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External Quality Assurance System (EQAS) of the WHO Global *Salmonella* Surveillance and Laboratory Support Project (Global Salm-Surv) Results from 2001

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Abstract

An international collaborative study on serotyping and antimicrobial susceptibility testing of eight *Salmonella enterica* strains was performed to enhance the capacity of national and regional reference laboratories in WHO Global Salm-Surv to conduct *Salmonella* serotyping and antimicrobial susceptibility testing. A total of 103 laboratories in 60 countries participated. For serotyping, 78% of the results were correct. For susceptibility testing, 91% of the results were in agreement with the expected results. However, 17% 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 worldwide, leading to millions of cases of diarrheal illness each year in developed as well as developing countries. Furthermore, there is a growing concern over increasing resistance to antimicrobial therapies in *Salmonella*; recently, the multiresistant *Salmonella* clone "DT104" has spread among several countries and continents. In order to enhance member countries capacities to detect and respond to *Salmonella* problems, as well as to improve global surveillance of *Salmonella*, WHO has recently launched an international *Salmonella* surveillance and laboratory support project - "WHO Global Salm-Surv".

To support laboratories participating in WHO Global Salm-Surv, an External Quality Assurance System (EQAS) has been established. The EQAS supports the assessment of the quality of serotyping and antimicrobial susceptibility testing of *Salmonella* in all participating laboratories.

The EQAS program is organised by the Danish Veterinary Institute (DVI) in collaboration with the WHO and the Centers for Disease Control and Prevention, Atlanta. In 2000 the EQAS was arranged for a limited number of WHO Global Salm-Surv laboratories, namely 44. The EQAS in 2001 included a total 103 WHO Global Salm-Surv laboratories and covered serotyping and susceptibility testing of eight *Salmonella*.

Materials and methods

EQAS was announced on the WHO Global Salm-Surv listserver, and interested laboratories were invited to apply. A total of 115 laboratories were enrolled in the EQAS 2001. In February an official invitation was sent either by e-mail or by fax to all WHO Global Salm-Surv members. The invitations were sent again in March if no answers were received.

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An interactive Web database was established for entry of the laboratories test results through a password protected site at the WHO Global Salm-Surv homepage on the Internet.

Eight *Salmonella* isolates and the *E. coli* reference strain were sent to all laboratories (Table 1). The *Salmonella* strains mainly represented the O:4, O:7, O:8 and O:9 antigenic serogroups. The eight strains had different antimicrobial susceptibility patterns. All strains were lyophilised and packed in double containers, before they were shipped to the participants. The strains were received by the participants within one to fourteen days, with the exception of two parcels which were delivered after approximately one month.

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 2000 were included with the strains (Appendix 2). In addition, the test-form, questionnaire, an evaluation questionnaire, username and password for the interactive Web database were sent by e-mail 1-2 days later.

Laboratories were instructed to subculture strains on agar plates as soon as possible after receipt and store them at refrigerator temperature until the serotyping and susceptibility tests could be performed. The test results were supposed to be recorded on the attached form and entered within 60 days in the EQAS Web database or sent by fax or e-mail to DVI for manual data entry in the database.

Participation in the WHO EQAS was free of charge except for each institution´s own expenses for analysis. The laboratories were requested to use the serotyping methods and susceptibility testing methods routinely performed in the laboratory. The strains were tested against as many as possible of the following antimicrobials: Ampicillin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfonamides, tetracycline, trimethoprim and sulfonamides and trimethoprim in combination.

Immediately after entering the results in the EQAS Web database, an individual report with the obtained zone diameters/MIC values, the obtained and expected results and comments to deviating results was generated by the computer and shown on the screen. If a participant was not able to enter the results or had a slow Internet connection, the results were entered by DVI and sent as files by e-mail to the participant. Afterwards, the participants were still able to access the EQAS Web database with their username and password and see their own evaluation report for 2001.

Results

Ninety-two laboratories recorded antimicrobial susceptibility test results using disk diffusion zone diameters, eight laboratories reported MIC results and three laboratories reported a combination of the two methods.

A total of 103 (90%) of the 115 enrolled laboratories reported their results in the EQAS database, or by e-mail, fax or mail between April 6th and October 9th. No results were received from 12 laboratories. The 103 laboratories were from 60 countries: Argentina, Australia, Bolivia, Bosnia-Herzegovina, Brazil, Cambodia, Canada, Chile, China, Costa Rica, Croatia, Cuba, Cyprus, Czech, Egypt, Estonis, Finland, France, Georgia, germany, Greece, Hungary, Iceland, India, Indonesia, Ireland, Israel, Italy, Japan,

Jordan, Kuwait, Latvia, Lebanon, Madagascar, Malaysia, Malta, Mauritius, Mexico, Moldovia, New Zealand, Oman, Papua New Guinea, Philippines, Poland, Rep. Korea, Romania, Slovenia, South Africa, Spain, Sri Lanka, Thailand, Tunesia, Turkey, United States and Uruguay.

Questionnaire

A mean of 1,346 *Salmonella* strains (range 0-20,258) were analysed yearly in the 57 laboratories who reported their numbers; these strains were either isolated in the laboratory or received from other laboratories for further analyses. On average, 66% (range 0-100%) of *Salmonella* strains were serotyped. Furthermore, an average of 551 strains per laboratory (range 0-5,279) was tested for antimicrobial susceptibility.

Serotyping

Sixty-four laboratories (62%) serotyped all eight isolates, 23 laboratories serotyped less than eight strains and five of these laboratories serogrouped the remaining strains. Eighteen laboratories only serogrouped the isolates. Of these, six laboratories serogrouped eight strains and one laboratory serogrouped seven strains. The remaining 11 laboratories serogrouped less than seven strains. Of the 622 serotyping results, 80% were determined correctly. The mean number of correct serotyping results per laboratory was six (75%) of the eight strains. The results of serotyping are given in Table 1. For the common serotypes *Salmonella* Typhimurium, *Salmonella* Infantis and *Salmonella* Enteritidis, the serotyping results deviated in six, eight and ten cases, respectively (<10% of laboratories). The strains belonging to other less common serotypes were incorrectly serotyped by 18 - 38% of the laboratories.

Table 1. The correct serotype of the tested strains, the number of obtained deviations among the total number of laboratories and the incorrect serotypes identified from each strain.

Strain	WHO2.1	WHO2.2	WHO2.3	WHO2.4	WHO2.5	WHO2.6	WHO2.7	WHO2.8
Serovar	Agona	Dublin	Infantis	Kottbus	Typhimu-rium	Newport	Hadar	Enteritidis
Correct serotype	1,4,[5],12: f,g,s:[1,2]	1,9,12,[Vi]: g,p: -	6,7,14: r: 1,5	6,8: e,h:-	1,4,[5],12: i: 1,2	1,8,20: e,h: 1,2	6,8: z10: e,n,x	1,9,12,[f]:g, m,[p]: [1,7]
Number of deviations	14	25	8	29	6	15	15	10
Number of laboratories serotyping this strain	78	79	81	77	81	79	73	74
List of deviating results[1]	Agona or Derby 1 California 1 Derby 7 Enteritidis 1 Essen 2 Kingston 1	Agona 2 Derby 1 Dublin or Rostock 3 Enteritidis 14 Il neasden 1 Moscow 1 Regent 1 Togo 1 Typhi 1	Colindale 1 Lorrita 1 Oritamerin 1 Thompson 1 Virchow 1	Newport 12 Tshiongwe 10 Ferruch 2 Kalumburu 1 Stourbridge 1 Hidalgo 1 Ferruch or Kottbus 1	Gloucester 1 Kiel 1 Lagos 1 Sandiego 1	Bardo 1 Ferruch 1 Newport or Bardo 1 Rechovot 1 Tshiongwe 8	Chailey 1 Hidalgo 1 Istanbul 1 Mapo 1 Quiniela 1 Tshiongwe 8	Berta 1 Blegdam 1 Dublin 2 Kottbus 1 Seremban 1 Typhimuri-um 1

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

Of the 64 laboratories serotyping all eight *Salmonella* strains, 41 laboratories serotyped seven or eight strains correctly (Table 2).

Table 2. Number of laboratories serotyping 0 to 8 *Salmonella* strains correctly.

Number of correct serotyped strains	Number of laboratories (%) in 2000	Number of laboratories (%) in 2000 (if eight strains were serotyped)	Number of laboratories (%) in 2001	Number of laboratories (%) in 2001 (if eight strains were serotyped)
8	9(27)	9(30)	32(37)	32(50)
7	9(27)	9(30)	13(15)	9(14)
6	3(9)	4(13)	9(10)	9(14)
5	3(9)	1(3)	10(11)	7(11)
4	3(9)	4(13)	4(5)	2(3)
3	2(6)	1(3)	7(8)	2(3)
2	3(9)	1(3)	4(5)	1(1)
1	1(3)	0(0)	4(5)	1(2)
0	1(3)	1(3)	4(5)	1(2)
In total	34	30	87	64

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 guideline M7-A5. On average, 7.3 out of 8.8 tested antimicrobials were inside the QC range (Table 3). In 43 (48%) of laboratories, all the results of the *E. coli* reference strain were within range. The expected MIC values are given in Table 4.

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

Antimicrobial	Percentage of laboratories inside the QA range in 2000 (No. of laboratories[2])	Percentage of laboratories inside the QA range in 2001 (No. of laboratories[2])
Ampicillin	73 (37)	81 (79 of 97)
Chloramphenicol	63 (38)	80 (78 of 97)
Ciprofloxacin	80 (35)	86 (83 of 97)
Gentamicin	77 (39)	88 (87 of 99)
Nalidixic acid	65 (37)	86 (64 of 74)
Kanamycin	81 (36)	86 (75 of 87)
Streptomycin	78 (36)	88 (71 of 81)

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

Sulfonamides	47 (19)	66 (35 of 53)
Tetracycline	58 (42)	78 (75 of 96)
Trimethoprim	70 (31)	78 (39 of 50)
Sulfonamides and trimethoprim		86 (77 of 90)

The numbers of correct antimicrobial susceptibility tests were 83% (763 of 922) for laboratories performing both disk diffusion and/or MIC determinations, 82% (695 of 843) for laboratories performing only disk diffusion and 86% (68 of 79) for laboratories performing only MIC determination.

Table 4. The MIC values and R/I/S categories of the eight *Salmonella* strains and the *E. coli* reference strain in EQAS 2001.

	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)	Tmp/ Sulfa[3]
7278893-2 WHO2.1	≤1 S	8 S	≤0.03 S	≤1 S	≤16 S	≤4 S	8 S	>512 R	≤2 S	≤4 S	≤1 S
9912013-2 WHO2.2	≤1 S	4 S	≤0.03 S	≤1 S	≤16 S	≤4 S	8 S	≤32 S	≤2 S	≤4 S	≤1 S
9961191-4 WHO2.3	>32 R	8 S	≤0.03 S	≤1 S	≤16 S	≤4 S	64 R	>512 R	>32 R	≤4 S	≤1 S
7261632-1 WHO2.4	≤1 S	4 S	0.125 S	≤1 S	≤16 S	>128 R	16 I	≤32 S	≤2 S	≤4 S	≤1 S
7222569-1 WHO2.5	>32 R	>64 R	≤0.03 S	≤1 S	≤16 S	≤4 S	64 R	>512 R	>32 R	≤4 S	≤1 S
98-24475-1 WHO2.6	≥32 R	64 R	≤0.03 S	1 S	>64 R	4 S	≥64 R	≥512 R	≥32 R	≥32 R	>8 R
9973011-2 WHO2.7	≤2 S	4 S	≤0.03 S	≤1 S	≤16 S	8 S	8 S	≤32 S	≤2 S	≤4 S	≤1 S
98-74091-5 WHO2.8	2 S	8 S	≤0.03 S	≥32 R	32 I	4 S	≥64 R	≥512 R	2 S	2 S	≤1 S
E. coli 25922 ATCC	2-8[1] / S	2-8[1] / S	0.004-0.016[1] /S	0.25-1[1] / S	1-4[1] / S	1-4[1] / S	4-16[2]	Sulfisoxazole: 8-32[1] / S	0.5-2[1] / S	0.5-2[1] / S	≤0.5[1]

[1] NCCLS standard, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 5th ed. Approved standard. M7-A5 NCCLS, Wayne, Pennesylvania.
[2] Developed by the manufacturer of the Sensititre® panels.
[3] Values are for trimethoprim.

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 72 susceptibility tests; 66 (91%) of the obtained R/I/S results were in complete agreement with the reference (Table 4).

In the individual report for each laboratory, the deviations were divided into minor and major deviations with comments provided for each. 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 an S - R deviation a major deviation. In total 7,409 antimicrobial susceptibility tests were performed. Of these, 6,753 (91.2%) results were in agreement with the reference, 5.8% were minor deviations and 3.0% were major deviations. The percentage of major deviations for each antimicrobial agent is shown in Table 5.

Table 5. Number of tests and the percentage of major deviations for each antimicrobial agent in 2000 and 2001.

	Ampi-cillin (10 µg)	Chloram-phenicol (30 µg)	Cipro-floxacin (5 µg)	Genta-micin (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)	Trime-thoprim + sulfona mides
Total no of determina-tions in 2000	343	343	334	343	312	328	312	248	335	295	
% major deviations in 2000	6.1	3.8	1.2	5.0	4.5	1.8	3.5	4.8	6.0	2.7	
Total no of determina-tions in 2001	793	785	784	792	595	697	643	412	775	398	728
% major deviations in 2001	4.0	1.8	0.6	1.1	2.0	1.4	7.0	4.4	6.7	1.5	2.1

The distribution of laboratories with different numbers of minor and major deviations is shown in Fig. 1. Forty-three laboratories had no major deviations, while only ten laboratories have no minor deviations. Six laboratories were responsible for 76 of the 221 major deviations.

The percentage of R, I and S results for each strain and antimicrobial are given in Table 6. If the laboratory used disks with a different concentration of antimicrobials than specified in the NCCLS guideline M2-A7, the results are not included in the calculation of the percentage of interpretations as R, I or S.

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

	Ampicil-lin (10 µg)	Chloram fenicol (30 µg)	Cipro-floxacin (5 µg)	Genta-micin (10 µg)	Kanami-cin (30 µg)	Nalidi-xic acid (30 µg)	Strepto-mycin (10 µg)	Sulfon-amides (300 µg)	Tetracy-cline (30 µg)	Trime-thoprim (5 µg)	TMP+ Sulfon-amides (5 µg)
WHO2.1	5/6/**89**	2/1/**97**	1/0/**99**	0/1/**99**	3/7/**91**	1/6/**93**	11/22/**67**	**94**/4/2	6/24/**69**	2/0/**98**	2/0/**98**
WHO2.2	2/3/**95**	1/0/**99**	0/0/**100**	1/1/**98**	1/0/**99**	1/3/**95**	23/29/**49**	10/2/**88**	1/8/**91**	0/2/**98**	0/2/**98**
WHO2.3	**99**/0/1	2/1/**97**	1/0/**99**	0/2/**98**	3/8/**89**	1/3/**95**	**94**/5/1	**94**/4/2	**85**/2/13	2/0/**98**	2/0/**98**
WHO2.4	5/3/**92**	1/0/**99**	0/0/**100**	0/2/**98**	1/8/**91**	**98**/1/1	10/42/**48**	17/4/**79**	6/17/**77**	4/0/**96**	4/0/**96**
WHO2.5	**99**/0/1	**98**/0/2	3/24/**73**	0/2/**98**	3/5/**92**	**99**/0/1	**95**/2/2	**96**/2/2	**98**/1/1	0/2/**98**	0/2/**98**
WHO2.6	**99**/1/0	**99**/0/1	0/0/**100**	2/1/**97**	**99**/0/1	1/6/**93**	**99**/0/1	**100**/0/0	**98**/2/0	**98**/2/0	**98**/2/0
WHO2.7	6/2/**92**	1/0/**92**	0/0/**100**	3/2/**95**	4/11/**85**	2/18/**79**	14/33/**53**	8/4/**88**	5/15/**80**	2/0/**98**	2/0/**98**
WHO2.8	13/6/**81**	4/6/**89**	0/0/**100**	**95**/2/3	46/**35**/18	2/6/**92**	**92**/4/4	**98**/2/0	22/27/**51**	2/0/**98**	2/0/**98**

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

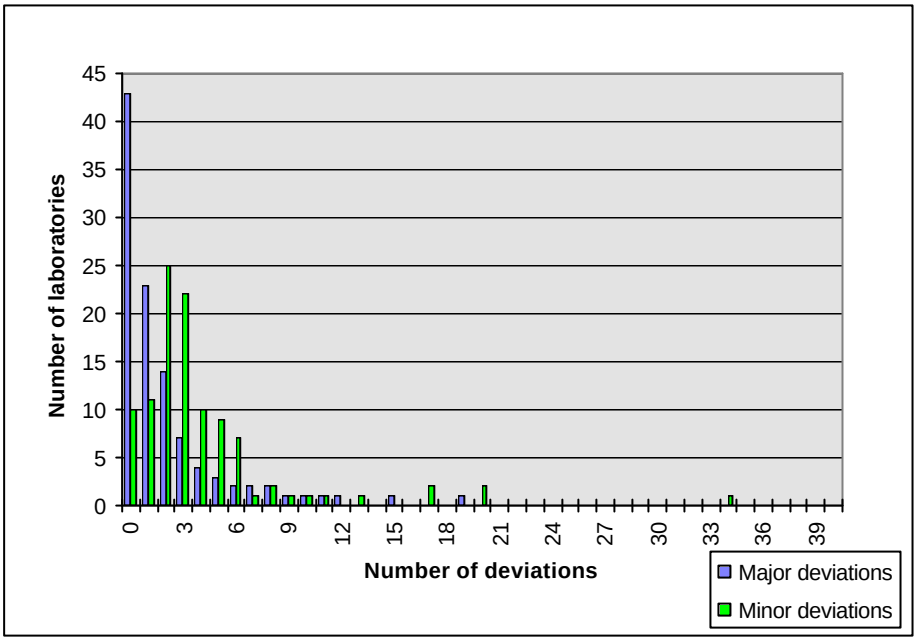

Figure 1. The distribution of laboratories with different numbers of minor and major deviations.

Evaluation of the EQAS

Based on the answers to the EQAS evaluation questionnaires from 33 laboratories, the written materials (the announcement, the EQAS welcoming letter, the EQAS reporting form and the individual reports) were satisfactory (4%), good (36%) or very good (60%). The organisation of the EQAS, the information describing EQAS and how participation met the expectations of the participants and were evaluated as satisfactory (4%), good (35%) or very good (61%). In addition, the laboratories found it important (20%) or very important (80%) to participate in the EQAS. The Web database was evaluated as satisfactory (2%), good (17%) and very good (81%) by 42 participants answering the questionnaire.

Discussion

Thirty-two out of 87 laboratories (37%) serotyped all eight strains correctly and thirteen (15%) of the laboratories had only a single deviation. However, nineteen laboratories (22%) had difficulties in serotyping five or more of the eight strains. This might be due to limited antisera of good quality or lack of experience. This strongly emphasises 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, 17% 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 approximately 4 mm and the pH of the agar after autoclaving should be 7.2-7.4. If quality control strains were routinely included in the laboratories that doesn't use them regularly, it is likely to improve the results considerably.

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In general, laboratories that tested low numbers of strains in 2000 had a poorer performance compared to laboratories that tested many strains, indicating that routine and experience plays an important role in insuring consistency and quality in the conduct of laboratory tests.
Participation in the EQAS was regarded as very important by nearly all the participating laboratories.

In the next EQAS, WHO Global Salm-Surv intends to invite all WHO Global Salm-Surv laboratoriesto participate. The next EQAS had commenced early 2002. Web based data entry of the EQAS results will also be available in 2002 to enable the laboratories to receive feedback on their performance immediately.

Only 27% of the laboratories serotyped all eight strains correctly and antimicrobial susceptibility testing revealed 8.8% deviating results, of which 5.8% were minor deviations and 3.0% were major deviations. Therefore, this EQAS demonstrated a real need both for a quality assurance system and for further training of the laboratories participating in GSS.