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Results of Use of WHO Global Salm-Surv External Quality Assurance System for Antimicrobial Susceptibility Testing of *Salmonella* Isolates from 2000 to 2007▽

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An international External Quality Assurance System (EQAS) for antimicrobial susceptibility testing of *Salmonella* was initiated in 2000 by the World Health Organization (WHO) Global Salm-Surv in order to enhance the capacities of national reference laboratories to obtain reliable data for surveillance purposes worldwide. Seven EQAS iterations have been conducted from 2000 to 2007. In each iteration, participating laboratories submitted susceptibility results from 10 to 15 antimicrobial agents for eight *Salmonella* isolates and an *Escherichia coli* reference strain (ATCC 25922). A total of 287 laboratories in 102 countries participated in at least one EQAS iteration. A large number of laboratories reported results for the *E. coli* ATCC 25922 reference strain which were outside the quality control ranges. Critical deviations for susceptibility testing of the *Salmonella* isolates varied from 4% in 2000 to 3% in 2007. Consistent difficulties were observed in susceptibility testing of amoxicillin-clavulanic acid, cefotaxime, ceftazidime, streptomycin, sulfonamides, and tetracycline. Regional variations in performance were observed, with laboratories in central Asia, Africa, and the Middle East not performing as well as those in other regions. Results from the WHO Global Salm-Surv EQAS show that most laboratories worldwide are capable of correctly performing antimicrobial susceptibility testing of *Salmonella* isolates, but they also indicate that further improvement for some laboratories is needed. In particular, further training and dissemination of information on quality control, appropriate interpretive criteria (breakpoints), and harmonization of the methodology worldwide through WHO Global Salm-Surv and other programs will contribute to the generation of comparable and reliable antimicrobial susceptibility data (D. M. A. Lo Fo Wong, R. S. Hendriksen, D. J. Mevius, K. T. Veldman, and F. M. Aarestrup, Vet. Microbiol. 115:128–139, 2006).

Salmonellae are among the most important food-borne pathogens, leading to millions of cases of diarrheal illness and thousands of hospitalizations and deaths each year worldwide. Infections with resistant *Salmonella* spp. are associated with increased morbidity and mortality (8), and there is growing concern for the increasing resistance to antimicrobial agents among the *Salmonella* spp. Correctly performed antimicrobial susceptibility testing is essential to provide data for surveillance of antimicrobial resistance and direct efforts to mitigate antimicrobial resistance.

In January 2000, the World Health Organization (WHO) launched an international effort, called WHO Global Salm-Surv (WHO-GSS), to enhance laboratory-based surveillance of *Salmonella* infections and other food-borne diseases and promote prevention and control activities. Enhancing worldwide surveillance of antimicrobial resistance in *Salmonella* spp. is a key objective of WHO-GSS.

To support laboratories that participate in WHO-GSS, an External Quality Assurance System (EQAS) was established in 2000 (9). Among other topics, the EQAS includes an assessment of the quality of antimicrobial susceptibility testing of *Salmonella* spp. in participating laboratories. Iterations of the EQAS are organized yearly by Denmark's National Food Institute (DTU Food) in collaboration with the WHO, the United States' Centers for Disease Control and Prevention (CDC), and France's Institute Pasteur (IP). The WHO-GSS EQAS is a self-evaluating system; participants receive a report which itemizes deviations. The report should be used by the participant to evaluate if the current methodologies are accurate, adequate, and reliable. The goal is to have all laboratories perform *Salmonella* antimicrobial susceptibility testing with a maximum of 10% total deviations (minor, major, or very major deviations) and a maximum of 5% critical deviations (major or very major deviations). Here we report the results of the first seven

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TABLE 1. Percentage of participating laboratories with deviations for the quality control strain for each antimicrobial agent tested per the EQAS iteration from 2000 to 2007

Antimicrobial agent	Interval of the quality control strain[a]		2000 EQAS (n = 44)		2001 EQAS (n = 107)		2002 EQAS (n = 114)		2003 EQAS (n = 144)		2004 EQAS (n = 140)	
	MIC (μg/ml)	Disks (mm)	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]
AMP	2–8	16–22	27	37	19	97	16	109	14	140	10	132
AUG	2–8	8–24									13	117
CAZ	0.06–0.5	25–32										
CHL	2–8	21–27	37	38	20	97	15	107	22	137	13	128
CIP	0.004–0.016	30–40	20	35	14	97	14	108	9	138	8	132
CTX	0.03–0.12	29–35									18	111
EFX[d]	0.008–0.03	32–40										
GEN	0.25–1	19–26	23	39	12	99	12	108	9	138	10	134
KAN	1–4	17–25	19	36	14	87	11	79	12	103		
NAL	1–4	22–28	35	37	14	74	14	102	16	132	9	126
POD	0.25–1	23–28										
SMX	8–32	15–23	53	19	34	53	26	57	17	82	16	84
STR	4–16[b]	12–20	22	36	12	81	11	82	9	105	6	110
SXT	≤0.5/9.5	23–29			14	90	12	102	14	129	11	120
TET	0.5–2	18–25	42	42	22	96	13	102	19	137	13	129
TMP	0.5–2	21–28	30	31	22	50	11	66	14	79	9	87
XNL	0.25–1	26–31										
FFC[d]	2–8	22–28										

[a] See references 3 and 4.
[b] Quality control range developed by the manufacturer of Sensititre.
[c] Total number of laboratories performing the test.
[d] EFX, enrofloxacin; FFC, florfenicol.

iterations of antimicrobial susceptibility testing of the WHO-GSS EQAS conducted from 2000 to 2007.

MATERIALS AND METHODS

An invitation to participate in each EQAS iteration was announced through the WHO-GSS Listserv, which is received by e-mail or facsimile by all WHO-GSS members (http://www.who.int/salmsurv/activities/bulletin_board/en/). In 2007, there were 1,027 WHO-GSS members from 152 countries, which included microbiologists and epidemiologists from 142 national reference (animal, food, or public health) laboratories from 90 countries. Many WHO-GSS members attended at least 1 of the 50 WHO-GSS international training courses held at the 14 training sites between 2000 and 2007. These courses included training for antimicrobial susceptibility testing of *Salmonella* isolates. WHO-GSS EQAS participation is free of charge, but each participating laboratory covered the expenses associated with the testing of the isolates in their laboratory. The WHO-GSS EQAS is estimated to cost $40,000 per iteration to organize.

Eight strains of *Salmonella* were selected for each EQAS iteration. Strains were obtained from the isolate collection of DTU Food. The same *Salmonella enteritidis* strain was included in 2000, 2001, 2004, 2006, and 2007; all other strains were included in only one EQAS iteration. *Salmonella* strains were inoculated onto agar stab cultures for shipping to participating laboratories. Participating laboratories also received a lyophilized reference strain as a quality control strain for susceptibility testing (*Escherichia coli* ATCC 25922 [CCM 3954]) in each EQAS shipment. The reference strain was purchased from the Czech Collection of Microorganisms (CCM), Czech Republic.

Antimicrobial susceptibility testing of the *Salmonella* strains was performed at DTU Food and verified at the CDC; the obtained MICs served as the reference standard. The following antimicrobials were used: ampicillin (AMP), amoxicillin-clavulanic acid (AUG), cefotaxime (CTX), cefpodoxime (POD), ceftazidime (CAZ), ceftiofur (XNL), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SMX), tetracycline (TET), trimethoprim (TMP), and sulfonamides-trimethoprim (SXT). MIC determination was performed using the Sensititre system from TREK Diagnostic Systems Ltd., with the exception of CTX, CAZ, and SXT. These exceptions were tested using Etest from AB-Biodisk, Sweden. Interpretive criteria (breakpoints) for each iteration were established using currently available versions of CLSI documents M07-A7 (1), M100-S16 (2), and M31-A2 (3). Exceptions were the following antimicrobials, for which epidemiological cutoff values were used: CIP and GEN according to EUCAST (http://www.eucast.org/) and STR according to DTU Food (http://www.crl-ar.eu/).

Testing instructions and a "participating laboratory record sheet" were copied to a compact disc and included with the *Salmonella* agar stab cultures and reference strain in double-pack containers (classified as UN 6.2) and sent to the participating laboratories according to the International Air Transport Association regulations as "Biological Substance Category B" (classified as UN 3373). Prior to shipping, each participating laboratory was informed about the dispatched parcels and the air waybill number for tracking the parcel and picking it up at the airport. Import permits were necessary for shipping the parcels to several countries.

Members of participating laboratories were instructed to follow the enclosed testing instructions, subculture the *Salmonella* strains, and propagate the quality control strain prior to performing the susceptibility method that was routinely used by each laboratory. In addition, laboratories were advised to maintain the quality control strain for future proficiency tests. After completion of the susceptibility testing of the *Salmonella* strains and the quality control strain, participating laboratories were instructed to record the obtained results, using MICs or zone diameter in millimeters, and categorize each of the *Salmonella* strains as either resistant, intermediate, or susceptible against each tested antimicrobial agent, using the breakpoints routinely used in each laboratory, on the participating laboratory record sheet. Participating laboratories were instructed to submit results via the WHO-GSS website (using a secured individual log-in) or by sending the completed participating laboratory record sheet by facsimile to DTU Food.

After results were submitted, participating laboratories received an individual report; those that submitted results online received an instant report via the secure website. The individual reports for the participating laboratories reported all deviations from the expected values and suggestions of how to either solve or investigate the problem. For the quality control strain, deviations were defined as values that exceeded the quality control range of the strain. Deviations of the antimicrobial susceptibility results were categorized as minor, major, or very major. A minor deviation was defined as an intermediate strain that had been classified as susceptible or resistant (i.e., a change from intermediate to susceptible or intermediate to resistant) or vice versa. A major deviation was defined as a susceptible strain that had been classified as resistant (i.e., a change from susceptible to resistant). A very major deviation was defined as a resistant strain that had been classified as susceptible (i.e., a change from resistant to susceptible). Total deviations are the number of all deviations; critical deviations are the numbers of major and very major deviations.

RESULTS

A total of 287 laboratories in 102 countries participated in at least one of the seven iterations of EQAS from 2000 to 2007; participation included 44 laboratories in 35 countries in 2000, 108 laboratories in 59 countries in 2001, 119 laboratories in 66 countries in 2002, 147 laboratories in 76 countries in 2003, 152

TABLE 1—*Continued*

2006 EQAS (n = 137)						2007 EQAS (n = 126)					
All		MIC		Disk		All		MIC (n = 23)		Disk (n = 102)	
% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]	% Laboratories	Total no. of laboratories[c]
14	133	5	20	16	113	11	124	0	23	14	101
9	116	6	17	10	99	8	102	0	17	9	85
15	96	20	10	14	86	9	92	0	8	10	84
18	126	13	16	19	110	14	123	0	21	17	102
8	127	11	19	8	108	12	121	13	23	12	98
21	115	30	10	20	105	16	104	30	10	15	94
63	19	0	1	67	18						
14	131	17	18	14	113	6	124	5	22	7	102
20	122	19	16	20	106	7	120	0	21	8	99
12	39	25	4	11	35	9	47	0	6	10	41
29	74	33	9	29	65	22	64	15	13	24	51
11	106	14	14	10	92	6	97	0	15	7	82
19	122	19	16	19	106	13	107	0	14	15	93
12	125	12	17	12	108	7	117	0	20	8	97
17	74	13	8	17	66	10	67	O	9	12	58
22	32	0	9	30	23	11	35	0	12	17	23
						0	13	0	5	0	8

laboratories in 80 countries in 2004, 143 laboratories in 75 countries in 2006, and 143 laboratories in 73 countries in 2007. The average number of participating laboratories per EQAS iteration between 2000 and 2007 was 122, with 139 laboratories participating in at least three iterations and 102 laboratories participating in at least four iterations. The participating laboratories included veterinary, food, and public health national reference laboratories and other hospital laboratories. One or more institutions participated in at least one EQAS iteration from the following countries: Albania, Argentina, Australia, Barbados, Belarus, Bolivia, Bosnia and Herzegovina, Botswana, Brazil, Bulgaria, Cambodia, Cameroon, Canada, Central African Republic, Chile, China, Colombia, Costa Rica, Croatia, Cyprus, the Czech Republic, Democratic Republic of the Congo, Cuba, Denmark, Ecuador, Egypt, Estonia, Finland, France, Gabon, Georgia, Germany, Greece, Guatemala, Honduras, Hungary, Iceland, India, Indonesia, Ireland, Israel, Italy, Cote d'Ivoire, Jamaica, Japan, Jordan, South Korea, Kuwait, Laos, Latvia, Lebanon, Lithuania, Luxembourg, Macedonia, Madagascar, Malaysia, Malta, Mauritania, Mauritius, Mexico, Moldova, Morocco, New Caledonia, New Zealand, The Netherlands, Nigeria, Nicaragua, Norway, Oman, Panama, Papua New Guinea, Paraguay, Peru, the Philippines, Poland, Romania, Russia, Saudi Arabia, Serbia and Montenegro, Senegal, Seychelles, Singapore, Slovakia, Slovenia, Sri Lanka, South Africa, Spain, Sudan, Suriname, Syria, Taiwan, Thailand, Trinidad and Tobago, Tunisia, Turkey, Uganda, Ukraine, the United Kingdom, the United States, Uruguay, Venezuela, and Vietnam.

A total of 856 participating laboratory record sheets were received from participating laboratories during the seven EQAS iterations. Among the 856 record sheets, the proportion of susceptibility results determined by disk diffusion was 88% (range, 83% to 93% for each iteration). For the quality control strain, susceptibility results for at least one antimicrobial agent were reported on 95% of the 856 record sheets (range, 88% to 100% for each iteration). The average number of antimicrobial agents with susceptibility results for the quality control strain per participating laboratory increased from 8.6 in 2001 to 11.6 in 2007. The proportions of participating laboratories with one or more deviations for the quality control strain were 30% in 2000, 17% in 2001, 14% in 2002, 14% in 2003, 11% in 2004, 11% in 2006, and 44% in 2007. There was a higher proportion

TABLE 2. Annual and overall average number of antimicrobial agents tested by participating laboratories, percent correct results, and percent minor, major, and very major deviations in the EQAS from 2000 to 2007[a]

Yr	No. of laboratories participating in each EQAS iteration	Avg no. of antimicrobial agents tested by participating laboratories	% Correct test results	% Minor deviations (S to I or I to R switch)	% Major deviations (S to R switch)	% Very major deviations (R to S switch)	% Critical deviations (R to S and S to R switch)	% Total deviations
2000	44	9.1	92	4	4	0	4	8
2001	108	8.9	91	6	2	1	3	9
2002	119	8.9	92	6	2	1	3	9
2003	147	9.3	92	4	2	2	4	8
2003[b]	147	8.1	93	4	3	0	3	7
2004	152	10.2	93	4	2	1	3	7
2006	143	11.2	88	8	3	1	4	12
2007	143	10.8	93	4	2	1	3	7
Total[b]	129	9.6	91	6	2	1	3	9

[a] S, susceptible; I, intermediate; R, resistant.
[b] We have excluded data from one strain (*Salmonella* serovar Panama) which may have lost resistance due to transport or storage stress.

TABLE 3. Annual and overall number of tests performed, percent total deviations, percent critical deviations, and percent major and total deviations for each antimicrobial agent in the EQAS from 2000 to 2007

Antimicrobial	2000 EQAS (n = 44)			2001 EQAS (n = 108)			2002 EQAS (n = 119)			2003 EQAS[a] (n = 147)		
	Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations
AMP	343	6	8	822	4	7	918	2	3	1,019	2	4
CHL	343	4	7	814	2	3	903	2	3	996	1	2
CIP	334	1	6	813	1	4	911	0	2	995	0	1
GEN	343	4	5	821	2	4	905	2	16	993	2	2
KAN	312	4	16	623	2	7	680	2	10	738	2	6
NAL	328	1	4	726	2	8	885	2	4	947	1	4
SMX	248	3	5	431	6	9	495	4	4	615	4	5
STR	312	4	12	679	7	27	718	4	34	768	9	39
SXT				757	2	5	724	7	10	929	2	2
TET	335	6	13	804	7	18	861	3	7	995	4	11
TMP	295	1	1	416	1	2	499	3	3	582	1	1
AUG												
CAZ												
XNL												
CTX												
POD												
Total	3,193	3	8	7,706	3	9	8,499	3	9	9,577	3	7

[a] We have excluded data from one strain which may have lost resistance due to transport or storage stress in 2003.

of deviations for the quality control strain for most antimicrobial agents in 2000 and 2007 than in all other years (Table 1). There was an almost steady decline in deviations from 2000 to 2007 for AMP (from 27% in 2000 to 11% in 2007), CIP (from 20% in 2000 to 12% in 2007), and TET (from 42% in 2000 to 7% in 2007).

A high proportion of participating laboratories reported deviations in susceptibility results for at least nine antimicrobial agents for one of the *Salmonella* isolates (an isolate of *Salmonella* serovar Panama) included in the 2003 EQAS. Participating laboratories reported 251 total deviations (13 minor, 18 major, and 220 very major deviations) in susceptibility results for the *Salmonella* Panama isolate, which accounted for 34% of the total deviations in susceptibility results in the 2003 iteration; the very major deviations with this isolate were shown for AMP (23%), CHL (22%), GEN (27%), KAN (20%), STR (13%), sulfonamides (15%), TMP (17%), SXT (23%), and TET (16%). Based on these details, the *Salmonella* Panama isolate was excluded from further analysis.

After excluding the *Salmonella* Panama isolate, there were a total of 67,229 susceptibility results for *Salmonella* strains reported on the 856 record sheets from participating laboratories in the seven EQAS iterations. The average number of antimicrobial agents with susceptibility results for each *Salmonella* strain was 9.6; by year, the average number of antimicrobial agents with susceptibility testing for each *Salmonella* strain was fairly consistent, from 8.1 in 2003 to 11.2 in 2006 (Table 2). During the seven EQAS iterations, there were a total of 6,051 deviations (4,034 minor, 1,345 major, and 672 very major deviations). During the seven EQAS iterations, overall, 92% of the susceptibility results for *Salmonella* strains were correct; 5% of results had a minor deviation, 2% had a major deviation, and 1% had a very major deviation, resulting in 8% total deviations and 3% critical deviations. From 2000 to 2007, the annual percentage of total deviations (7% to 12%) in susceptibility results was almost doubled. However, the critical deviations (3% to 4%) were largely unchanged. Similarly, for the *Salmonella enteritidis* isolate tested in 2000, 2001, 2004, 2006, and 2007, there was little change in total deviations (20.3%, 16.9%, 6.5%, 7.5%, and 8.3%) or critical deviations (7.2%, 5.2%, 2.4%, 3.3%, and 2.9%).

Each year, the percentage of critical deviations in susceptibility results for CIP, CHL, GEN, KAN, NAL, SXT, and TMP was low compared with that for AUG, SMX, STR, and TET (Table 3). The annual percentage of critical deviations in susceptibility results for each antimicrobial agent was largely unchanged from 2000 to 2007.

The overall percentage of critical deviations varied by region, with a high percentage of critical deviations in central Asia and the Middle East (range, 6 to 10%), Africa (range, 2 to 10%), South America (range, 1 to 5%), and Southeast Asia (range, 2 to 7%) (Table 4). In central Asia and the Middle East, Africa, South America, and Southeast Asia, 21, 29, 34, and 40 laboratories participated in 42%, 34%, 39% and 33% of the iterations during all 7 years, respectively.

DISCUSSION

Results from the first seven iterations of the WHO-GSS EQAS indicate that most laboratories worldwide are capable of correctly performing antimicrobial susceptibility testing of *Salmonella* isolates. Nevertheless, some laboratories did not meet the WHO-GSS goal of <10% total deviations and <5% critical deviations in susceptibility results. Our data suggest that several factors contributed to the observed deviations. Many laboratories did not perform adequate internal quality control procedures. Although we did not ascertain the actual breakpoints used in each laboratory, based on the susceptibility results it appears that some laboratories did not use appropriate breakpoints, which results in deviations. Furthermore, several antimicrobial agents offered particular difficulties (e.g., in the measuring of inhibition zones). Finally, there are important regional differences in the capacities of laboratories.

When performing antimicrobial susceptibility testing, it is essential to include reference strains for internal quality control (5). When appropriately utilized, the reference strain will provide quality control for both the method and the reagents. If results for the quality control strain are not within the ex-

TABLE 3—*Continued*

2004 EQAS (n = 152)			2006 EQAS (n = 143)			2007 EQAS (n = 143)			Overall EQAS from 2000–2007[a] (n = 856)		
Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations	Total no. of determinations	% Critical deviations	% Total deviations
1,178	3	5	1,092	2	3	1,114	5	7	6,486	3	5
1,159	2	2	1,060	3	15	1,105	0	6	6,380	2	5
1,162	0	1	1,110	2	6	1,101	1	1	6,426	1	3
1,201	2	3	1,078	3	7	1,111	3	4	6,452	3	6
									2,468	3	10
1,130	1	4	1,035	2	6	1,092	2	3	6,143	2	5
734	5	8	649	6	7	678	5	6	3,850	5	6
947	1	21	896	5	22	875	4	26	5,195	5	26
1,051	3	4	996	3	5	971	3	3	5,428	3	5
1,122	5	11	1,054	9	20	1,047	4	11	6,218	5	13
729	2	2	607	1	2	583	1	2	3,711	1	2
973	6	12	950	9	22	908	6	17	2,831	7	17
			769	7	11	830	1	1	1,599	4	6
			225	2	9	258	0	6	483	1	8
995	0	14	956	7	15	914	1	2	2,865	3	10
			305	1	26	389	4	16	694	3	21
12,381	3	7	12,782	4	12	12,976	3	7	67,229	3	9

pected parameters, results for the test organisms should not be reported. A reference stain, *E. coli* ATCC 25922, was included with each iteration of the EQAS program. A high number of laboratories reported results outside the quality control range for this isolate. These results typically arise from inadequate standardization of methodologies, improper storage of disks, which may cause the disks to lose potency, or repeated subculturing of strains, which may lead to loss of plasmids carrying resistance genes. For these laboratories, deviations in antimicrobial susceptibility testing can likely be remedied by improving quality control practices.

Accurate antimicrobial susceptibility testing also requires the use of appropriate criteria (breakpoints) for interpreting susceptibility results. Several national and international committees have established breakpoints for susceptibility testing. The most widely used are those provided by CLSI (http://www.clsi.org/), which publishes methods for susceptibility testing and tables with clinical breakpoints (both MIC tables and zone diameter determinants) as approved by the U.S. Food and Drug Administration (FDA). In Europe, the European Committee for Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/) provides epidemiological cutoff values and clinical breakpoints for MIC determination. In addition to the committees mentioned above, many countries have established national standards and breakpoints (6).

Because the use of different standards and breakpoints will result in different susceptibility results for some isolates, there is a need for international harmonization of standards and breakpoints when comparing results between countries (7).

Susceptibility testing is particularly difficult for certain antimicrobial agents. In the WHO-GSS EQAS, a high percentage of deviations was observed with AUG, CTX, CAZ, SMX, STR, and TET. Problems associated with AUG are often due to a "breakpoint phenomenon," where many strains have values close to the breakpoint, causing some strains to be read as intermediate and others as resistant (6). In addition, beta-lactamase-producing strains may have reduced susceptibilities to AUG that are sometimes difficult to interpret. No specific guidelines on how to interpret the cephalosporins were disseminated with the WHO-GSS instructions; thus, some laboratories may have followed CLSI guidelines, which indicate that all members of the cephalosporin class should be interpreted as resistant if one member of the class tests as resistant. STR often poses a challenge in susceptibility testing as many strains have zone diameters or MICs near the breakpoint. TET usually causes few deviations, but it accounted for 9% of the deviations in 2006, which may relate to the change in CLSI breakpoints for TET in 2006. SMX deviations may have been caused by a high thymidine and thimine content in the medium or difficulty in the interpretation of sulfonamide breakpoints (1). Excessive levels of thymidine or thimine have been shown to antagonize the effects of SXT. Additionally, while most antimicrobials produce clear, definitive zones of inhibition, it is not uncommon to observe light growth near the sulfonamide breakpoint. As such, it is recommended that sulfonamide zone diameters be measured from the point of 80% inhibition, not from the point of complete inhibition typically utilized for other classes (3).

Results from the WHO-GSS EQAS also demonstrate important regional differences in antimicrobial susceptibility results. Particular focus is required for Africa, central Asia, and the Middle East. The laboratories' continuous participation in the WHO-GSS EQAS in these regions is low, and only a few training courses have been conducted by WHO-GSS. In addition, unpublished data from a survey conducted in 2007 indicate that the availability of reagents for many laboratories in developing countries poses a challenge as resources are limited.

There were several limitations of this study. Although most laboratories participated in more than one EQAS iteration, identical isolates were not included in each iteration and the overall degree of difficulty of the susceptibility testing of the isolates varied in each iteration. Therefore, it is difficult to determine whether or not there has been improvement in the accuracy of susceptibility testing over time. Furthermore, the EQAS results of the participating laboratories cannot be used to assess the performance and accuracy of the selected susceptibility testing method. Observed differences between participating laboratories may be due to many additional factors other than differing susceptibility testing methods. To deter-

TABLE 4. Number of laboratories, by year and region, that have deviating results[a]

Region(s)	Yr[b]	No. of laboratories	% Correct test results	% Minor deviations (S to I or I to R switch)	% Major deviations (S to R switch)	% Very major deviations (R to S switch)	% Critical deviations	% Total deviations
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8
	2002	10	94.3	4.1	1.0	0.6	1.6	5.7
	2003	13	86.9	6.6	2.8	3.7	6.5	13.1
	2004	11	85.7	7.2	5.2	1.9	7.1	14.3
	2006	20	85.8	7.5	4.1	2.7	6.8	14.3
	2007	16	90.7	4.4	4.0	0.9	4.9	9.3
Central Asia and the Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3
	2002	6	83.4	9.8	6.6	0.2	6.8	16.6
	2003	8	89.9	4.5	4.0	1.6	5.6	10.1
	2004	10	87.5	6.7	5.5	0.3	5.8	12.5
	2006	7	79.2	10.5	9.8	0.5	10.3	20.8
	2007	8	87.8	5.0	6.2	1.1	7.3	12.2
Caribbean	2001	2	83.5	9.5	7.0	0.0	7.0	16.5
	2002	1	95.8	4.2	0.0	0.0	0.0	4.2
	2003	8	91.7	6.4	1.5	0.5	2.0	8.4
	2004	8	94.1	3.1	1.9	0.9	2.8	5.9
	2006	5	92.1	5.4	1.6	1.0	2.6	8.0
	2007	4	95.0	3.1	0.9	0.9	1.8	5.0
China	2001	4	98.9	0.8	0.0	0.3	0.3	1.1
	2002	3	96.0	4.0	0.0	0.0	0.0	4.0
	2003	8	90.1	3.6	2.8	3.6	6.4	10.0
	2004	8	96.0	3.2	0.7	0.1	0.8	4.0
	2006	6	89.6	7.0	2.9	0.5	3.4	10.4
	2007	10	98.3	1.1	0.3	0.2	0.5	1.6
Europe	2001	47	91.3	5.7	2.7	0.3	3.0	8.7
	2002	57	92.7	5.2	1.2	0.9	2.1	7.3
	2003	64	92.9	3.8	1.0	2.3	3.3	7.1
	2004	58	93.5	4.3	1.4	0.8	2.2	6.5
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7
North America	2001	4	95.8	3.8	0.0	0.4	0.4	4.2
	2002	3	90.5	6.9	0.6	2.0	2.6	9.5
	2003	7	93.4	5.2	0.0	1.4	1.4	6.6
	2004	9	94.2	4.2	1.8	0.0	1.8	6.0
	2006	8	94.8	2.9	1.0	1.3	2.3	5.2
	2007	10	95.4	2.9	0.8	0.8	1.6	4.6
Oceanic countries	2001	6	91.8	4.7	2.7	0.9	3.6	8.2
	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
Russia	2001	1	81.9	15.3	2.8	0.0	2.8	18.1
	2002	1	84.5	9.9	5.6	0.0	5.6	15.5
	2003	1	100.0	0.0	0.0	0.0	0.0	0.0
	2004	4	91.2	6.6	1.5	0.7	2.2	8.8
	2006	5	87.4	8.2	2.7	1.7	4.4	12.6
	2007	8	88.9	5.8	4.8	0.4	5.2	11.0
South America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2
	2002	13	93.7	4.6	0.7	1.0	1.7	6.3
	2003	12	90.8	4.2	2.0	3.0	5.0	9.2
	2004	17	94.4	4.7	0.8	0.1	0.9	5.6
	2006	16	88.7	6.3	4.5	0.6	5.1	11.3
	2007	17	94.9	1.8	1.9	1.4	3.3	5.0
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9
	2002	18	89.0	8.1	1.4	1.6	3.0	11.0
	2003	17	87.4	5.2	4.7	2.7	7.4	12.6
	2004	16	92.8	4.4	2.3	0.5	2.8	7.2
	2006	15	90.0	8.1	1.2	0.8	2.0	10.0
	2007	20	93.9	4.0	1.4	0.7	2.1	6.1

[a] S, susceptible; I, intermediate; R, resistant.
[b] No data available for 2000.

mine whether one susceptibility testing method is more precise than another, participating laboratories should apply both susceptibility testing methods to the same isolates in a series of trials.

The WHO-GSS EQAS is a key component of the capacity-building efforts of WHO-GSS. Consistent sources of deviations in antimicrobial susceptibility results identified in the WHO-GSS EQAS are being addressed through the various activities

of WHO-GSS. Laboratories participating in EQAS receive a deviation report aimed at correcting the sources of deviations. For example, if deviations are limited to a few antimicrobial agents, the laboratory is instructed to evaluate the expiration date of these agents and whether or not proper storage conditions for these agents have been fulfilled. The laboratory is instructed to completely evaluate all parameters of their methodology, if deviations are found for several agents and occur with both the *Salmonella* strains and the reference strain. If deviations occur with a *Salmonella* strain but not the reference strain, the laboratory is instructed to evaluate their breakpoints, e.g., the low number of laboratories that show the reference strain exceeding the quality control range for POD from 2006 to 2007 compared with the higher number of laboratories showing minor deviations for the same antimicrobial (Tables 1 and 3). This example illustrates that some laboratories obtain minor deviations during testing, especially when testing intermediate-resistance isolates, as a result of minor differences in the breakpoint being applied. The curricula of the WHO-GSS international training courses have also been revised to address several of the consistent deviations observed in EQAS. In particular, additional attention is provided to internal quality control methods and the use of appropriate interpretive criteria. Training has also been enhanced for the antimicrobial agents with the most observed deviations. Addressing regional differences will be more challenging but will involve additional training courses in selected regions.

Conclusion. This study showed that there is a continuing need to improve antimicrobial susceptibly testing and that this need appears to be greater in specific regions. We see an essential need to harmonize the methodologies worldwide and provide standardized guidelines for antimicrobial susceptibility testing, particularly for certain antimicrobial agents. Many laboratories reported results for the quality control strain which were outside of the expected range. We feel that it is crucial to emphasize the importance of optimizing methodologies based on internal quality control testing and stress the need to withhold test results when quality control results are outside expected parameters. There is also a need to disseminate, via WHO-GSS and other programs, the latest breakpoint guidelines and to strengthen awareness of performing and interpreting internal quality control as well as to identify the barriers for antimicrobial susceptibility testing in each individual laboratory.

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