WHO Collaborating Center for Foodborne Disease Surveillance Atlanta, USA

World Health Organization Department of Communicable Disease Surveillance and Response Geneva, Switzerland

Danish Veterinary Institute Copenhagen, Denmark

Pasteur Institute, Paris, France

Health Canada, Guelph, Ontario, Canada

Results from EQAS 2002 - the External Quality Assurance System 2002 of the WHO Global *Salmonella* Surveillance and Laboratory Support Project (Global Salm-Surv)

Petersen A., Aarestrup F. M., Jensen A. B., Lo Fo Wong D., Evans M. C., Angulo F., Imhoff B., Binsztein N, Braam P. , Jouan M., Wegener H. C. and Seyfarth A.M.

ISBN: 978-87-92158-40-6

February 2003

Abstract

An international external quality assurance program 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 (WHO GSS). In 2002 a total of 117 laboratories from 67 countries participated. For serotyping, almost 90 % of the results were correct. For susceptibility testing, 91 % of the results were in agreement with the expected results, and 86 % of the performed tests with the reference strain *E. coli* ATCC 25922 were inside the quality control range specified by NCCLS guidelines.

Comparing the results of EQAS 2002 to the results from 2000 and 2001 showed a clear improvement in the laboratories capacity to accurately serotype and susceptibility test *Salmonella*. We attribute this improvement to the training provided by WHO GSS, the EQAS and the high-quality *Salmonella* typing antisera, provided to most participants as a part of the WHO GSS.

Introduction

Salmonella is one of the most important foodborne pathogens worldwide, leading to millions of cases of diarrheal illness each year in developing as well as industrialized countries. Furthermore, there is a growing concern for the increasing resistance to antimicrobial therapies in *Salmonella*. Recently, the multiresistant *Salmonella* clone "DT104" has spread among several countries and continents. In addition infections with resistant *Salmonella* are associated with increased morbidity and mortality.

In order to enhance the member countries capacity to detect and respond to *Salmonella* problems, as well as to improve global surveillance of *Salmonella*, WHO launched an international *Salmonella* surveillance and laboratory support project in January 2000, the "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 participating laboratories.

The EQAS program is organised by the Danish Veterinary Institute (DVI) in collaboration with WHO, Institut Pasteur and Centers for Disease Control and Prevention in Atlanta and has been

performed annually since 2000. Each EQAS has so far involved serotyping and susceptibility testing of eight *Salmonella* strains.

The first EQAS in 2000 was arranged for only a limited number of WHO Global Salm-Surv laboratories, 44. In 2001 the EQAS covered 103 laboratories and finally the EQAS in 2002 included a total of 117 WHO Global Salm-Surv laboratories.

Materials and methods

The EQAS was announced on the WHO Global Salm-Surv listserver, and laboratories not previously participating were encouraged to apply. A total of 132 laboratories were enrolled in the EQAS 2002.

In 2001 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. In 2002 this database was extended to make entry of the answers of an evaluation questionnaire possible as well.

Eight *Salmonella* isolates and an *E. coli* reference strain were sent to all laboratories. The *Salmonella* strains represented different serogroups (Table 1) and antimicrobial susceptibility patterns (Table 4). All strains were shipped as stab cultures according to the IATA Dangerous Goods Regulations, 43rd ed. 2002 for shipment of infectious substances affecting humans. A test form for results and a questionnaire with general questions about methods, yearly numbers of isolations etc. was enclosed.

An evaluation questionnaire and information about username and password for the Web database were sent by e-mail together with further information about AWB number, flight company, expected arrival etc.

Laboratories were instructed to subculture strains on agar plates as soon as possible after receipt and store them at refrigerator temperature. The test results were requested 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.

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 and susceptibility testing methods routinely performed in the laboratory. The strains were tested against as many as possible of the following antimicrobials: Ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), gentamicin (Gen), kanamycin (Kan), nalidixic acid (Nal), streptomycin (Str), sulphonamide (Su), tetracycline (Tet), trimethoprim (Tmp) and finally the combination of sulphonamide and trimethoprim (T/S).

Immediately after entering the results in the Web database, an individual evaluation report on obtained and expected results with comments to deviating results was generated by Oracle Portal software and displayed on the screen. If a participant was not able to enter the results, this was done by DVI.

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Results

A total of 117 (88.6 %) of 132 laboratories enrolled in the EQAS 2002 reported their results. Of the 15 laboratories not reporting their data, at least four laboratories never received their strains because of problems in customs.

The 117 laboratories represented 67 countries: Albania, Argentina, Australia, Bolivia, Bosnia-Herzegovina, Botswana, Brazil, Bulgaria, Cambodia, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, Czech, Dom. Rep. of Congo, Denmark, Ecuador, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, India, Indonesia, Ireland, Israel, Italy, Japan, Jordan, Korea, Kuwait, Latvia, Lebanon, Lithuania, Madagascar, Malaysia, Malta, Morocco, Mauritius, Mexico, Moldovia, Netherlands, New Zealand, Oman, Papua New Guinea, Peru, Philippines, Poland, Romania, Senegal, Slovakia, Slovenia, South Africa, Spain, Sri Lanka, Thailand, Tunisia, Turkey, Uruguay, Venezuela and Vietnam.

A mean of 1,413 *Salmonella* strains (range 1-25,976) were analysed yearly in the 63 laboratories who reported their numbers. On average, 82 % (range 0-100%) of the *Salmonella* strains were serotyped.

Serotyping

A total of 97 laboratories (82.9 %) performed serotyping. Of these, 78 laboratories (80.4 %) serotyped all eight strains. Nine laboratories performed serogrouping or incomplete typing.

Of 723 serotyping results, 648 (89.6 %) were determined correctly. The results of serotyping are given in Table 1. The number of deviations range from 4 % for *Salmonella* Typhimurium to 17 % for *Salmonella* Virchow. For the common serotypes *Salmonella* Typhimurium, *Salmonella* Derby and *Salmonella* Enteritidis incorrect results were reported in four, six and seven percent of cases, respectively.

With a serotyping reaction of strain WHO3.4 as 1,3,19:g,s,t:- it is not possible to distinguish *Salmonella* Senftenberg from *Salmonella* Dessau. In this case it is normal practice to record the result as *Salmonella* Senftenberg. Therefore, in cases where *Salmonella* Dessau was reported, results were not recorded as deviations.

Of 97 laboratories serotyping, 52 % correctly serotyped all eight strains, and further 32 % had seven or six strains correctly serotyped. Table 2 shows the number of laboratories with respectively 0, 1, 2,..,8 correct serotypings in 2002 compared to previous years.

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Table 1. List of *Salmonella* serotypes sent to the participants and number and types of deviations.

Strain	Correct serotype		Labs sero-typing the strain	% devi-ations	List of deviating results
WHO 3.1	Virchow	6,7: r: 1,2	95	17	Infantis 9, Colindale 5, Galiema 1, Senegal 1, Thompson 1
WHO 3.2	Derby	1,4,[5],12: f,g: [1,2]	91	6	Agona 3, Essen 1, Fyris 1, Kiel 1
WHO 3.3	Weltevreden	3,10,[15]: r: z6	91	6	Elisabethville 3, Seegefeld 1, Ughelli 1, Wilmington 1
WHO 3.4	Senftenberg	1,3,19: g,[s],t: -	89	12	Westhampton 6, Catanzaro 1, Maiduguri 1, Kingston 1, Rideau 2, Suberu 1
WHO 3.5	Typhimurium v. Copenhagen	1,4,12: i: 1,2	96	4	Agona 1, Choleraesuis 1, Kingston 1, Lagos 1
WHO 3.6	Manhattan	6,8: d: 1,5	90	12	Blockley 1, Bovismorbificans 1, Duesseldorf 1, Dunkwa 2, Isangi 2, Kottbus 1, Muenchen 2, Newport 1, Yovokome 1
WHO 3.7	Enteritidis	1,9,12,[f]:g,m,[p]: [1,7]	95	7	Dublin 2, Essen 1, Kapemba 1, Nitra 1, Senftenberg 1, Typhimurium 1
WHO 3.8	Bovismorbificans	6,8,20:r,[i]: 1,5	92	8	Hidalgo 1, Hindmarsh 4, Infantis 1, Kentucky 1, Tallahassee 1

Table 2. Number of correct serotypings in relation to number of laboratories for EQAS 2002 compared to previous years.

Number of correct serotypes	EQAS 2000		EQAS 2001		EQAS 2002	
	Number of laboratories		Number of laboratories		Number of laboratories	
	n	%	n	%	n	%
8	9	26	32	37	50	52
7	9	26	13	15	17	18
6	3	9	9	10	14	14
5	3	9	10	11	3	3
4	3	9	4	5	2	2
3	2	6	7	8	3	3
2	3	9	4	5	6	6
1	1	3	4	5	1	1
0	1	3	4	5	1	1
In total	N = 34	100 %	N = 87	100 %	N = 97	100 %

Logistic regression analysis was performed to test, whether there was a significant difference in over-all performance between 2001 and 2002. Dependent variable was defined as number of laboratories with certain number of correct answers out of total number of participating laboratories. Independent variable was an interaction term between number of correct answers and year of participation. There was a significant increase in number of laboratories correctly identifying all 8 serotypes from 2001 to 2002 (p=0.0437).

Antimicrobial susceptibility testing

A total of 117 laboratories reported their susceptibility data. Of these, 105 laboratories performed disk diffusion and 6 laboratories MIC-determinations. Six laboratories reported both methods.

If testing is correctly standardized and performed, the results for the *E. coli* ATCC 25922 reference strain are supposed to be inside the quality control (QC) ranges specified by NCCLS. In 52 % of the laboratories, all the results for the *E. coli* reference strain were within range. For the remaining laboratories a mean of 2.5 tests were out of range.

QC ranges and number of laboratories inside range compared to previous years are shown in Table 3. Of 1,022 reference tests performed, 86.3 % (882) were within range.

Table 3. Results within the NCCLS QC range for reference strain *E. coli* ATCC 25922.

Anti-microbial	QC range[1] *E. coli* ATCC 25922		Laboratories inside QC range		
			EQAS 2000	EQAS 2001	EQAS 2002
	MIC (ug/ml)	Disks (mm)	% (N)[3]	% (N)[3]	% (N)[3]
Amp	2-8	16-22	73 (37)	81 (97)	84 (109)
Chl	2-8	21-27	63 (38)	80 (97)	85 (107)
Cip	0.004-0.016	30-40	80 (35)	86 (97)	86 (108)
Gen	0.25-1	19-26	77 (39)	88 (99)	88 (108)
Kan	1-4	17-25	81 (36)	86 (87)	89 (79)
Nal	1-4	22-28	65 (37)	86 (74)	86 (102)
Str	4-16[2]	12-20	78 (36)	88 (81)	89 (82)
Su	8-32	15-23	47 (19)	66 (53)	74 (57)
Tet	0.5-2	18-25	58 (42)	78 (96)	87 (102)
Tmp	0.5-2	21-28	70 (31)	78 (50)	89 (66)
T/S	=0.5/9.5	23-29		86 (90)	88 (102)

[1] *NCCLS standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing; 12th Informational suppl. NCCLS document M100-S12, Wayne, Pennesylvania.*

[2] *QC range developed by the manufacturer of Sensititre â*

[3] *The number of laboratories performing the test*

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The results of antimicrobial susceptibility testing of eight Salmonella strains were categorised as resistant (R), intermediate (I) or susceptible (S) according to the breakpoints normally used in the laboratories. The expected resistance pattern for the strains are listed in Table 4, and the results (percentage of R/I/S) for each strain and antimicrobial are given in Table 5.

Table 4. Expected resistance pattern for the *Salmonella* strains EQAS 2002.

Strain	Resistance pattern	Strain	Resistance pattern
WHO 3.1	Chl Nal Str Su Tet Tmp T/S	**WHO 3.5**	Amp Chl Gen Kan^{I} Nal Str Su Tet Tmp T/S
WHO 3.2	Amp Chl Nal Str Su Tet	**WHO 3.6**	None resistance
WHO 3.3	Chl Str Su Tet Tmp T/S	**WHO 3.7**	Chl Gen^{I} Str^{I} Su Tet
WHO 3.4	Str^{I} *)	**WHO 3.8**	Str Su Tet Tmp T/S

*) *Intermediary resistance*

Table 5. Susceptibility test results (% R/I/S) of eight *Salmonella* strains in 117 laboratories.

Strain	Amp	Chl	Cip	Gen	Kan	Nal	Str	Su	Tet	Tmp	T/S
WHO3.1	3/1/**96**	**100**/0/0	1/3/**96**	1/2/**97**	1/2/**96**	**100**/0/0	**87**/9/3	**100**/0/0	**97**/0/3	**99**/0/1	**100**/0/0
WHO3.2	**98**/0/2	**100**/0/0	1/6/**94**	1/1/**98**	2/9/**89**	**99**/0/1	**95**/5/0	**100**/0/0	**95**/1/4	0/0/**100**	**40**/20/40
WHO3.3	1/2/**97**	**94**/4/2	0/1/**99**	1/1/**98**	1/1/**98**	1/1/**98**	**48**/43/9	**100**/0/0	**98**/1/1	**97**/1/1	**100**/0/0
WHO3.4	1/3/**96**	1/2/**97**	0/1/**99**	2/1/**97**	1/5/**94**	0/7/**93**	14/**44**/42	13/3/**83**	8/16/**76**	1/0/**99**	3/1/**96**
WHO3.5	**96**/0/4	**98**/0/2	1/9/**90**	**67**/17/17	**66**/28/6	**98**/0/2	**79**/17/3	**100**/0/0	**99**/0/1	**94**/0/6	**98**/0/2
WHO3.6	3/1/**96**	4/1/**95**	0/1/**99**	1/0/**99**	1/4/**95**	6/6/**89**	14/41/**45**	12/3/**85**	7/13/**81**	6/0/**94**	5/1/**94**
WHO3.7	4/1/**95**	**94**/2/4	1/1/**98**	**66**/15/19	4/9/**88**	3/3/**94**	30/**41**/29	**97**/0/3	**97**/1/2	4/0/**96**	5/4/**91**
WHO3.8	6/1/**93**	3/3/**94**	0/1/**99**	2/1/**97**	1/5/**94**	3/5/**92**	**91**/6/3	**100**/0/0	**98**/1/1	**99**/1/0	**99**/0/1

Bold: Expected interpretation. Grey cell: < 90 % hit correct interpretation.

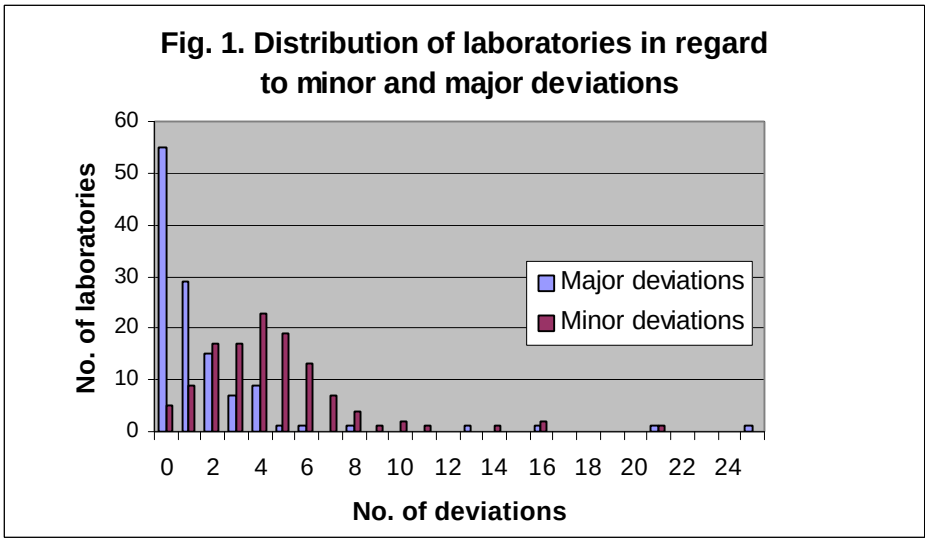
In total 8,554 antimicrobial susceptibility tests were performed. Of these, 91.2 % (7,799) were in agreement with the expected results, 6.4 % were minor deviations and 2.5 % were major deviations.

Results were regarded as deviations if they were incorrectly interpreted as resistant, intermediate or sensitive. I-S or I-R deviations were called minor deviations, while S-R or R-S deviations were called major. In the individual evaluation reports major deviations were further devided into very major (measuring sensitive when resistant) or just major deviations (measuring resistant when sensitive). This further specification is not included in this report. The percentage of major deviations for each antimicrobial agent is shown in Table 6 for year 2002 and previous years.

The distribution of laboratories in regard to the number of minor and major deviations is shown in Fig. 1. All in all, 55 laboratories had no major deviations and 5 laboratories had no deviations at all. Six laboratories were responsible for 89 of the 210 major deviations.

Table 6. Number of tests and percentage of major deviations for each antimicrobial.

Anti-microbial	EQAS 2000		EQAS 2001		EQAS 2002	
	Total no. of determinations	% major deviations	Total no. of determinations	% major deviations	Total no. of determinations	% major deviations
Amp	343	6.1	793	4.0	918	2.9
Chl	343	3.8	785	1.8	911	1.8
Cip	334	1.2	784	0.6	911	0.5
Gen	343	5.0	792	1.1	905	2.8
Kan	312	4.5	595	2.0	680	1.5
Nal	328	1.8	697	1.4	893	2.1
Str	312	3.5	643	7.0	734	4.2
Su	248	4.8	412	4.4	503	3.6
Tet	335	6.0	775	6.7	869	3.3
Tmp	295	2.7	398	1.5	507	3.0
T/S			728	2.1	731	2.3



Evaluation of the EQAS by participating laboratories

The evaluation of the EQAS program was based on a respons of 61 laboratories to the EQAS evaluation questionnaire. Written materials (announcement, welcoming letter, reporting form and individual evaluation reports) were evaluated as satisfactory (5 %), good (42 %) and very good (52 %). Organisation of the EQAS, information describing EQAS and fulfilment of expectations for the participants were evaluated as satisfactory (5 %), good (40 %) or very good (55 %). In addition, 29 % of the laboratories found it important and 71 % found it very important to participate in the EQAS. The Web database was evaluated as satisfactory (8 %), good (35 %) and very good (58 %).

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Discussion

This year more than half of the laboratories (52 %) serotyped all eight strains correctly and further 18 % had only one single serotype deviation. The percentage of correct serotyping results was 90 % compared to 80 % in EQAS 2001. These results indicate an improvement in the ability of serotyping compared to last year in spite of increasing difficulty. Improvement is partly believed to be a consequence of providing last years participants in WHO Global Salm-Surv training courses with small amounts of high-quality antisera. Of course, we hope that attendance at the training courses and repeated participation in EQAS also contributed to improving performance of individual laboratories.

The percentage of correct antimicrobial susceptibility test results was 91.2 % -exactly the same as last year. Most participants had very few deviations, while a few laboratories had a lot of deviations. In some cases, this could be explained by use of expired disks or use of disks with low potency.

Deviations were especially frequent for testing of aminoglycosides, tetracycline and suphonamides. Among others, testing of these antimicrobials is known to be highly influenced by variations in media conditions such as cationic concentration, acidity and agar depth (specified by the NCCLS guidelines). Also misreading of sulphonamide- and trimethoprim results because of the delayed bacterial response to these antimicrobials (viewed as pinpoint growth inside inhibition zone or continuous growth after MIC endpoint) may have influenced the outcome.

When performing antimicrobial susceptibility testing, it is very important to include reference strains for internal quality control. The fact that 15 % of the performed tests with the *E. coli* reference strain were outside the quality control range shows that the tests were not in perfect control in all laboratories. Routine testing of quality control strains in laboratories not using them regularly would probably improve results considerably.

In general, laboratories reporting high numbers of strains tested yearly seem to have a better performance compared to laboratories testing only few strains, indicating that routine and experience plays an important role in insuring consistency and quality in the conduct of laboratory tests.

We are looking forward to the next round of EQAS in 2003 and hope for just as many participants or even more. We are pleased to experience that participation was regarded as very important by nearly all the participating laboratories.