phenotype:
- CTX or CAZ > 1 mg/L AND
- MER ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

ESBL+AmpC-phenotype:
- CTX or CAZ > 1 mg/L AND
- MER ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

AmpC-phenotype:
- CTX or CAZ > 1 mg/L AND
- MER ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- No synergy for CTX : CTX/Cl nor CAZ : CAZ/Cl
  (note, presence of ESBLs is not excluded)

Carbapenemase-phenotype:
- MER > 0.12 mg/L
  (note, presence of ESBLs or AmpCs is not excluded)

Other-phenotype:
- Not covered by any of the above categories AND
- CTX, CAZ, FOX, or MER > interpretative criteria as susceptible in Table 1 (i.e. exhibits reduced susceptibility)

No ESBL-, AmpC-, or carbapenemase:
- CTX, CAZ, FOX, and MER ≤ interpretative criteria as susceptible in Table 1 (i.e. exhibits susceptibility)

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but is not requested as part of this WHO GFN EQAS.
4 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the web database. For entering your results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a 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’.

**Results must be submitted no later than 28th February 2018.**

If you do not have access to the Internet, or if you experience difficulties in entering your results, please contact the EQAS Coordinator directly, explaining the issues that occur.

All results will be summarized in a report which will be publicly available. Individual results will be anonymous and will only be forwarded to the official GFN Regional Centre in your region.

We are looking forward to receiving your results.

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

Susanne Karlsmose Pedersen
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby - DENMARK
Tel: +45 3588 6601
E-mail: suska@food.dtu.dk

Direct communication with the EQAS organisers must be in English.

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please carefully read these instructions before entering the web page. Remember that you need by your side the completed test forms and the breakpoint values you used.

In general, you can browse back and forth in the pages of the database. Always remember to save your input before leaving a page.
1) Enter the WHO Collaborating Centre website (from http://www.antimicrobialresistance.dk), then
   a. Click on ‘EQAS’
   b. Click on the link for the interactive database (http://eqas.food.dtu.dk/who)
   c. Write your username and password in lower-case letters and click on ‘Login’.
      You can find your username and password in the letter following your strains.
      Your username and password will remain unchanged in future trials. Do not hesitate to
      contact us if you experience problems with the login.

2) Click on ‘Materials and methods’
   a. Fill in the fields relative to brand of antisera (very important because we would like to
      compare results obtained with different brands of antisera)
   b. Fill in the fields relative to the method used for antimicrobial susceptibility testing
   c. Enter the brand of materials, e.g. Oxoid
   d. Fill in the field asking whether your institute serves as a national reference laboratory
   e. In the comment field, report which antisera you think is required to complete your
      serotyping, if relevant
   f. Click on ‘Save and go to next page’ – ALWAYS remember to save each page before
      leaving it!

3) In the data entry page ‘Routinely used breakpoints’
   a. Fill in the fields relative to the breakpoints used routinely in your laboratory to determine
      the antimicrobial susceptibility category. Remember to use the operator keys in order to
      show – equal to (=), less than (<), less or equal to(≤), greater than (>), or greater than or
      equal to (≥).

4) In the data entry pages ‘Salmonella strains 1-8’,
   a. SELECT the serogroup (O-group) from the drop-down list, DO NOT WRITE – Wait a few
      seconds – the page will automatically reload, so that the drop-down list in the field
      “Serotype” only contains serotypes belonging to the chosen serogroup.
   b. SELECT the serotype from the drop-down list – DO NOT WRITE – wait a few seconds
      and you can enter the antigenic formula (e.g. 1,4,5,12:i:1,2)
   c. Enter the zone diameters in mm or MIC values in µg/ml. Remember to use the operator
      keys to show e.g. equal to (=), etc.
   d. Enter the interpretation as R (resistant), I (intermediate) or S (susceptible)
   e. If you performed confirmatory tests for ESBL production, select the appropriate result.
   f. If relevant, fill in the field related to comments (e.g. which antisera you miss for complete
      serotyping)
   g. Click on ‘Save and go to next page’

If you did not perform these tests, please leave the fields empty
5) In the data entry page ‘E. coli reference strain’:
   a. Enter the zone diameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to (=), etc.
   b. Click on ‘Save and go to next page’

6) The next page is a menu that allows you to review the input pages and approve your input and finally see and print the evaluated results
   a. Browse through the input pages and make corrections if necessary. Remember to click on ‘save and go to next page’ if you make any corrections.
   b. Approve your input. Be sure that you have filled in all the results before approval, as **YOU CAN ONLY APPROVE ONCE!** The approval blocks your data entry into the interactive database, but allows you to see the evaluated results.
   c. As soon as you have approved your input, an evaluation report will appear.

7) After browsing all pages in the report, you will find a new menu. You can choose ‘EQAS 2017 start page’, ‘Review evaluated results’ (a printer friendly version of the evaluation report is also available) or ‘Go to WHO GFN homepage’.

   **End of entering your data – thank you very much!**
SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1 PURPOSE AND REFERENCES

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

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

2 DEFINITION OF TERMS

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

Reference Stock Culture: 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.

Working Stock Cultures: 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.

Subcultures (Passages): 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.

3 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 (after 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.
WHO Collaborating Centre
External Quality Assurance System (EQAS)

4 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.

5 FREQUENCY OF TESTING

Weekly vs. daily testing
Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.
- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three 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

If five acceptable QC results are available, no additional days of QC-testing are needed.
If the problem cannot be resolved, continue daily testing until the errors are identified.
Repeat the 30 days validation before resuming weekly testing.
INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES


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