

External Quality Assurance System (EQAS) of the WHO Global *Salmonella* Surveillance and Laboratory Support Project (Global Salm-Surv)

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Abstract

To assure the quality of methods for serotyping and antimicrobial susceptibility testing among laboratories in WHO Global Salm-Surv, an international collaborative study on serotyping and antimicrobial susceptibility testing of eight *Salmonella enterica* strains was performed. A total of 44 laboratories in 35 countries participated. For serotyping, 76% of the results were correct. For susceptibility testing, 92% of the results for the eight *Salmonella* strains were in agreement with the expected results. However, 28% of the performed tests with the *E. coli* ATCC 25922 reference strain were out of the quality control range specified by NCCLS guidelines.

Introduction

Salmonella is one of the most important foodborne pathogens world wide. Internationally, there is a growing concern over the increasing resistance in *Salmonella*; recently, the multiresistent *Salmonella* clone "DT104" has spread among several countries and continents. To improve world wide surveillance, WHO has recently launched an international *Salmonella* surveillance and laboratory support project - "Global Salm-Surv".

To support laboratories participating in this WHO global *Salmonella* surveillance network, an External Quality Assurance System (EQAS) was initiated. This EQAS will support the assessment of the quality of serotyping and antimicrobial susceptibility testing of *Salmonella* in all participating laboratories.

The EQAS program was organised by the Danish Veterinary Laboratory (DVL) in collaboration with the WHO Headquarter and Centers for Disease Control and Prevention, Atlanta. The first cycle of the EQAS covered serotyping and susceptibility testing of eight *Salmonella* isolates by 44 laboratories participating in Global Salm-Surv.

Materials and methods

The EQAS was announced on the Global Salm-Surv listserver, and interested laboratories were asked to apply. A total of 50 laboratories were enrolled in the first cycle of the EQAS. On February 29th, an official letter of participation was sent by e-mail if an e-mail address was given; otherwise, the message was sent by fax. The messages were sent again on March 9th or 15th if no answers were obtained.

A total of eight *Salmonella* isolates were sent to all laboratories (Table 1). The *Salmonella* strains mainly represented the O:4 antigenic serogroup and had different antibiotic susceptibility patterns. All strains were freeze dried and packed in double containers. On April 3rd, 43 of these parcels were sent to the participants with a courier firm, and seven parcels were sent April 17th after acceptance

of participation. The parcels were received by the participants after two to fourteen days. However, in one case it took more than three months because the parcel was held in customs.

A test form for the results and a questionnaire with general questions about methods used and numbers of *Salmonella* isolations, serotypings and susceptibility tests performed in 1999 were included in the parcel. The test form and questionnaire were also sent by e-mail approximately a week later to participants with an e-mail address.

On arrival, cultures were subcultured on agar plates and stored until the serotyping and susceptibility tests could be performed. The test results were supposed to be recorded on the attached form and returned within 30 days by fax or e-mail to the Danish Veterinary Laboratory.

Participation in the WHO EQAS was free of charge except costs of performing the analyses. The laboratories were requested to use the serotyping methods and susceptibility testing methods routinely performed in the laboratory. Disk diffusion was used in 42 laboratories (95%) and MIC determination was used in three laboratories. One laboratory used both disk diffusion and MIC determination. The strains were tested against the following antimicrobials: Ampicillin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfonamides, tetracycline and trimethoprim. Not all the laboratories were able to test for susceptibility towards all the antimicrobials.

A total of 44 of the 50 enrolled laboratories returned the reply form with laboratory results between April 10th and June 6th. No results were received from six laboratories. The 44 laboratories were from a total of 35 countries: Argentina, Australia, Bulgaria, Cambodia, China, Cyprus, Czech, Egypt, Estonia, Greece, Guatemala, Hungary, Japan, Kuwait, Laos, Latvia, Lebanon, Malaysia, Mauritius, Mexico, New Zealand, Oman, Poland, Rep. Korea, Rep. Moldova, Slovenia, South Africa, Spain, Sri Lanka, Syria, Thailand, Turkey, Uganda, Uruguay and Vietnam.

An individual report was prepared for each participant. The report consisted of two sections. The first section contained a summary of the answers to general questions about the number of performed tests and the methods and materials for serotyping and susceptibility testing. The other section contained the obtained zone diameters/MIC values, the obtained and expected results, and comments to the deviating results. In total, 36 EQAS reports and EQAS evaluation questionnaires were sent on June 23rd. The 36 EQAS reports covered all the laboratories that had sent their results before May 29th with the exception of two laboratories. The remaining eight reports were sent to the participants on August 16th.

Results

Questionnaire

A mean of 1786 *Salmonella* strains (range 30 - 26095) were analysed yearly in the participating laboratories; these strains were either isolated in the laboratory or received from other laboratories for further analyses. On average, 1005 (range 9 - 3579) *Salmonella* strains were serotyped and 341 strains (range 5 - 3000) were tested for antimicrobial susceptibility in the laboratories that responded to these questions in the questionnaire.

Serotyping

Thirty-four laboratories (77%) serotyped the eight isolates. Three laboratories serotyped two to six strains and serogrouped the remaining because of lack of antisera. Serogrouping of all the eight *Salmonella* strains was performed in two laboratories. Five laboratories neither serotyped nor serogrouped the strains. Of the 216 serotyping results, a total of 76% were determined correctly. The mean of correct serotyping results was six correctly serotyped strains per laboratory (75%). The results of serotyping are given in Table 1.

Table 1. The correct serotypes of the tested strains, the total number of obtained deviations among the participating laboratories and the incorrect serotypes found for each strain.

Strain	99-23688-1	98-22876-3	99-21292-3	98-24475-1	98-24527-3	99-22292-3	99-22829-1	98-74091-5
Serovar	Typhimurium	Typhimurium	Bredeney	Newport	Saintpaul	Schwarzengrund	Heidelberg	Enteritidis
Correct serotype	4,5,12: i: 1,2	1,4,5,12: i: 1,2	1,4,12: 1,v: 1,7	6,8: e,h: 1,2	1,4,12: e,h: 1,2	1,4,12,27: d: 1,7	1,4,12: r: 1,2	9,12: g,m: -
Number of deviations	3	4	11	13	11	12	11	4
Number of laboratories serotyping this strain	36	36	37	36	36	36	35	37
List of deviating results ¹	1,4,(5),12: i: 1,5 4: i: enx 1,4,12: i: z6	(-): e,h: 1,2 4,5: e,h: R1 1,4,5,12:i: 1,w 1,4,12,27: i: 1,w 1,4,(5),12: i: 1,5	4,[5],12: a: 1,7 1,12:e,h:1,7 4,12: f,g 1,4,12,27: i: 1,w (-): e,h: 1,7 1,4,12:b:1,2 Copenhagen 4: 1,v: 1,2 1,4,5,12: 1,v: 1,2/1,5? 1,4,12:i:1,2 4,5,12:i:1,2	(-):e,h:1,2 6,7:e,h: 6,7:e,h: enz15 6,7:e,h:1,2 6,8:e,h: enz15 (2 laboratories) 6,8:e,h:1,5 6,8:z4 8:e,h:1,2 (4 laboratories) 8:e,h:enz15	4,[5],12:e,h:enz15 1,4,12:e,h: enx (3 laboratories) 4,12:e,h:1,7 (2 laboratories) 4,12:i: enz15 4:e,h:e,n 4:e,h:enz15 4,27:e,h:2 4,5:a:1,2	4:b:1,6 1,13,23:d: 1,7 1,4,12,27:d: 1,2 4:d:1,2 1,4,12,19:d: 1,7 Cairo 4:d:- 1,3,19:d:1,5 1,4,12:d:1,2 4,5,12,27:d: 1,7 4,12:f,g 1,4,12,27:i: 1,w	1,4,[5],12: a:1,2 1,4,12:b:1,2 6,7:b:z35 4:d:1,2 4,12,27:d: 1,7 1,4,12:e,h: 1,2 (-):e,h:1,2 4,12:f,g 1,4,(5),12:i: 1,2 4:i:1,2 1,4,12,27:r: 1,7	1,9,12:d:1,5 9,12:g,m,q:- 9:g:- 9,12:e,h:1,2

For two strains of *S. Typhimurium* and one strain of *S. Enteritidis* (strains 99-23688-1, 98-74091-5 and 98-74091-5) the serotyping results deviated in five, four and three cases, respectively. This suggests that nearly all laboratories were able to serotype the most common *Salmonella* strains, *S.*

¹ The numbers in brackets represent the number of laboratories that had obtained the antigenic formula.

Typhimurium and *S. Enteritidis*. However, the strains belonging to other serotypes were incorrectly serotyped by 30 - 33% of the laboratories.

Of the 34 laboratories serotyping all eight *Salmonella* strains, 18 laboratories serotyped seven or eight strains correctly (Table 2).

Table 2. Number of laboratories serotyping from eight to nil *Salmonella* strains correctly.

Number of correct serotyped strains	Number of laboratories (%)
8	9(27)
7	9(27)
6	3(9)
5	3(9)
4	3(9)
3	2(6)
2	3(9)
1	1(3)
0	1(3)

Antimicrobial susceptibility testing of the reference strain

In the individual report for each participating laboratory, the results of the *E. coli* ATCC 25922 reference strain were compared with the quality control range specified in the NCCLS guidelines M31-A, M2 and M7. On average, two out of ten tested antimicrobials were out of range (Table 3). In only fourteen laboratories (32%), all the results of the *E. coli* reference strain were within range. Diagrams showing the distribution of obtained zone diameters and quality assurance range for the *E. coli* reference strain are given in Appendix 1. The results from three laboratories testing the reference strain with MIC determination are not included, but the expected MIC values are given in Table 4.

Table 3. Percentage of results of the *E. coli* reference strain ATCC 25922 inside the quality control range.

Antimicrobial	Percentage of laboratories inside the QA range (No. of laboratories ²)
Ampicillin	73 (37)
Chloramphenicol	63 (38)
Ciprofloxacin	80 (35)
Gentamicin	77 (39)
Nalidixic acid	65 (37)
Kanamycin	81 (36)
Streptomycin	78 (36)
Sulfonamides	47 (19)
Tetracycline	58 (42)
Trimethoprim	70 (31)

² Total number of laboratories using disks with the amount of diffusible antimicrobial specified in the NCCLS guidelines.

Table 4. The MIC values and R/I/S categories of the eight *Salmonella* strains and the *E. coli* reference strain used for the EQAS.

	Ampi- cillin (10 µg)	Chloram- phenicol (30 µg)	Cipro- floxacin (5 µg)	Gentami- cin (10 µg)	Kanamy- cin (30 µg)	Nalidixic acid (30 µg)	Strepto- mycin (10 µg)	Sulfon- amides (300 µg)	Tetra- cycline (30 µg)	Trimetho- -prim (5 µg)
99-23688-1	1 S	8 S	0.03 S	1 S	≤16 S	4 S	8 S	32 S	2 S	≤4 S
98-22876-3	≥32 R	64 R	≤0.03 S	1 S	≤16 S	≥128 R	≥64 R	≥512 R	≥32 R	≥32 R
99-21292-3	≥32 R	4 S	0.25 S	1 S	≤16 S	≥128 R	≥64 R	≥512 R	≥32 R	≥32 R
98-24475-1	≥32 R	64 R	≤0.03 S	1 S	>64 R	4 S	≥64 R	≥512 R	≥32 R	≥32 R
98-24527-3	≥32 R	64 R	≤0.03 S	1 S	≤16 S	4 S	≥64 R	≥512 R	≥32 R	≥32 R
99-22292-3	1 S	4 S	≤0.03 S	1 S	≤16 S	4 S	≥64 R	≥512 R	≥32 R	≥32 R
99-22829-1	≥32 R	64 R	≤0.03 S	1 S	≤16 S	4 S	≥64 R	≥512 R	≥32 R	≥32 R
98-74091-5	2 S	8 S	≤0.03 S	≥32 R	32 I	4 S	≥64 R	≥512 R	2 S	2 S
<i>E. coli</i> 25922 ATCC	2-8 ¹ / S	2-8 ¹ / S	0.004- 0.016 ² / S	0.25-1 ¹ / S	1-4 ¹ / S	1-4 / S	4-16 ³	Sulfisoxa zole: 8- 32 ¹ / S	0.5-2 ¹ / S	0.5-2 ² / S

Antimicrobial susceptibility testing of the eight *Salmonella* strains

The results of antimicrobial susceptibility testing were categorised as resistant (R), intermediate (I) or susceptible (S) according to the breakpoint values normally used in the different laboratories. Not all laboratories performed all the specified tests. On average, each laboratory performed 75 susceptibility tests; 68 (92%) of the obtained R/I/S results were in total agreement with the reference (Table 4).

In the individual report for each laboratory, the deviations were divided into minor and major deviations and commented. A result is regarded as a deviation if it is incorrectly interpreted as resistant, intermediate or sensitive. An I - S or an I - R deviation is called a minor deviation and a S - R deviation a major deviation. In total 3151 antimicrobial susceptibility tests were performed. Of these 2890 (91.7%) results were in agreement with the reference, 4.5% were minor deviations and 3.8% were major deviations. The percentage of major deviations of each antimicrobial agent is shown in Table 5.

Table 5. Number of tests and the percentage of major deviations for each antimicrobial agent.

	Ampi- cillin (10 µg)	Chloram- phenicol (30 µg)	Cipro- floxacin (5 µg)	Gentami- cin (10 µg)	Kanamy- cin (30 µg)	Nalidixic acid (30 µg)	Strepto- mycin (10 µg)	Sulfon- amides (300 µg)	Tetra- cycline (30 µg)	Trimetho- -prim (5 µg)
Total no of determina- tions	343	343	334	343	312	328	312	248	335	295
% major deviations	6.1	3.8	1.2	5.0	4.5	1.8	3.5	4.8	6.0	2.7

The percentage of R, I and S results for each strain and antimicrobials are given in Table 6. When calculating the percentage of interpretations as R, I or S, results from using disks with other

concentrations of diffusible amount of antimicrobials than those specified in the test form are not included.

Table 6. Results of susceptibility tests (% R/I/S) of the eight *Salmonella* strains in 44 laboratories. Bold indicates expected results.

	Ampicillin (10 µg)	Chloramphenicol (30 µg)	Ciprofloxacin (5 µg)	Gentamicin (10 µg)	Kanamycin (30 µg)	Nalidixic acid (30 µg)	Streptomycin (10 µg)	Sulfonamides (300 µg)	Tetracycline (30 µg)	Trimetho-prim (5 µg)
99-23688-1	15/2/ 84	5/7/ 88	0/2/ 98	5/2/ 93	3/8/ 90	0/5/ 95	13/51/ 36	15/8/ 77	21/21/ 57	7/0/ 93
98-22876-3	100 /0/0	100 /0/0	2/29/ 69	5/2/ 93	5/5/ 90	98 /0/2	97 /0/3	96 /4/0	100 /0/0	13/3/ 83
99-21292-3	100 /0/0	7/7/ 86	0/12/ 88	2/2/ 95	5/5/ 90	98 /0/2	97 /0/3	100 /0/0	100 /0/0	100
98-24475-1	100 /0/0	98 /2/0	0/0/ 100	5/0/ 95	97 /0/3	2/5/ 93	95 /5/0	100 /0/0	100 /0/0	100 /0/0
98-24527-3	100 /0/0	100 /0/0	0/0/ 100	5/0/ 95	5/3/ 92	0/5/ 95	95 /5/0	100 /0/0	100 /0/0	100 /0/0
99-22292-3	12/5/ 84	7/5/ 88	5/0/ 95	7/2/ 91	5/5/ 90	5/2/ 93	95 /3/3	96 /0/4	100 /0/0	100 /0/0
99-22829-1	98 /0/2	98 /0/2	0/0/ 100	2/2/ 95	8/3/ 90	0/0/ 100	95 /0/5	100 /0/0	100 /0/0	100 /0/0
98-74091-5	21/10/ 69	10/5/ 85	2/0/ 98	100 /0/0	51/ 36 /13	2/7/ 90	95 /3/3	96 /0/4	27/32/ 42	3/0/ 97
<i>E. coli</i> ATCC 25922	21/12/ 67	6/6/ 88	0/7/ 93	3/3/ 94	7/7/ 87	0/3/ 97	7/30/ 63	11/11/ 78	10/10/ 81	10/0/ 91

R, resistant strains, I, intermediate strains, S, sensitive strains.

Diagrams showing the distribution of obtained zone diameters for each strain and antimicrobial and the breakpoint values are given in Appendix 1.

The distribution of laboratories with different numbers of minor and major deviations is shown in Fig. 1. Seventeen laboratories have no major deviations, while only one laboratory had no minor deviation. Five laboratories were responsible for 79 of the 132 major deviations in total.

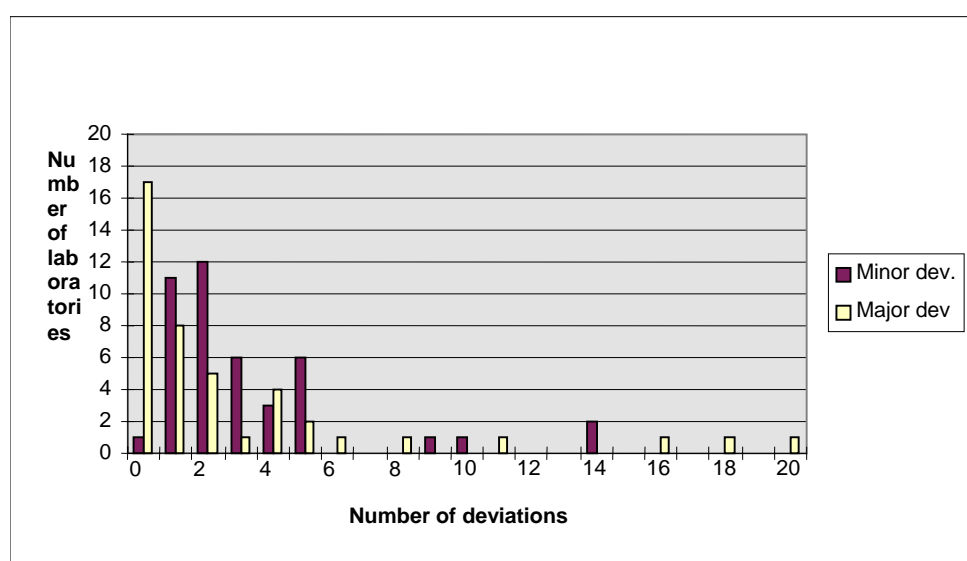


Figure 1. The distribution of laboratories with different numbers of minor and major deviations.

Evaluation of the EQAS

Based on the answers of the EQAS evaluation questionnaires from 33 laboratories, the written materials (the announcement, the EQAS welcoming letter, the EQAS report form and the individual reports) were satisfactory (1%), good (39%) or very good (60%). The organisation of the EQAS, the information of the EQAS and how participating in the EQAS met the expectations of the participants were evaluated as satisfactory (1%), good (16%), very good (54%) or excellent (29%). In addition, the laboratories found it important (23%) or very important (77%) to participate in the EQAS. Only a few laboratories found that the number of tested strains was either too large or too small or that the time for analysing the strains was too short.

Discussion

Nine out of 34 laboratories (26%) serotyped all eight strains correctly and nine (26%) of the laboratories had only one mistake. However, ten laboratories (29%) had difficulties in serotyping five or more of the eight strains. This might be due to a limited antisera of good quality or lack of experience. Since any world-wide surveillance will be based on results from a large number of different laboratories, this strongly emphasise the need for training of these laboratories.

When performing antimicrobial susceptibility testing it is very important to include reference strains for internal quality control. However, 28% of the performed tests with the *E. coli* reference strain were outside the quality control range, showing that the tests were not in perfect control. In some laboratories the obtained zone diameters were much smaller or larger than the expected zone diameters. In some cases, this could be explained by the use of expired disks or by improper incubation conditions. In other cases, the deviations seemed to be caused by a non uniform agar depth or a media pH that was not in agreement with the NCCLS guidelines. According to these guidelines, the agar depth should be 3-4 mm and the pH of the agar after autoclaving should be 7.2-7.4. If quality control strains are routinely included in some laboratories not using these regularly, this is likely to improve the results considerably.

Participation in the EQAS was regarded as very important by nearly all the participants, and several of them did not take part in any other Quality Assurance System of *Salmonella* serotyping. Some laboratories would like to receive help in serotyping or phage typing of different *Salmonella* strains. This is possible, as DVL as a reference laboratory is offering these services free of charge to Global-Salm-Surv members on a limited scale. An agreement between DVL and the laboratory has to be made in advance. Contact: faa@svs.dk. Because of limited financial means, some laboratories had to use antisera or disks with exceeded expiration dates. Indeed, seven laboratories would like the WHO to supply them with antisera of a good quality; some participants also wanted disks and the last issues of the NCCLS guidelines and the Kauffmann-White scheme.

In the future, Global Salm-Surv intends to include all member laboratories in the EQAS. The next cycle of the EQAS will start in the beginning of 2001. The EQAS cycles will likely be repeated once every year. In order to make the evaluation of the EQAS results less time consuming, Web-based reporting of the results will be facilitated. This will also enable the laboratories to compare their results to the correct results and evaluate the performance of the laboratory at once.

Twelve of the participants in the first cycle of the EQAS had attended a WHO laboratory training course on serotyping and antimicrobial resistance in food borne pathogens in Thailand November 1999. The course included serotyping and disk diffusion with emphasis on using a Quality Control strain. These participants had a markedly better score than the rest of the participants (Data not shown). As no tests have been performed before these participants participated in the EQAS it cannot be excluded that the participants in the training course in Thailand in general had better facilities and were more skilled in making these analyses than the rest of the participants. It will be interesting to follow the improvement of the results of the participating laboratories in the next rounds of the EQAS to estimate the benefit of the EQAS.

As only 26% of the laboratories serotyped all eight strains correctly and antimicrobial susceptibility testing revealed 8.3% deviating results, of which 4.5% were minor deviations and 3.8% were major deviations, this EQAS demonstrated a real need both for a quality assurance system and for further training of some of the participants.

