Ability of Laboratories To Detect Emerging Antimicrobial Resistance: Proficiency Testing and Quality Control Results from the World Health Organization's External Quality Assurance System for Antimicrobial Susceptibility Testing

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The accuracy of antimicrobial susceptibility data submitted by microbiology laboratories to national and international surveillance systems has been debated for a number of years. To assess the accuracy of data submitted to the World Health Organization by users of the WHONET software, the Centers for Disease Control and Prevention distributed six bacterial isolates representing key antimicrobial-resistance phenotypes to approximately 130 laboratories, all but one of which were outside of the United States, for antimicrobial susceptibility testing as part of the World Health Organization's External Quality Assurance System for Antimicrobial Susceptibility Testing. Each laboratory also was asked to submit 10 consecutive quality control values for several key organism-drug combinations. Most laboratories were able to detect methicillin (oxacillin) resistance in *Staphylococcus aureus*, high-level vancomycin resistance in *Enterococcus faecium*, and resistance to extended-spectrum cephalosporins in *Klebsiella pneumoniae*. Many laboratories, particularly those using disk diffusion tests, had difficulty in recognizing reduced susceptibility to penicillin in an isolate of *Streptococcus pneumoniae*. The most difficult phenotype for laboratories to detect was reduced susceptibility to vancomycin in an isolate of *Staphylococcus epidermidis*. The proficiency testing challenge also included a request for biochemical identification of a gram-negative bacillus, which most laboratories recognized as *Enterobacter cloacae*. Although only a small subset of laboratories have submitted their quality control data, it is clear that many of these laboratories generate disk diffusion results for oxacillin when testing *S. aureus* ATCC 25923 and *S. pneumoniae* ATCC 49619 that are outside of the acceptable quality control range. The narrow quality control range for vancomycin also proved to be a challenge for many of the laboratories submitting data; approximately 27% of results were out of range. Thus, it is important to establish the proficiency of laboratories submitting data to surveillance systems in which the organisms are tested locally, particularly for penicillin resistance in pneumococci and glycopeptide resistance in staphylococci.

Resistance to a variety of antimicrobial agents is emerging in bacterial pathogens throughout the world (8, 21, 50). Increases in the prevalence of penicillin resistance in *Streptococcus pneumoniae* (13, 36, 39, 47), methicillin resistance in *Staphylococcus aureus* (1, 3, 38), vancomycin resistance in enterococci (5, 9, 16, 28), extended-spectrum β-lactamase-production in enteric gram-negative bacilli (2, 18, 37), and fluoroquinolone resistance in *Neisseria gonorrhoeae* (17) are just a few examples of the rising problem of resistance documented by both national and international surveillance systems in the past few years. The antimicrobial susceptibility testing data collected by the various surveillance systems are generated in several different fashions. In some systems, bacterial isolates are sent to a central laboratory for testing using a standardized reference method (12, 43), and the data are compiled and maintained by the central laboratory. In other systems, the Etest method is used by participating laboratories to test isolates locally, and the data are forwarded to a central database (10, 35). A third approach to surveillance is to collect the antimicrobial susceptibility testing data directly from microbiology laboratories by either a direct computer link (40) or through diskettes sent to a central laboratory in which standardized computer database software such as the WHONET is used (49). These systems usually employ a series of data checks and quality control filters before the data are entered into the larger database. The final surveillance system consists of the microbiology laboratories throughout the world that perform routine antimicrobial susceptibility testing of bacterial isolates but that do not belong to one of the above systems (40). This vast network, which generates tens of thousands of susceptibility results daily, is a significant source of resistance data. It is often through this system of astute clinical microbiologists that new resistant organisms are detected (30).

The proliferation of surveillance systems internationally has raised the issue of the accuracy of the data collected, particularly for systems in which testing occurs outside of a central site. The need to obtain and review the quality control data

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from noncentralized laboratories or to assess the competence of laboratories both before data collection begins and during testing has resulted in the call for wider proficiency testing of laboratories.

In 1995, the World Health Organization (WHO) initiated a program of quality control and proficiency testing focused on antimicrobial susceptibility testing to assist laboratories in both developed and developing countries to assess the accuracy of their antimicrobial susceptibility testing data. The goals of the program were (i) to assist laboratories, particularly those that were WHONET software users, in evaluating the quality of their antimicrobial susceptibility testing data, (ii) to validate the susceptibility testing data submitted to WHO and the WHO Collaborating Center for Surveillance of Antimicrobial Resistance in Boston, (iii) to provide guidance for laboratories wishing to develop quality assurance programs, and (iv) to provide laboratories with organisms that manifest novel antimicrobial resistance so that they could confirm that their susceptibility testing methods were capable of detecting the emerging resistance patterns. The program, which began with 17 laboratories, all of which were located outside of the United States, has evolved into the WHO External Quality Assurance System for Antimicrobial Susceptibility Testing (WHO-EQAS), which now includes approximately 130 laboratories in 42 countries (including one laboratory in the United States). Several countries have set up national quality assurance systems further distributing the proficiency testing strains to an additional 200 laboratories. Herein, we describe the results reported by approximately 130 laboratories for the first six proficiency testing challenges.

MATERIALS AND METHODS

Organism challenges. Three sets of two organisms each were sent to approximately 130 laboratories in 42 countries over a 3-year period (1996 through 1999). Most of the laboratories were WHONET software users or laboratories that had participated in WHO laboratory training programs. Some laboratories participated in only one or two of the challenges, and additional laboratories were continually added to the list of participants throughout the study period. New regulations disallowing the importation of infectious substances into various countries also limited the participation of several laboratories. Thus, the number of results and the antimicrobial agents tested for each organism in this report vary. The list of countries represented among the participating laboratories is shown in Table 1 by WHO region. The organisms were tested multiple times at the Centers for Disease Control and Prevention (CDC) by broth microdilution and disk diffusion using National Committee for Clinical Laboratory Standards (NCCLS) methods (32–34) to establish the reference MIC and disk diffusion values. In some cases (e.g., for methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus faecium*, and erythromycin-resistant *S. pneumoniae*), PCR assays for *mecA*, *vanA*, and *mefE*, respectively, were used to confirm the resistance mechanisms (54). A data collection sheet was provided with each organism, requesting information on the antimicrobial susceptibility testing method used (MIC or disk diffusion), source of media and reagents, interpretive criteria used (e.g., NCCLS [32–34], the Comité de l'Antibiogramme de la Société de Française Microbiologie [CA-SFM] [22], or the British Society for Antimicrobial Chemotherapy [BSAC] [57]), and disk potency. The data sheets included a suggested set of antimicrobial agents to test and provided space to fill in additional drugs that were tested in the laboratory. Laboratories were asked to provide both quantitative results (MICs or zone diameters) and the qualitative interpretations (i.e., susceptible, intermediate, or resistant [S, I, or R, respectively]) for each antimicrobial agent tested. Data sheets were returned to CDC for analysis. The data were entered into a SAS data set (SAS, Cary, N.C.). Because NCCLS, CA-SFM, and BSAC often use conflicting interpretive criteria, only laboratories using NCCLS methods and interpretive criteria were considered for this manuscript, to make comparison of data feasible. If the laboratory provided an incorrect interpretation (i.e., an interpretive error) for an MIC or disk diffusion result, the correct interpretation (S, I, or R) was entered into Tables 3, 5, 6, 7, 8, or 9 (see

TABLE 1. Number of participating laboratories by WHO region and country

Region/country	No. of laboratories[a]
African Region	
Algeria	1
Kenya	4
South Africa	1
Sudan	1
Uganda	1
Eastern Mediterranean Region	
Jordan	1
Kuwait	2
Lebanon	1
Saudi Arabia	15
European Region	
Bulgaria	2
Croatia	15
Czech Republic	2
Finland	19
Greece	1
Hungary	1
Italy	1
Poland	1
Russian Federation	1
Slovakia	1
Turkey	1
Region of the Americas	
Argentina	24
Colombia	1
Guatemala	1
United States	1
Uruguay	3
Venezuela	3
Southeast Asian Region	
Hong Kong	1
India	2
Indonesia	1
Myanmar	1
Sri Lanka	1
Thailand	2
Western Pacific Region	
China	11
Japan	59
Korea	1
Malaysia	1
Mongolia	1
Philippines	1
Viet Nam	2

[a] Because some laboratories joined the system later than others and some completed testing of only the first four organisms, the number of participating laboratories listed exceeds 130.

below), and the error was noted in a footnote to these tables. The interpretive errors from laboratories using disk diffusion are included in Table 10 below, and interpretive errors from laboratories using MIC methods are listed in Table 11 below.

RESULTS

Organism challenges. The antimicrobial susceptibility testing methods used by participating laboratories for each of the challenge organisms are shown in Table 2.

Challenge WHO-1. The first challenge strain (WHO-1) was an isolate of *Klebsiella pneumoniae* that produced a TEM-3 extended-spectrum β-lactamase (ESBL) (48) (Table 3). One hundred and thirty laboratories reported results; 63.9% (83 of 130) used a disk diffusion method, and 36.1% (47 of 130) used

TABLE 2. Antimicrobial susceptibility testing methods used by participating laboratories by organism[a]

Strain	No. of results	No. of participating laboratories that used method							
		Disk diffusion	Broth microdilution[a]	MIC 2000[a]	MicroScan[a]	Sceptor[a]	Sensititre[a]	Vitek[a]	MIC method not identified
WHO-1	130	83	2	7	23	9	2	3	1
WHO-2	127	75	3	7	25	9	2	5	1
WHO-3	122	72	4	7	23	8	2	5	1
WHO-4	128[b]	109	2	4	1	5	1	0	6
WHO-5	130	106	2	3	12	1	2	3	1
WHO-6	130	105	2	0	19	1	1	2	0

[a] Manufacturers: Broth microdilution, no manufacturer or method specified; MIC 2000, Eiken Ltd., Tokyo, Japan; MicroScan, Dade Behring, West Sacramento, Calif.; Sceptor, Becton Dickinson Microbiology Systems, Cockeysville, Md.; Sensititre, Trek Diagnostics, Westlake, Ohio; Vitek, bioMérieux, Hazelwood, Mo.
[b] Forty laboratories performed the oxacillin screen test and determined the penicillin MIC of the organism using the Etest method.

an MIC method (Table 2). The extended-spectrum cephalosporins and monobactams tested most frequently by participating laboratories are shown in Table 4. Six laboratories (four disk diffusion users and two MIC users) failed to test any extended-spectrum cephalosporins or aztreonam. Overall, 5.4% (7 of 130) laboratories (all disk diffusion users) reported WHO-1 to be susceptible to all extended-spectrum cephalosporins. For individual drugs, 91.4% of 105 laboratories reported an intermediate or resistant result for ceftazidime, 86.9% of 92 laboratories reported an intermediate or resistant result for cefotaxime, and 81.2% of 16 reported an intermediate or resistant result for ceftriaxone. On the other hand, 11.1% (5 of 45) of laboratories testing cephalothin, a narrow-spectrum cephalosporin, reported the organism incorrectly as susceptible to this agent. Of the nine laboratories reporting ceftazidime-susceptible results, six also reported susceptible results for cefotaxime or ceftriaxone. Only 2 of the 130 laboratories specifically reported the isolate as an "ESBL-producing strain." None changed the results for extended-spectrum cephalosporins or aztreonam from susceptible to resistant based on detection of resistance to another extended-spectrum cephalosporin, as suggested by NCCLS (34). Failure to test extended-spectrum cephalosporins ($n = 6$) and reporting of susceptible results for all extended-spectrum cephalosporins ($n = 7$) (this includes the five laboratories that reported susceptible results for cephalothin) were considered unacceptable results.

Challenge WHO-2. The second challenge strain (WHO-2) was a methicillin (oxacillin)-resistant, *mecA*-positive *S. aureus* (MRSA) (Table 5). One hundred twenty-seven laboratories reported results; 59.1% (75 of 127) reported disk diffusion results, and 40.9% reported MIC results. The MRSA strain demonstrated high-level resistance to oxacillin, erythromycin, clindamycin, and tetracycline. Approximately 91% of laboratories recognized the strain as an MRSA. Only one laboratory reported both penicillin and oxacillin as susceptible results, but eight other laboratories reported the strain as oxacillin or methicillin susceptible. These were considered unacceptable results. Three additional challenge strains of *S. aureus* were sent to the nine laboratories that reported the strain as oxacillin susceptible. Of the five laboratories returning results, four were successful in differentiating the two MRSA strains from the susceptible *S. aureus* strain.

Challenge WHO-3. The third challenge (WHO-3) was a vancomycin-resistant strain of *E. faecium* (VRE; previously referred to as NJ-1 [55]) that was also borderline resistant to penicillin but (using NCCLS breakpoints) susceptible to ampicillin. Of the 122 laboratories that tested this organism, 59.0% used disk diffusion and 41.0% used an MIC method. Only 2.6% (3 of 117) of laboratories testing vancomycin failed to recognize this strain, which contained the *vanA* resistance gene, as highly resistant to vancomycin (Table 6). While 80.3% of laboratories correctly reported the strain as penicillin resistant, 21.0% incorrectly reported it as ampicillin resistant using

TABLE 3. Results of testing *K. pneumoniae* (WHO-1)

Antimicrobial agent (no. of labs that tested)[a]	Reference result (interpretive category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in μg/ml)		
	MIC in μg/ml	Disk zone diameter in mm	S	I	R	S	I	R
Aztreonam (9)	32 (R)	16 (I)	3 (28–32)	1 (20)[b]	0	0	1 (16)	4 (>16–>32)
Cefazolin (74)	>32 (R)	7 (R)	0	0	24 (6–14)	0	0	50 (>16–>128)
Cefotaxime (92)	>64 (R)	12 (R)	6 (28–34)	18 (15–22)[b,c]	32 (6–14)	6 (4–8)	8 (16–32)[d]	22 (>32)
Ceftriaxone (16)	>64 (R)	12 (R)	3 (27–32)	0	13 (6–13)[e]	0	0	0
Ceftazidime (105)	128 (R)	11 (R)	9 (18–30)[d]	4 (15–17)[f]	57 (6–14)	0	4 (16)[d]	31 (>16–>32)
Cephalothin (45)		6 (R)	5 (18–22)	2 (15–16)	35 (6–14)	0	0	3 (32–>256)

[a] Labs returning results, 130.
[b] One incorrectly called susceptible.
[c] Seven incorrectly called resistant.
[d] One incorrectly called resistant.
[e] One incorrectly called intermediate.
[f] Two incorrectly called resistant.

TABLE 4. Extended-spectrum cephalosporins and monobactams
tested against WHO-1 by 130 laboratories

Antimicrobial agent(s) tested	No. of laboratories	No. of laboratories reporting all extended-spectrum cephalosporins as susceptible
Ceftazidime, cefotaxime	68	4
Cefotaxime only	18	
Ceftazidime, ceftriaxone	14	1
Ceftazidime only	14	
No extended-spectrum cephalosporins nor monobactams	6	
Aztreonam, ceftazidime	4	
Aztreonam, ceftazidime, cefotaxime	4	
Aztreonam, cefotaxime	1	
Ceftazidime, cefotaxime, ceftriaxone	1	1
Ceftriaxone only	1	1

NCCLS interpretive criteria. Three of 39 (7.7%) laboratories
reported the isolate incorrectly as beta-lactamase positive. As
the beta-lactam results were on the borderline and represented
an unusual phenotype, for this challenge, reporting vancomy-
cin-susceptible results ($n = 3$) was considered unacceptable
performance.

Challenge WHO-4. The fourth challenge (WHO-4) was an
erythromycin-resistant (*mefE*-positive) strain of *S. pneumoniae*
with reduced susceptibility to penicillin. The penicillin MIC
varied from 0.06 μg/ml (susceptible) to 0.12 μg/ml (interme-
diate), but it invariably produced a zone diameter of 14 to 17
mm around a 1-μg oxacillin disk, which, according to NCCLS
guidelines, indicates that an MIC test should be performed to
determine if the isolate is susceptible or resistant to penicillin.
Of the 89 laboratories that performed an oxacillin screen test,
35.9% reported a value of ≥20 mm, which indicates suscepti-
bility to β-lactam agents (Table 7). Only 59.4% (76 of 128) of
laboratories reported an MIC result for penicillin. Although
NCCLS does not recommend penicillin or cephalosporin disk
diffusion tests for pneumococci, two laboratories reported re-
sults for a 1-U penicillin disk, and six reported results for a
10-U penicillin disk. In addition, 21 laboratories reported val-
ues for 30-μg cefotaxime disks, and 15 reported values for
30-μg ceftriaxone disks. Thirteen laboratories (10.2%) incor-
rectly reported the organism as susceptible to erythromycin,
and 2 reported it incorrectly as intermediate or resistant to
clindamycin. Reporting oxacillin zone diameters of ≥20 mm, a

penicillin MIC of ≥2 μg/ml or reporting erythromycin-suscep-
tible results was considered unacceptable performance for this
organism ($n = 40$).

Challenge WHO-5. The fifth challenge (WHO-5) was an
Enterobacter cloacae strain that was sent for identification
and antimicrobial susceptibility testing. It was resistant to all
penicillins, cephamycins, and cephalosporins, including extend-
ed-spectrum cephalosporins, but was susceptible to the amino-
glycosides, trimethoprim-sulfamethoxazole, ciprofloxacin, imi-
penem and meropenem (4, 42). Few laboratories had problems
in recognizing the β-lactam resistance in this strain (Table 8),
although 6.1% of laboratories (8 of 130) failed to identify this
organism as an *Enterobacter* species. Susceptible results for
ampicillin or extended-spectrum cephalosporins were consid-
ered to be unacceptable performance ($n = 3$). Because the
focus of the survey was on susceptibility testing results and not
identification, misidentifications were not considered as unac-
ceptable performance.

Challenge WHO-6. The sixth challenge (WHO-6) was a glyco-
peptide-intermediate strain of *Staphylococcus epidermidis* (20).
The reference MIC of vancomycin for this organism was 8 μg/
ml, and the MIC of teicoplanin was 16 μg/ml. Of the 130 lab-
oratories testing this organism, 105 (80.8%) used disk diffusion,
and 19.2% used an MIC method. Ninety-seven (74.6%) of 130
laboratories testing vancomycin reported this isolate as vanco-
mycin susceptible (Table 9), which was considered unaccept-
able performance. This included a laboratory that reported a
teicoplanin zone diameter of 6 mm, which also would be con-
sidered incorrect (since it is an unusually small zone diameter).
Four laboratories that tested vancomycin by disk diffusion re-
ported zone diameters of 6 to 14 mm, which are unusually
small for this strain (52). The two reports of vancomycin zone
diameters of 6 mm were also considered unacceptable perfor-
mance.

**Overall performance and interpretation and reporting er-
rors.** Of the 74 laboratories that sent results on at least five of
the six challenge organisms, 17 had fully acceptable results, 33
reported unacceptable results for a single challenge, 20 re-
ported unacceptable results for 2 challenges, and 4 reported
unacceptable results for 3 challenges. None reported unaccept-
able results for four or more of the challenges.

In addition to the unacceptable results reported above,
which primarily represent very major testing errors (where the

TABLE 5. Results of testing for *S. aureus* WHO-2

Antimicrobial agent (no. of labs that tested antimicrobial agent)[a]	Reference result (category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in μg/ml)		
	MIC in μg/ml	Disk zone diameters in mm	S	I	R	S	I	R
Erythromycin (121)	>8 (R)	6 (R)	1 (25)	3 (17–22)[b,c]	68 (6–13)	0	0	49 (>4–>32)
Oxacillin (117)	32 (R)	6 (R)	9 (13–23)	1 (12)	57 (6–10)	0	0	50 (>4–>64)
Penicillin (99)	128 (R)	7 (R)	1 (32)	0	64 (6–28)	0	0	34 (>8–>16)[b]
Trimethoprim-sulfa-methoxazole (106)	≤0.25 (S)	21 (S)	68 (16–35)[d,e]	0	0	37 (0.12–<2)	0	1 (5)[b]

[a] Labs returning results, 127.
[b] One incorrectly called susceptible.
[c] Two incorrectly called resistant.
[d] One incorrectly called intermediate.
[e] One incorrectly called resistant.

TABLE 6. Results of testing for *E. faecium* WHO-3

Antimicrobial agent (no. of labs that tested antimicrobial agents)[a]	Reference result (category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in µg/ml)		
	MIC in µg/ml	Disk zone diameter in mm	S	I	R	S	I	R
Ampicillin (95)	4 (S)	19 (S)	36 (17–36)[b]	0	19 (6–16)[c]	39 (1–8)[d]	0	1 (>4)
Penicillin (71)	32 (R)	6 (R)	4 (15–20)[e]	0	32 (6–13)	10 (8)	0	25 (>1–>16)
Teicoplanin (26)	256 (R)	6 (R)	1 (17)	3 (11–12)[e]	19 (6–10)	1 (0.25)	0	2 (>16–32)
Vancomycin (117)	512 (R)	6 (R)	3 (21–30)	0	63 (6–12)	0	0	51 (>8–64)[d]
β-Lactamase (39)	Negative				36 negative, 3 positive			

[a] Labs returning results, 122.
[b] Three incorrectly called intermediate.
[c] One incorrectly called susceptible.
[d] One incorrectly called intermediate.
[e] One incorrectly called resistant.

reference result was reported as resistant, and the proficiency test result was reported as susceptible), 61 of the S, I, or R disk diffusion categorical interpretations (Table 10) and 13 of the MIC categorical interpretations (Table 11) provided by the participating laboratories for the six challenge organisms were incorrect. These represent 5 very major errors, 15 major errors, and 54 minor errors. The majority of errors were reported for the *S. epidermidis* isolate (*n* = 24). Beta-lactam testing of the *K. pneumoniae* isolate also resulted in a large number of reporting errors. Some of the errors with oxacillin testing of the *S. epidermidis* isolate probably reflect recent changes in the NCCLS breakpoint for this organism-drug combination (34).

Quality control data. Each of the laboratories participating in the WHO-EQAS system was asked to submit 10 consecutive quality control results for *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. pneumoniae* ATCC 49619 for analysis. Data were available from approximately 35 laboratories using NCCLS quality control ranges. Figure 1 demonstrates that many of the quality control results from oxacillin disk diffusion tests for *S. aureus* ATCC 25923 fell outside the specified control range. Of the 654 data points submitted by 35 laboratories, 16 values (2.5%) (from 3 different laboratories) were below the range and 162 values (24.8%) (from 22 laboratories) were above the range. By comparison, 130 of 705 disk

diffusion results (18.4%) reported by 38 laboratories were out of range when testing vancomycin against the *S. aureus* control (Fig. 2). Twenty-eight laboratories reported values for vancomycin that were below the NCCLS quality control range, while 11 laboratories reported values beyond the upper end of the range. For oxacillin testing of *S. pneumoniae* ATCC 49619, 30 laboratories reported 167 of 606 of their results (27.6%) above the designated range, and 15 laboratories reported 92 results (15.2%) below the range (Fig. 3). For erythromycin testing of *S. pneumoniae* ATCC 49619, 41 of 677 values (6.1%) from 16 laboratories were below the range, and 149 values (22.0%) were above the range (Fig. 4). For *E. coli* ATCC 25922, ceftazidime results were more often in control. Of 500 reported values, only 19 (3.8%) were below the designated range, and 45 (9.0%) were above the range (Fig. 5).

DISCUSSION

The WHO-EQAS was designed to enhance the ability of laboratories to detect organisms with emerging antimicrobial resistance patterns and to ensure that the laboratories' reporting strategies for antimicrobial resistance were accurate. Overall, approximately 20% of laboratories reported fully acceptable results, while the number reporting unacceptable results

TABLE 7. Results of testing for *S. pneumoniae* WHO-4

Antimicrobial agent (no. of labs that tested antimicrobial agent)[a]	Reference result (category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in µg/ml)		
	MIC in µg/ml	Disk zone diameter in mm	S	I	R	S	I	R
Clindamycin (115)	0.12 (S)	22 (S)	94 (19–40)	1 (18)[b]	0	19 (≤0.12–≤0.25)		1 (>8)
Erythromycin (128)	4 (R)	10 (R)	9 (21–34)[c]	6 (16–19)	94 (6–15)[b,d]	4 (≤0.5)	0	15 (1–16)[e]
Penicillin (76)	0.06 (S)	na[f]				61 (0.007–0.06)	14 (0.12–1.0)[g]	1 (≥2.0)
Oxacillin screen (89)	na[f]	15	32 (20–28)[h]		57 (6–19)[i]			

[a] Labs returning results, 128.
[b] One incorrectly called susceptible.
[c] One incorrectly called intermediate.
[d] Two incorrectly called intermediate.
[e] Three incorrectly called intermediate.
[f] na, not applicable.
[g] Three incorrectly called susceptible.
[h] Results are for zone diameters of ≥20 mm.
[i] Results are for zone diameters of ≤19 mm. A result of ≤19 mm is not necessarily resistant; NCCLS recommends that a penicillin MIC test be performed.

TABLE 8. Results of testing for *E. cloacae* WHO-5

Antimicrobial agent (no. of labs that tested antimicrobial agent)[a]	Reference result (category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in μg/ml)		
	MIC in μg/ml	Disk zone diameter in mm	S	I	R	S	I	R
Ampicillin (124)	>64 (R)	6 (R)	1 (22)	0	100 (5–8)	1 (4)	0	22 (>16–>128)
Cefotaxime (81)	64 (R)	8 (R)	0	1 (17)	64 (6–13)	0	2 (32)	14 (>32–128)
Ceftazidime (111)	>128 (R)	6 (R)	0	0	98 (6–13)	0	0	13 (>16–>128)
Ceftriaxone (45)	>64 (R)	8 (R)	1 (22)	0	42 (6–12)	0	0	2 (64)
Ciprofloxacin (109)	≤0.06 (S)	30 (S)	100 (22–37)[b]	0	1 (6)	8 (0.15–<1)	0	0
Gentamicin (115)	≤0.25 (S)	20 (S)	93 (15–28)[b,c]	0	0	22 (0.12–<1)	0	0
Imipenem (121)	1–2 (S)	22 (S)	96 (17–29)[d]	0	1 (6)	24 (0.5–<4)[e]	0	0
Tobramycin (77)	0.5 (S)	18 (S)	66 (16–28)	1 (13)	1 (11)	9 (0.12–<4)	0	0
Trimethoprim-sulfa-methoxazole (118)	≤0.12 (S)	24 (S)	97 (16–32)[b,c]	0	2 (6)	19 (0.001–<4)	0	0
Identification (126)	*E. cloacae*		114 correct, 4 *Enterobacter* species, 8 incorrect					

[a] Labs returning results, 130.
[b] One incorrectly called intermediate.
[c] Two incorrectly called resistant.
[d] Two incorrectly called intermediate.
[e] One incorrectly called resistant.

for three or more of the challenges was low (<5%). Most laboratories had problems with the *S. epidermidis* and *S. pneumoniae* isolates (see below).

Because of ongoing changes in the interpretive criteria for antimicrobial susceptibility testing (e.g., NCCLS, BSAC, or CA-SFM), it is possible to perform a test correctly but report inaccurate results, particularly if old guidelines or criteria are used. There were 74 instances in this study in which the results of the susceptibility tests were interpreted incorrectly, which, in a clinical setting, would have resulted in inaccurate information being transmitted to the patient's chart. The goal of quality assurance is to detect and correct problems such as these in a continuous fashion to improve the accuracy of testing, record keeping, and reporting (41, 44). The WHO-EQAS proficiency testing program was designed specifically to help laboratories determine whether all aspects of their current antimicrobial susceptibility testing methods are accurate. To this end, feedback letters detailing the reporting errors were sent to each participating laboratory as part of the quality assurance aspect of the study.

For the proficiency testing portion of the WHO-EQAS, it was gratifying to observe that most laboratories were able to detect methicillin resistance in *S. aureus* and high-level vancomycin resistance in *E. faecium*. Only a few laboratories had problems detecting resistance to the extended-spectrum cephalosporins mediated by the chromosomal AmpC β-lactamase in the *E. cloacae* isolate; additional laboratories also had problems identifying this isolate biochemically, but the two groups of laboratories did not completely overlap. Assessing the accuracy of bacterial identification was not a major goal of this

TABLE 9. Results of testing for *S. epidermidis* WHO-6

Antimicrobial agent (no. of labs that tested antimicrobial agent)[a]	Reference result (category)		No. of labs reporting disk diffusion results (range of zone diameters in mm)			No. of labs reporting MIC results (range of MICs in μg/ml)		
	MIC in μg/ml	Disk zone diameter in mm	S	I	R	S	I	R
Ciprofloxacin (87)	>8 (R)	6 (R)	2 (26–30)	1 (20)[b]	82 (6–10)	1 (1)	0	1 (64)
Clindamycin (117)	0.25 (S)	26 (S)	90 (22–42)	2 (19)[c]	0	25 (<0.12–0.5)	0	0
Erythromycin (124)	0.5 (S)	28 (S)	92 (24–42)	7 (19–22)[d]	0	22 (≤0.2–0.5)	3 (1)	0
Gentamicin (116)	16 (R)	10 (R)	9 (15–30)[b]	4 (13–14)[e]	77 (6–12)[f]	0	0	26 (>8–32)
Oxacillin (127)	>16 (R)	6 (R)	14 (18–30)	0	86 (6–16)[g,h]	0	0	27 (>4–>256)
Penicillin (121)	>2 (R)	10 (R)	0	0	93 (6–25)	0	0	28 (8–64)
Rifampin (90)	≤0.12 (S)	40 (S)	77 (23–48)	0	3 (6–10)	10 (<0.008–<1)		
Teicoplanin (49)	16 (I)	14 (S)	30 (14–24)[f]	10 (11–13)[e]	1 (6)	1 (4)	3 (8–16)	4 (>16–32)
Trimethoprim-sulfa-methoxazole (121)	76/4 (R)	6 (R)	2 (30–32)	5 (12–15)[b]	88 (6–10)	1 (2)	0	25 (>2–160)
Vancomycin (127)	8 (I)	18 (S)	92 (15–34)	0	4 (6–14)[c]	5 (1–4)	26 (8–16)	0

[a] Labs returning results, 130.
[b] One incorrectly called resistant.
[c] One incorrectly called susceptible.
[d] Three incorrectly called susceptible.
[e] Two incorrectly called resistant.
[f] One incorrectly called intermediate.
[g] Seven incorrectly called susceptible.
[h] Three incorrectly called intermediate.

TABLE 10. Interpretation errors by disk diffusion users

Antimicrobial agent	No. and category of errors for bacterial strain:					
	K. pneumoniae WHO-1	S. aureus WHO-2	E. faecium WHO-3	S. pneumoniae WHO-4	E. cloacae WHO-5	S. epidermidis WHO-6
Ampicillin			1 very major 3 minor			
Aztreonam	1 minor					
Cefotaxime	8 minor					
Ceftazidime	1 major 2 minor					
Ceftriaxone	1 minor					
Ciprofloxacin					1 minor	1 minor
Clindamycin				1 minor		1 minor
Erythromycin		3 minor		1 very major 3 minor		3 minor
Gentamicin					2 major 1 minor	1 major 3 minor
Imipenem					2 minor	
Oxacillin						7 major 3 minor
Penicillin			1 major			
Teicoplanin			1 minor			3 minor
Trimethoprim-sulfamethoxazole	1 major 1 minor				1 major 1 minor	1 minor
Vancomycin						1 very major

program, but since NCCLS recommends testing for ESBL production among *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca* isolates, we wanted to determine if most laboratories could differentiate *E. cloacae* from *K. pneumoniae*.

The ESBL-producing *K. pneumoniae* was one of the first isolates sent to most participating laboratories and preceded publication of the NCCLS guidelines for ESBL detection (34). While approximately 88% of laboratories reported that the *K. pneumoniae* isolate was resistant to at least one extended-spectrum cephalosporin, only 1.5% reported that it was specifically an extended-spectrum β-lactamase producer. None of the laboratories modified the interpretations of the other cephalosporins to "resistant" as currently suggested by NCCLS (34). Based on feedback from participating laboratories, the issue of ESBL reporting is a source of confusion for many laboratories

and may take additional educational efforts before improvement will be seen (11, 53).

The key problem areas identified in our surveys were detection of reduced susceptibility to penicillin in pneumococci and detection of reduced susceptibility to glycopeptides in staphylococci. Approximately 36% of the laboratories reported oxacillin zone diameters of ≥20 mm for the *S. pneumoniae* strain WHO-4, indicating a failure to detect reduced susceptibility to penicillin. Of those laboratories that did report a zone diameter of ≤19 mm, only 68.2% reported an MIC result. Previous reports have noted that the oxacillin screening test for pneumococci is very sensitive and rarely fails to detect penicillin-resistant pneumococci. However, the test lacks specificity, often yielding zone diameters of ≤19 mm for strains of pneumococci for which the penicillin MICs are 0.03 to 0.06

TABLE 11. Interpretation errors by MIC users

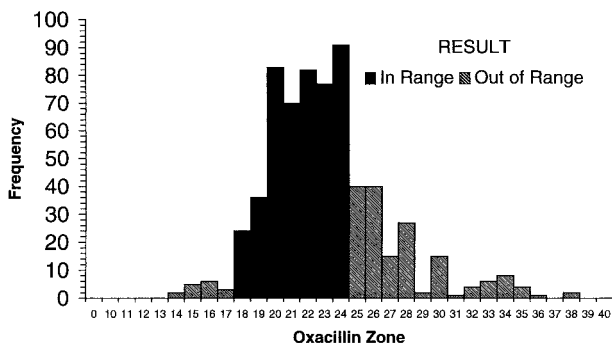| Antimicrobial agent	No. and category of errors for bacterial strain:					
	K. pneumoniae WHO-1	S. aureus WHO-2	E. faecium WHO-3	S. pneumoniae WHO-4	E. cloacae WHO-5	S. epidermidis WHO-6
Ampicillin			1 minor			
Aztreonam						
Cefotaxime	1 minor					
Ceftazidime	1 minor					
Ceftriaxone						
Ciprofloxacin						
Clindamycin						
Erythromycin				3 minor		
Gentamicin						
Imipenem					1 major	
Oxacillin						
Penicillin		1 very major		3 minor		
Teicoplanin						
Trimethoprim-sulfamethoxazole		1 very major				
Vancomycin			1 minor			

FIG. 1. Histogram showing quality control results for disk diffusion testing of *S. aureus* ATCC 25923 against oxacillin. The NCCLS quality control range against which results were compared was 18 to 24 mm. Data are all results received as of 30 August 1999 and tested by NCCLS methods.
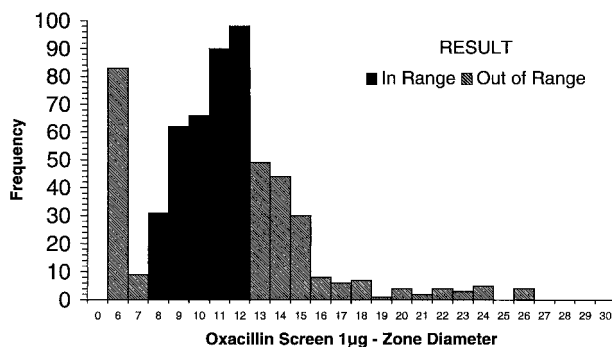

FIG. 3. Histogram showing quality control results for disk diffusion testing of *S. pneumoniae* ATCC 49619 against oxacillin. The NCCLS quality control range against which results were compared was 8 to 12 mm. Data are all results received as of 24 August 1999 and tested by NCCLS methods.

µg/ml (15, 27). For this reason, the NCCLS has recommended that all pneumococci yielding oxacillin zone diameters of ≤19 mm should be tested for penicillin resistance using an MIC method (32). The range of penicillin MICs reported for this isolate was also remarkably wide, from 0.007 to 2.0 µg/ml. The modal penicillin MIC reported at CDC was 0.06 µg/ml; thus, several laboratories significantly overcalled the resistance level of this organism. Of the 76 laboratories reporting a penicillin MIC result, 40 used Etest and the other 36 used a variety of methods (Table 2). Proficiency testing results from pneumococcal challenges conducted in United States laboratories as collected in recent surveys by the College of American Pathologists (CAP) showed similar testing problems with β-lactam drugs, indicating that testing of pneumococci for susceptibility to penicillin and cephalosporins in the United States is far from optimal (14). One of the key problems recognized in both the WHO-EQAS and CAP studies is the continued use of penicillin, cefotaxime, and ceftriaxone disks for pneumococcal testing. Although NCCLS has not approved interpretive criteria for these disks, laboratories continue to use them. The zone diameters and interpretations sent to the WHO-EQAS project indicate that a combination of gram-negative enteric, staphylococcal, and occasionally enterococcal breakpoints is being

used to interpret results, which frequently leads to under reporting of resistance. Clearly, a portion of the problem relates to the expense of performing MIC versus disk diffusion tests in many developing countries. While disk diffusion methods for β-lactam drugs were proposed many years ago (26), they have not been embraced by NCCLS because of their poor predictive values in multicenter studies. More recent attempts to optimize disk testing using multidisk systems have been encouraging (25), but the accuracy of the method has yet to be independently confirmed. Given the wide intermediate range for penicillin (0.1 to 1.0 µg/ml), it is very difficult to devise a disk diffusion test using a 2-U or 10-U penicillin disk that accurately differentiates susceptible and resistant isolates. Because the breakpoint of 0.1 µg/ml is so important for optimal therapy of pneumococcal meningitis (19, 24, 31, 56) and because high-level ceftriaxone- and cefotaxime-resistant pneumococci have emerged (45), the importance of using accurate MIC methods for penicillin and extended-spectrum cephalosporins cannot be overstated. Although the Etest method has been shown to be an accurate predictor of penicillin and cephalosporin resistance (29), it is often too expensive for widespread use in developing countries.

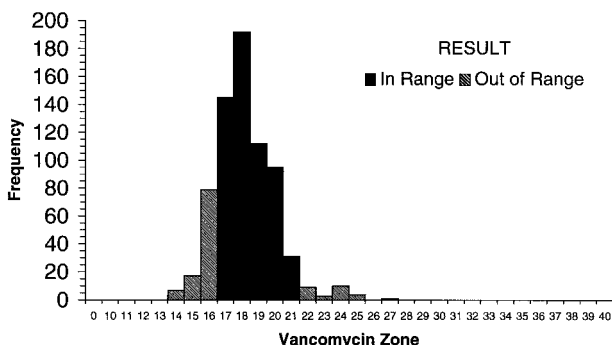The emergence of reduced susceptibility to vancomycin in

FIG. 2. Histogram showing quality control results for disk diffusion testing of *S. aureus* ATCC 25923 against vancomycin. The NCCLS quality control range against which results were compared was 17 to 21 mm. Data are all results received as of 25 August 1999 and tested by NCCLS methods.
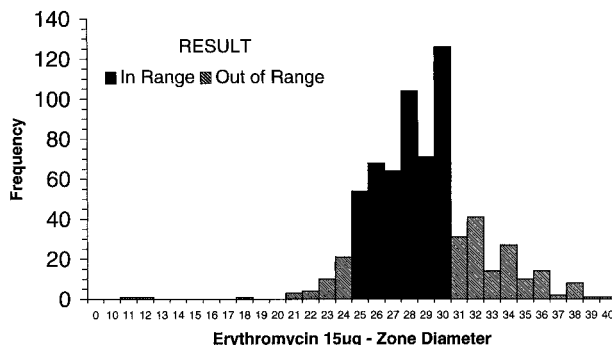

FIG. 4. Histogram showing quality control results for disk diffusion testing of *S. pneumoniae* ATCC 49619 against erythromycin. The NCCLS quality control range against which results were compared was 25 to 30 mm. Data are all results received as of 24 August 1999 and tested by NCCLS methods.
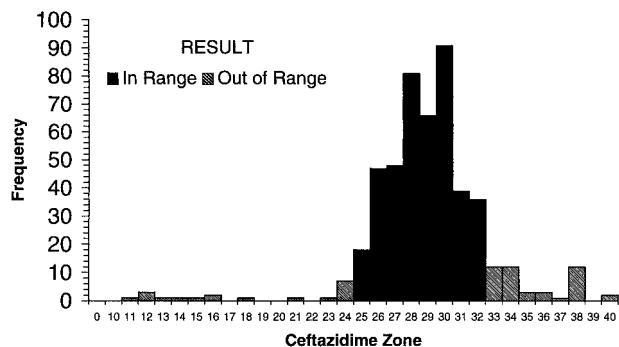
FIG. 5. Histogram showing quality control results for disk diffusion testing of *E. coli* ATCC 25922 against ceftazidime. The NCCLS quality control range against which results were compared was 25 to 32 mm. Data are all results received as of 30 August 1999 and tested by NCCLS methods.

S. aureus is a serious public health issue (23, 46). CDC and its advisory committees have published recommendations on preventing the spread of vancomycin-resistant organisms (7) and separate guidelines on preventing the development and spread of staphylococci with reduced susceptibility to vancomycin (6). In the WHO-EQAS survey, few laboratories were successful in detecting reduced susceptibility to vancomycin in the *S. epidermidis* isolate. This is primarily because such organisms cannot readily be detected by disk diffusion testing (52). Testing of staphylococci, particularly for vancomycin resistance, in laboratories where disk diffusion is used will require the use of an alternate method, such as brain heart infusion agar plates containing 6 μg of vancomycin per ml (developed for enterococci), which have been shown to detect most of the glycopeptide-intermediate staphylococci to date (52). This test, which is inexpensive to prepare, should allow laboratories to detect strains of staphylococci with emerging glycopeptide resistance. Many laboratories also had problems with oxacillin testing of this isolate. Recently, NCCLS changed the oxacillin breakpoints for coagulase-negative staphylococci (34, 51). Based on the results submitted to WHO-EQAS, it appears that many laboratories are unaware of this change.

Quality control testing ensures that the reagents and media and the technologist conducting the test are performing in a predictable and reliable fashion. Unfortunately, adherence to a quality control program is often lax in both developed and developing countries, where it is considered to be either too time consuming or too expensive to perform or both. This is probably why only 30 to 40 laboratories have sent quality control data to WHO-EQAS to date. The quality control data received from the participating laboratories showed many failures. However, it is encouraging that the laboratories continue to generate and use such data to optimize their testing methods. The data from the WHO-EQAS studies have been reviewed by NCCLS to determine whether some of the narrow ranges, such as that for oxacillin and *S. pneumoniae* ATCC 49619, require revision. Quality control testing is at the heart of good surveillance data and should be reviewed and verified prior to accepting data from sentinel laboratories participating in surveillance systems, particularly if such data are used to guide changes in empiric antimicrobial chemotherapy.

Overall, the results of the WHO-EQAS challenges have been encouraging. However, there is a clear need for educational programs that emphasize proper laboratory testing methods, the importance of quality control, and the basic concepts of quality assurance. The majority of participants in the WHO-EQAS used disk diffusion as their testing method. While this technique is suitable for routine surveillance for resistance in most nonfastidious pathogens, it does have its drawbacks, particularly for testing beta-lactam agents against pneumococci and glycopeptides against staphylococci. Through the use of alternate screening methods and a strict program of quality control to ensure the quality of the media, the emergence of resistance to these classes of drugs in these organisms should be detected. In conclusion, surveillance for antimicrobial resistance is critical, but the data must be validated prior to use.

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