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Results from the WHO GSS EQAS 2004

- The External Quality Assurance System of the WHO Global *Salmonella* Surveillance and Laboratory Support Project

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Abstract

An international external quality assurance program on serotyping and antimicrobial susceptibility testing of *Salmonella enterica* subsp. *enterica* strains is performed yearly to enhance the capacity of national and regional reference laboratories in the WHO Global *Salmonella* Surveillance and Laboratory Support Project (WHO Global *Salm*-Surv). Today, the project, launched in 2000, also includes other types of food borne pathogens than *Salmonella.* In 2004, the main part of the Regional Centres of WHO Global *Salm*-Surv established around the world, was involved in organizing the EQAS and shipping the strains to participants in their region.

In 2004, a total of 156 laboratories from 82 countries participated. When testing of the eight *Salmonella* strains, 80.9 % of all serotypings and 93.0 % of all susceptibility tests performed were correct. The performance was at the same level as in 2003. The results and comments from the participants indicate that the primary barrier for serotyping is lack of antisera and/or lack of high-quality antisera. Misreading of the Kauffmann-White scheme also seems to play a role.

The number of laboratories submitting Quality Control (QC) data for antimicrobial susceptibility testing (87% of the participants) and the number of correct QC testing results (89 %), have further improved compared to previous years. This indicates an increasing awareness of the importance of QC. Anyhow, inadequate standardization of the method used is still considered a barrier for high quality antimicrobial susceptibility data for at least 44% of the participating laboratories.

For identification of two strains of *Campylobacter*, a total of 109 laboratories participated and 83.6 % of all identifications were correct. For the blinded strain (*Shigella flexneri*) a total of 121 laboratories participated and 75.2 % submitted correct species identification. The results strongly indicate a need for protocols and quality assurance programs for identification procedures of other human pathogens.

It is concluded, that further improvement of global serotyping and antimicrobial susceptibility testing requires access to high-quality antisera and continuing focus on internal QC, and that there is a need for expanding the WHO Global *Salm*-Surv operational area to include all pathogens of human importance together with standardization of the methods needed for identification and typing.

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Introduction

In 2000, WHO launched an international *Salmonella* surveillance and laboratory support project, WHO Global *Salm*-Surv, in order to enhance the member countries capacity to detect and respond to *Salmonella* problems, as well as to improve the global surveillance of *Salmonella.* Today the WHO Global *Salm*-Surv embraces other important foodborne pathogens as well, and Regional Centers have been established around the world.

To support laboratories within the WHO Global *Salm*-Surv, an External Quality Assurance System (EQAS) is organized yearly. The EQAS supports the assessment of the quality of serotyping and antimicrobial susceptibility testing of *Salmonella* in participating laboratories. In 2003, the EQAS was extended to include *Campylobacter* and other food borne pathogens.

Salmonella and *Campylobacter* are among the most important food borne pathogens worldwide, leading to millions of cases of diarrhoeal illness every year in developing as well as industrialized countries. Furthermore, there is a growing concern for the increasing resistance to antimicrobial therapies in *Salmonella*. Infections with resistant *Salmonella* and *Campylobacter* are known to be associated with increased morbidity and mortality.

The EQAS 2004 was organized by the Danish Institute for Food and Veterinary Research, DFVF, in collaboration with Centers for Disease Control and Prevention, CDC, in Atlanta, WHO in Geneva, Institute Pasteur in Paris, the Public Health Agency in Canada and the WHO GSS Regional Centers.

Materials and methods

The EQAS 2004 was announced at the WHO Global *Salm*-Surv list server, and all interested laboratories were encouraged to sign up. A total of 180 laboratories were enrolled. Participation was free of charge except for each laboratories own expense for the analysis.

Bacterial strains were selected by the DFVF, except for the blank bacterial isolate selected and provided by the Public Health Agency in Canada. After repeated testing, the strains were sent to Institute Pasteur and CDC for verification of serotypes and resistance profiles, respectively. Shipping cultures were prepared; *Campylobacter* as lyophilized cultures and the remaining strains as stab cultures. Purity control and re-testing of the strains was performed. Finally, the strains were packed and shipped according to the IATA regulations of Dangerous Goods classified as “UN2814 Infectious substances, affecting humans”. Protocols were enclosed.

In 2004, the following Regional Centres (RC) and Steering Committee members supported the shipping by taking care of the distribution to participants in their own region: RC China in Beijing distributed to the Chinese participants, RC Thailand in Bangkok covered most of the Asian participants, Institute Pasteur shipped to the French speaking African countries, and CDC distributed the strains to the US and Canadian participants. The Public Health Agency of Canada shipped the strains to all the Caribbean and South- and MidAmerican countries, and the shipping in these regions was coordinated with the PAHO-EQAS in order to harmonize the two EQAS systems.

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The laboratories were requested to subculture the isolates upon arrival, to store the cultures at refrigerator temperature and to use the testing methods routinely used at their laboratory. A protocol for recovering lyophilized cultures was included.

The testing included:

1) Serotyping and susceptibility testing of eight *Salmonella* strains
2) Susceptibility testing of the included Quality Control strain *E. coli* ATCC 25922
3) Species identification of two thermophilic *Campylobacter* strains
4) Species identification of a blinded strain of non-toxin producing *Shigella flexneri* type 2a.

The results were submitted through a password protected Web database at the WHO Global *Salm*-Surv homepage. Immediately after data entry, an individual evaluation report with comments on deviating results was displayed on the screen. If participants were not able to enter their results, it was done by the DFVF. New participants were encouraged to fill in a questionnaire with general information about their laboratory.

The *Salmonella* serotypes included in the EQAS 2004 and the corresponding antimicrobial susceptibility patterns are presented in Table 1 and 4, respectively.

For antimicrobial susceptibility testing new compounds were included in EQAS 2004 in order to increase the awareness of the emergence and spread of Extended Spectrum Beta-Lactase (ESBL) producing strains of *Salmonella,* and of course to enhance the skills of detecting these strains. The strains were tested against as many as possible of the following antimicrobials: Ampicillin (Amp), amoxicillin/clavulanic acid 2:1 (Amx/Cl), cefotaxime (Ctx), chloramphenicol (Chl), ciprofloxacin (Cip), gentamicin (Gen), nalidixic acid (Nal), streptomycin (Str), sulphonamide (Su), tetracycline (Tet), trimethoprim (Tmp) and the combination of trimethoprim/sulphonamide 1:19 (T/S).

Results

Participants

A total of 156 of 180 enrolled laboratories (86,7 %) participated and submitted their results.

The 156 participating laboratories represented 82 countries: Albania, Argentina, Australia, Barbados, Belarus, Bosnia and Herzegovina, Brazil, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Costa Rica, Croatia, Cuba, Cyprus, Czech Republic, Democratic Republic of Congo, Denmark, Ecuador, Egypt, Finland, France, Georgia, Germany, Greece, Guatemala, Hungary, Iceland, India, Indonesia, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Jordan, Korea, Kuwait, Latvia, Lithuania, Luxembourg, Macedonia, Madagascar, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, Netherlands, New Zealand, Nicaragua, Oman, Paraguay, Peru, Philippines, Poland, Romania, Scotland, Senegal, Serbia and Montenegro, Seychelles, Singapore, Slovakia, Slovenia, Spain, Sri Lanka, Taiwan, Thailand, Trinidad and Tobago, Tunisia, Turkey, Ukraine, Uruguay, USA, Venezuela, Vietnam.

Of the 156 participating laboratories, 70 % also participated in 2003, and the same level of repeated participation was observed from 2002 to 2003.

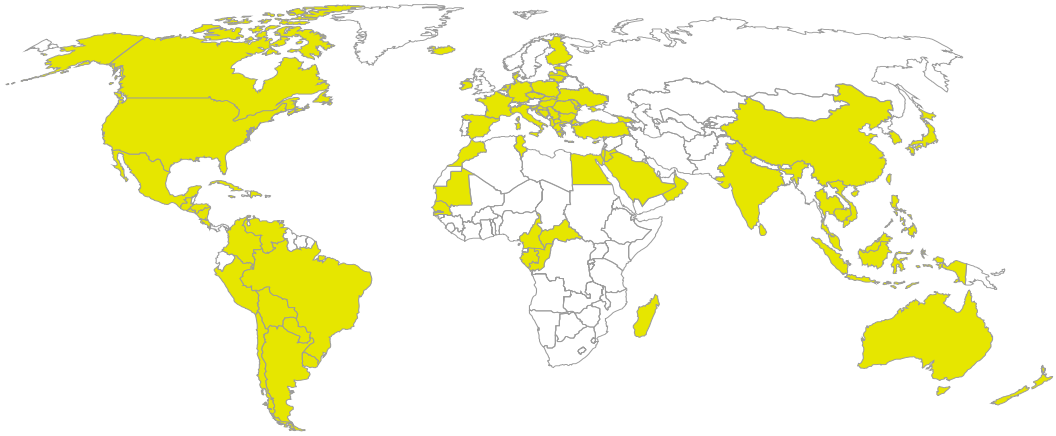

Figure 1. EQAS participation 2004

Serotyping

A total of 141 laboratories (90.4%) participated on serotyping or serogrouping. Of these, a total of 127 laboratories (90.1 %) performed at least one serotyping, and 14 laboratories (9.9 %) performed serogrouping or incomplete typing with no specification of the serovar. Of the 127 serotyping laboratories, 74 laboratories (58.3 %) reported serotyping results on all eight strains.

Of 867 serotypings, 701 results (80.9 %) were correct. Table 1 presents the serotyping results for each strain including a list of the deviations. Number of deviations ranged from 5.8 % for *Salmonella* Enteritidis to 37.9 % for *Salmonella* Chester.

Table 2 shows the number of laboratories with respectively 0, 1, 2,..,8 correct serotypings in 2004 compared to previous years. Of 127 serotyping laboratories, 41 (32.3 %) performed correct serotyping of all strains, and further 14 laboratories (11.0 %) had seven correctly serotyped strains.

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Table 1. List of *Salmonella* serotypes and deviations, 2004.

Strain	Correct serotype		No. of labs serotyping	% deviations	Deviating results (frequency presented if the deviation appears more than once)
WHO 5.1	Give	3,10[15][15,34]:[d]lv:1,7	114	25.4 %	London (9), Meleagridis (3), Parkroyal (3), Joal (2), Nchanga (2), Anatum, Assinie, Cannonhill, Elisabethville, Give/Newbrunswick, Litchfield, Nyborg, Ruzizi, Sinchew, Sinstorf
WHO 5.2	Braenderup	6,7,14:eh:enz$_{15}$	113	7.1 %	Larochelle (2), Norwich (2), Larose, Lomita, Newport, Sanjuan
WHO 5.3	Corvallis	8,20:z$_4$z$_{23}$:[z$_6$]	90	20.0 %	Chailey (5), Dabou (4), Albany (2), Albany/Corvallis, Ackwepe, Breda, Hindmarsh, Kallo, Kentucky, Noya
WHO 5.4	Heidelberg	1,4,[5],12:r:1,2	119	16.0 %	Magumeri (2), Remo (2), Typhimurium (2), Winneba (2), Africana, Agona, Fayed, Hidalgo, Kalamu, Kiel, Ljubljana, Paratyphi B, Saintpaul, Sandiego, Schwarzengrund
WHO 5.5	Chester	1,4,[5],12:eh:enx	116	37.9 %	Sandiego (27), Saintpaul (5), Abortusequi (3), Agona, Goldcoast, Haîfa, Kaapstad, Magumeri, Paratyphi B, Sarajane, Tennyson, Texas
WHO 5.6	Corvallis	8,20:z$_4$z$_{23}$:[z$_6$]	88	22.7 %	Albany (4), Dabou (4), Chailey (3), Bellevue (2), Newport (2), Rechovot (2), Bardo, Bovismorbificans, Corvallis/Albany
WHO 5.7	Mbandaka	6,7,14:z$_{10}$:enz$_{15}$	106	19.8 %	Djugu (6), Menden (4), Redba (2), Gabon, Infantis, Lindenburg, Lockleaze, Namibia, Omuna, Paratyphi C, Thompson
WHO 5.8	Enteritidis	1,9,12:gm:-	121	5.8 %	Blegdam (3), Berta, Gallinarum, Goverdhan, Typhi

Table 2. Number of correct serotypings in relation to number of laboratories, 2000-2004.

Number of correct serotypes	EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004	
	No of labs		No of labs		No of labs		No of labs	
	N	%	N	%	N	%	N	%
8	32	37	50	52	32	26	41	32.3
7	13	15	17	18	15	12	14	11.0
6	9	10	14	14	18	14	16	12.6
5	10	11	3	3	23	18	16	12.6
4	4	5	2	2	14	11	11	8.7
3	7	8	3	3	12	10	10	7.9
2	4	5	6	6	3	2	10	7.9
1	4	5	1	1	5	4	5	3.9
0	4	5	1	1	3	2	4	3.1
In total	87	100 %	97	100 %	125	100 %	127	100 %

Antimicrobial susceptibility testing

A total of 151 reported their susceptibility data. Disk diffusion was performed in almost 90 % of the laboratories, while E-test and broth MIC-determination was performed in 1 and 15 laboratories, respectively. One laboratory submitted data on both methods, but only data on the routinely used method (disk diffusion) is presented in this report.

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. 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.

The expected resistance patterns for the strains are listed in Table 3. The results (percentage of R/I/S) for each strain and antimicrobial are presented in Table 4, where figures in bold indicate the expected interpretation, and grey cells indicate where < 90 % of the results hit correct interpretation.

Table 3. Expected resistance for the *Salmonella* strains, EQAS 2004.

Strain	Expected resistance*	Strain	Expected resistance*
WHO 5.1	Str^{IR}	**WHO 5.5**	Sensitive to all tested antimicrobials
WHO 5.2	Amp^{R} Chl^{R} Str^{I} Tet^{R} Su^{R} Tmp^{R} T/S^{R}	**WHO 5.6**	Amp^{R} Ctx^{I} Chl^{R} Gen^{R} Nal^{R} Str^{R} Su^{R}
WHO 5.3	Sensitive to all tested antimicrobials	**WHO 5.7**	Tet^{R}
WHO 5.4	Amp^{R} Amx/Cla^{R} Ctx^{I} Chl^{R} Str^{R} Su^{R} Tet^{R}	**WHO 5.8**	Gen^{R} Str^{R} Su^{R}

** As determined by the DFVF using Sensititre (microdilution MIC) or E-test (cefotaxime). All results verified by the CDC.*

Table 4. Susceptibility test results (% R/I/S) of the *Salmonella* strains in 151 laboratories, 2004

Strain	Amp	Amx/Cl	Ctx	Chl	Cip	Gen	Nal	Str	Su	Tet	Tmp	T/S
5.1	3/3/**94**	1/1/**98**	0/3/**97**	1/0/**99**	0/0/**100**	2/2/**96**	3/4/**93**	**27**/**49**/24	8/7/**85**	6/6/**88**	1/0/**99**	3/0/**97**
5.2	**95**/0/5	13/10/**77**	1/1/**98**	**93**/1/6	1/0/**99**	5/1/**94**	2/2/**96**	26/**50**/24	**95**/0/5	**96**/0/4	**96**/0/4	**93**/0/7
5.3	2/1/**97**	0/1/**99**	1/2/**97**	1/1/**98**	0/0/**100**	1/1/**98**	2/4/**94**	4/16/**80**	8/4/**88**	6/8/**86**	0/1/**99**	1/0/**99**
5.4	**97**/0/3	**93**/3/4	19/**70**/11	**99**/0/1	0/0/**100**	1/0/**99**	0/3/**97**	**97**/3/0	**99**/0/1	**99**/0/1	1/1/**98**	5/0/**95**
5.5	4/2/**94**	2/1/**97**	1/4/**95**	1/0/**99**	0/1/**99**	1/1/**98**	1/3/**96**	3/8/**89**	7/1/**92**	6/6/**88**	2/0/**98**	2/0/**98**
5.6	**99**/0/1	21/31/**48**	58/**31**/11	**99**/0/1	2/1/**97**	**98**/1/1	**98**/1/1	**98**/1/1	**98**/0/2	4/7/**89**	3/0/**97**	2/3/**95**
5.7	3/3/**94**	1/0/**99**	0/2/**98**	1/1/**98**	0/1/**99**	3/1/**96**	0/4/**96**	1/24/**75**	10/9/**81**	**95**/1/4	2/0/**98**	2/0/**98**
5.8	5/6/**89**	2/2/**96**	1/2/**97**	1/1/**98**	0/1/**99**	**98**/0/2	1/4/**95**	**92**/5/3	**98**/0/2	10/23/**67**	1/0/**99**	2/1/**97**

Numbers in bold: % with expected interpretation. Grey cell: < 90 % of results hit correct interpretation

The percentage of correct results and percentage of minor and major deviations in 2004 compared to previous years are presented in Table 5. In total, 12,381 antimicrobial susceptibility tests were performed. Of these, 93.0 % (11,514) were in agreement with the expected results, 4.5 % were minor deviations and 2.5 % were major deviations.

Table 5. Susceptibility testing results from 2000 to 2004

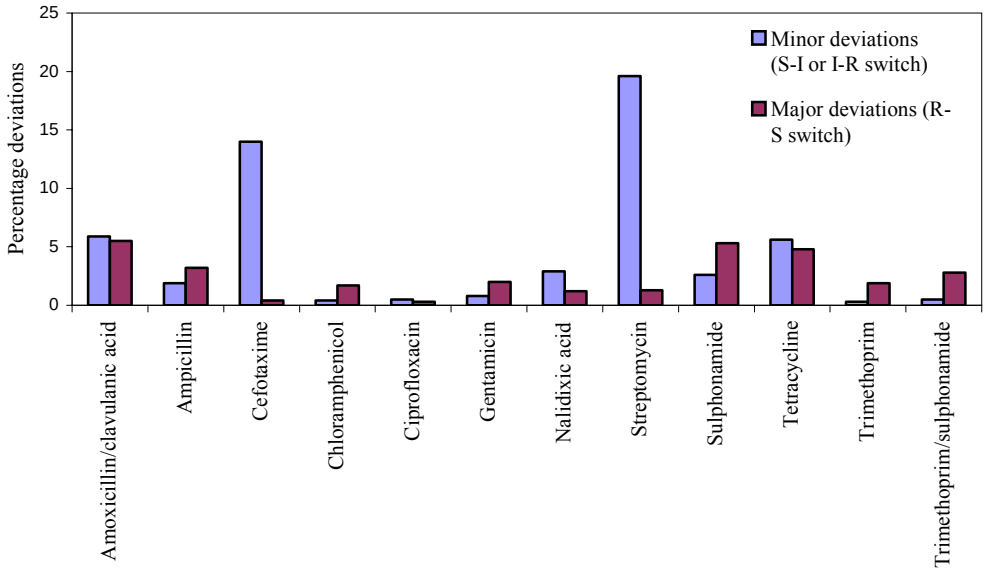
Year	All testings performed	Percentage correct results	Percentage minor deviations (S-I or I-R switch)	Percentage major deviations (R-S switch)
2000	3,151	91.7	4.5	3.8
2001	7,409	91.2	5.8	3.0
2002	8,554	91.2	6.4	2.5
2003	9,473	94.7	3.5	1.8
2004	12,381	93.0	4.5	2.5

The percentage of correct results and of major deviations for each antimicrobial is presented in Table 6, and the percentage of both minor and major deviations for each antimicrobial is presented in Figure 2.

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

Anti-microbial	EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004	
	Total no. of tests	% major deviations	Total no. of tests	% major deviations	Total no. of tests	% major deviations	Total no. of tests	% major deviations
Amp	793	4.0	918	2.9	1,005	1.6	1,178	3.2
Amx/Cl	-	-	-	-	-	-	973	5.5
Ctx	-	-	-	-	-	-	995	0.4
Chl	785	1.8	911	1.8	982	0.7	1,159	1.7
Cip	784	0.6	911	0.5	981	0.4	1,162	0.3
Gen	792	1.1	905	2.8	979	1.6	1,201	2.0
Kan	595	2.0	680	1.5	732	2.3	-	-
Nal	697	1.4	893	2.1	933	1.1	1,130	1.2
Str	643	7.0	734	4.2	761	4.3	947	1.3
Su	412	4.4	503	3.6	615	3.6	734	5.3
Tet	775	6.7	869	3.3	981	4.0	1,122	4.8
Tmp	398	1.5	507	3.0	582	0.5	7,29	1.9
T/S	728	2.1	731	2.3	922	0.5	1,051	2.8

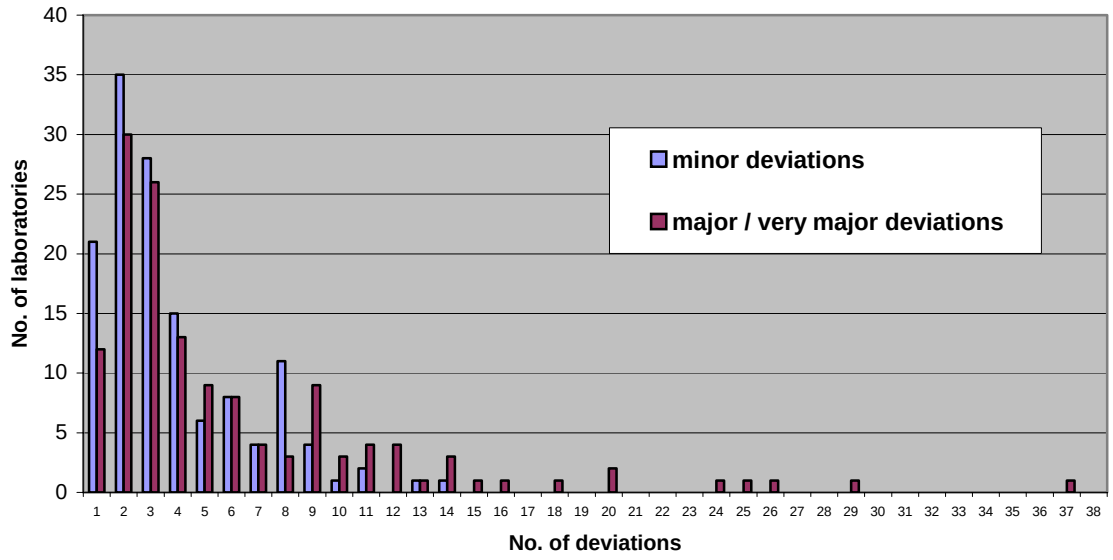
Figure 2. Percentage minor and major deviations by antimicrobial, 2004.



All in all, 12 laboratories (8.0%) had no deviations at all. A total of 67 laboratories (44.4%) had only minor deviations. Ten laboratories were responsible for 26.3% (228) of the total of 867 deviations. Figure 3 shows the distribution of laboratories in relation to number of minor deviations and number of major/very major deviations. If the major deviations are further divided into “very major” (measuring sensitive when resistant) and “major” deviations (measuring resistant when sensitive), it appears that all “very major” deviations derived from 24 laboratories and were

observed for almost all compounds tested.

Figure 3. Number of laboratories in relation to number of deviations



Quality Control (QC) testing

If testing is correctly standardized and performed in accordance to the guidelines given by the CLSI, the results for the *E. coli* ATCC 25922 QC strain are supposed to be inside the QC ranges given by the CLSI.

Of 156 laboratories performing antimicrobial susceptibility testing, a total of 136 laboratories (87%) reported QC data. For 76 of these laboratories (56 %), all results for the *E. coli* QC strain were correct. For the remaining 44 % of the laboratories a mean of 2.6 tests were out of range.

A total of 1,410 tests for QC were performed. Of these, 11.2 % (158 tests) were outside QC range. QC range and number of laboratories with incorrect QC results range compared to previous years are shown in Table 7.

Table 7. Quality Control results for testing of the reference strain *E. coli* ATCC 25922.

Anti-microbial	QC range[1] *E. coli* ATCC 25922		Laboratories outside QC range							
			EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004	
	MIC (ug/ml)	Disks (mm)	% of labs	N[3]	% of labs	N[3]	% of labs	N[3]	% of labs	N[3]
Amp	2-8	16-22	19	97	16	109	14	140	9.8	132

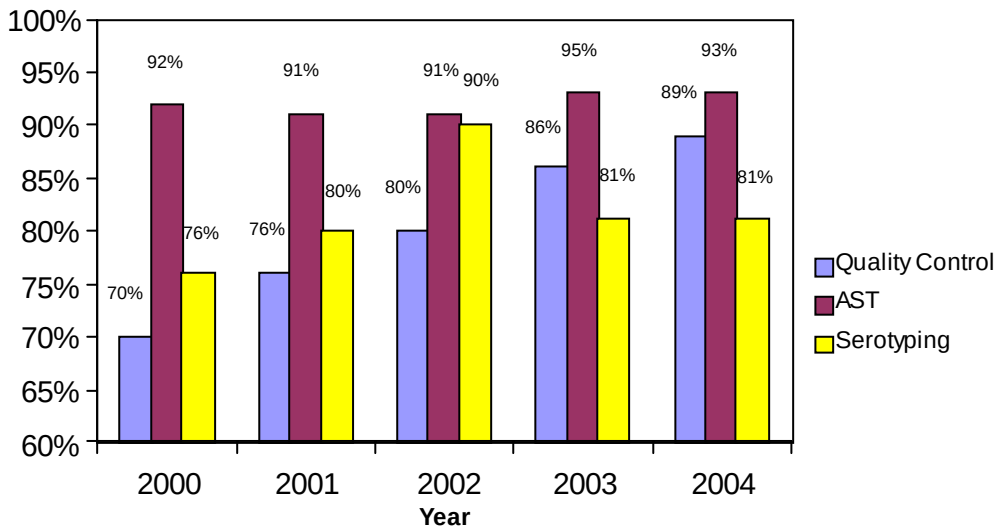
Amx/Cl			-	-	-		-		12.8	117
Ctx			-	-	-		-		18.0	111
Chl	2-8	21-27	20	97	15	107	22	137	13.3	128
Cip	.004-.016	30-40	14	97	14	108	9	138	8.3	132
Gen	0.25-1	19-26	12	99	12	108	9	138	10.4	134
Kan	1-4	17-25	14	87	11	79	12	103	-	-
Nal	1-4	22-28	14	74	14	102	16	132	8.7	126
Str	4-16[2]	12-20	12	81	11	82	9	105	5.5	110
Su	8-32	15-23	34	53	26	57	17	82	15.5	84
Tet	0.5-2	18-25	22	96	13	102	19	137	13.2	129
Tmp	0.5-2	21-28	22	50	11	66	14	79	9.2	87
T/S	≤0.5/9.5	23-29	14	90	12	102	14	129	10.8	120

[1] *CLSI standard, Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing; 12th Informational suppl. NCCLS document M100-S12, Wayne, Pennsylvania.*
[2] *QC range developed by the manufacturer of Sensititre®*
[3] *The total number of laboratories performing the test*

Selected results over time

The percentage correct results of the total number of testings of serotyping, antimicrobial susceptibility testing and quality control are summarized over time and presented in Figure 4.

Figure 4. Percentage correct results on Quality Control Antimicrobial Susceptibility Testing (AST) and Serotyping from 2000-2004.



Identification of the extra strains

For all laboratories participating in the EQAS 2004, the extra strains were included in the shipment, except for those labs that specifically requested not to be sent any *Campylobacters*.

A total of 109 laboratories (70.0 % of the participants) submitted their results on *Campylobacter* identification. All in all 93 laboratories submitted results on both strains, and 68 of these

laboratories performed correct species identifications for both the strains. Results of the testing are presented in table 8.

Table 8. Results for the identification of two Campylobacter isolates to species level.

Year	No. of participating labs	Expected result	No. of participants for each strain*	Percentage correct result per strain	**Deviations**	Overall % correct results
2003	97	C. jejuni	92	87.0 %	C. coli (9 labs) C. lari (3 labs)	85.0 %
		C. coli	92	83.7 %	C. jejuni (7 labs) C. lari (4 labs) C. upsaliensis (4 labs)	
2004	109	C. lari	95	80.0 %	C. coli (11 labs) C. jejuni (8 labs)	83.6 %
		C. jejuni	107	86.9 %	C. coli (8 labs) C. lari (4 labs) C. upsaliensis (2 labs)	

** The number of participants for each strain differs and is lower than the total number of participants due to unsuccessfully recovering of strains.*

The 109 participating laboratories represented 61 countries: Argentina, Australia, Barbados, Belarus, Brazil, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Costa Rica, Croatia, Cuba, Cyprus, Czech Republic, Denmark, Ecuador, Germany, Greece, Guatemala, Hungary, Iceland, India, Israel, Italy, Japan, Latvia, Lithuania, Luxembourg, Macedonia, Malaysia, Malta, Mauritius, Mexico, Moldova, Morocco, Netherlands, New Zealand, Oman, Paraguay, Peru, Philippines, Poland, Romania, Senegal, Serbia and Montenegro, Singapore, Slovakia, Slovenia, Spain, Taiwan, Thailand, Trinidad and Tobago, Turkey, Ukraine, Uruguay, USA, Venezuela.

Table 9. Results for the identification of the blinded bacterial isolate to species level.

Year	No. of participating labs	Expected result	Percentage correct results on species identification	Deviations
2003	115	*E. coli* (serotype O157)	99.1 %	*Pseudomonas putida* (1 lab)
2004	121	*Shigella flexneri* (type 2a)	75.2 % *	*E. coli* (2 labs) *Salmonella* Corvallis (1 lab) *Salmonella* Typhimurium (1 lab) *Citrobacter freundii* (1 lab)

** Another 20.7 % of the participants submitted "Shigella" as the result, with no indication of species or with two species suggested.*

A total of 121 laboratories submitted results on identification of the blinded bacterial sample, this year a *Shigella flexneri* type 2a isolate. Of these, 91 laboratories identified the strain as "Shigella flexneri", while 22 laboratories reported the strain as "Shigella" with no indication of the species. Further typing was reported by 68 laboratories, in 41 cases with indication of serotype 2a, and in further 20 cases with the indication type 2 only.

Discussion

In order to identify the barriers for serotyping, the level of difficulty in serotyping was considerably increased in 2003 and 2004. Thus, an extended spectrum of antisera and performance of additional biochemical testing was needed to perform complete serotyping for some of the strains. As a consequence the performance of serotyping decreased (Fig. 4). The list of deviating serotypings in Table 1 shows, that the most troubled strains in serotyping were *S.* Give, *S.* Corvallis and *S.* Chester, and that the most predominant deviating serovar only differ by one O-factor or one H-factor in first or second phase. These serotyping results together with comments from the participants strongly indicate that many of the laboratories lack the antisera needed for complete serotyping. It can be concluded that barrier number one for serotyping is lack of antisera and high-quality antisera. Also basic understanding of the Kaufmann-White serotyping scheme, and of *Salmonella* taxonomy appear to play a role.

The antimicrobial susceptibility testing performance on the *Salmonella* strains has remained at a relatively high level all years (91-95% correct results) with a tendency towards better performance in the period 2001-2003 (Table 5, Figure 4). In 2004, a total of 93.0 % of the tests were correct, and the percentage of minor and major deviations increased slightly (Table 5). Deviations were especially frequent for testing of tetracycline, streptomycin, sulphonamide and amoxicillin/clavulanic acid.

The testing of tetracyclines, aminoglycosides and sulphonamides is known to be highly influenced by variations in the media (acidity, cationic concentration, presence of antagonists). In addition, misreading of the sulphonamide testing results may also be of importance, since growth that should be ignored appears in a short period due to a delayed bacterial response. Testing of amoxicillin and clavulanic acid was introduced in 2004, and seems to be problematic. It is well-known that especially the testing of beta-lactamse producing strains can be difficult because they can show reduced MICs towards amoxicillin and clavulanic acid.

Strain 5.4 and 5.6 are ESBL (Extended Spectrum Beta-Lactamase) producing strains, and thus resistant to the 3rd generation cephalosporin, cefotaxime. Phenotypically, as seen in Table 4, when performing an initial screening for ESBLs with cefotaxime, the strains may appear intermediary resistant. In this report, the intermediary resistant result is taken as the reference value, since both the DFVF and the CDC confirmed this intermediary categorisation. Anyhow, some of the participants took the step to do a complete ESBL-confirmatory test, and found the strains to be ESBL-producing. In future, both intermediary and fully resistant results to cefotaxime should be taken as an indication on the presence of ESBLs, and testing of more than one cephalosporin should be included (procedure described by CLSI).

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When performing antimicrobial susceptibility testing, it is extremely important to include reference strains for internal quality control (QC). The results for the *E. coli* QC strain revealed that 158 (11.2 %) of the performed tests were outside the QC range given by CLSI, spread over 60 laboratories (44% of the laboratories reporting QC data). Anyhow, this is an improvement compared to previous years (Figure 4), and since also the fraction of participants performing internal QC has increased year by year (data not presented), there seems to be an increasing awareness of the importance of QC even though inadequate standardization of the method used is still an important barrier for antimicrobial susceptibility data of high quality. Unaware use of expired disks, improper storage, repeated subculturing of strains with loss of resistance genes as a consequence, are also plausible reasons for part of the incorrect results.

In conclusion, the results indicate a strong need for antisera at high quality and affordable prices, a further need for training in *Salmonella* serotyping and finally a further need for strengthen the awareness of performing internal QC and to learn how to intervene if results are out of control.

In future cycles of EQAS, the GSS Regional Centres will actively do a follow-up on individual results in their region, if needed, and by time, conduct regional-specific EQAS programs

We were pleased to experience that even more laboratories participated on identification of *Campylobacter* and *Shigella*. However, the results indicate a need for protocols and quality assurance programs for identification procedures of other human pathogens too, and highly supports the efforts to expand the WHO Global *Salm*-Surv operational area to include even more pathogens of human importance together with the methods needed for identification, typing and susceptibility testing.