The World Health Organization’s External Quality Assurance System Proficiency Testing Program Has Improved the Accuracy of Antimicrobial Susceptibility Testing and Reporting among Participating Laboratories Using NCCLS Methods

Jasmine M. Chaitram,1,2* Laura A. Jevitt,1,2 Sara Lary,1,2 Fred C. Tenover,1,2 and The WHO Antimicrobial Resistance Group3,4†

Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention,1 Atlanta, Georgia 30333; World Health Organization Collaborating Center for Global Antimicrobial Resistance Monitoring,2 Atlanta, Georgia 30333; World Health Organization, Geneva, Switzerland3; and World Health Organization Collaborating Center for Surveillance of Antimicrobial Resistance, Brigham and Women’s Hospital, Boston, Massachusetts 02115

Received 19 December 2002/Returned for modification 27 January 2003/Accepted 25 March 2003

A total of 150 laboratories in 33 countries that followed the NCCLS testing procedures participated in the World Health Organization’s External Quality Assurance System for Antimicrobial Susceptibility Testing (EQAS-AST) from January 1998 through March 2001. Laboratories tested seven bacterial isolates for antimicrobial resistance and reported the results to the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. The results were compared to the results generated at the CDC with the NCCLS broth microdilution and disk diffusion reference methods. Although there were few testing errors with Salmonella enterica subsp. enterica serovar Enteritidis, drugs that are not appropriate for therapy of Salmonella infections were tested and reported by 136 (91%) of 150 laboratories. In addition, 29 (20%) of 150 laboratories used the Staphylococcus aureus breakpoints to report oxacillin results for Staphylococcus saprophyticus. For a vanB-containing Enterococcus faecalis strain, 124 (83%) of 150 laboratories correctly reported vancomycin results that were \pm 1 doubling dilution from the reference MIC or \pm 3 mm from the reference disk diffusion result. Of the laboratories that tested Streptococcus agalactiae by disk diffusion, 17% reported nonsusceptible results for penicillin in error. While 110 laboratories (73%) tested the S. pneumoniae challenge isolate against a fluoroquinolone, 83% tested it against ciprofloxacin, for which there are no NCCLS interpretive criteria. Ten of 12 laboratories testing levofloxacin and 4 of 4 laboratories testing ofloxacin by an MIC method correctly reported resistant results for the isolate. Feedback letters sent to participating laboratories highlighted areas of susceptibility testing in individual laboratories that needed improvement. The positive impact of the feedback letters and the overall effectiveness of the EQAS program were documented in repeat testing challenges with pneumococci and staphylococci. The 31 and 19% increases in the numbers of laboratories using appropriate testing methods for pneumococci and staphylococci, respectively, in 2000 versus 1998 indicate that laboratory performance is improving.

Clinical microbiology laboratories, through routine antimicrobial susceptibility testing and participation in various surveillance programs, help monitor the development and spread of antimicrobial resistance in their communities. The accuracy of data generated by both formal and informal surveillance systems has been debated for several years, which has led to a call for more careful monitoring of laboratory performance through external quality assurance and proficiency testing programs (12, 15). Proficiency testing is an external quality assurance method in which laboratories are sent simulated clinical specimens or bacterial isolates for testing by routine laboratory methods. Proficiency testing provides data about the accuracy of susceptibility testing and can determine if a laboratory’s methods are sufficiently sensitive to detect novel resistance patterns. This method of quality assurance also allows a clinical laboratory’s performance to be assessed in comparison to reference methods and to other peer laboratories. Several reports suggest that providing feedback on proficiency testing results improves the quality of testing among clinical laboratories (7, 8, 17).

A previous report on the External Quality Assurance System for Antimicrobial Susceptibility Testing (EQAS-AST), coordinated by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), highlighted the types of testing errors that were common among participating laboratories (15). The goals of the present study were (i) to determine if laboratories are following the NCCLS guidelines for reporting results for salmonellae and coagulase-negative staphylococci (CoNS); (ii) to assess the accuracy with which laboratories test and report results for group B streptococci, vanB-containing enterococci, and fluoroquinolone-resistant pneumococci; and (iii) to determine if feedback letters with specific recommendations for modifying susceptibility
testing strategies improve the performance of laboratories when they are rechallenged with similar organisms.

**MATERIALS AND METHODS**

**Distribution.** The following organisms were sent to approximately 300 laboratories outside of the United States: vancomycin-intermediate *Staphylococcus epidermidis* and penicillin-susceptible *Streptococcus pneumoniae* in 1998, vanB-containing *Enterococcus faecalis* and fluoroquinolone-resistant *S. pneumoniae* in 1999, ampicillin- and tetracycline-resistant *Salmonella enterica* subsp. *enterica* serovar Enteritidis and penicillin-susceptible *S. pneumoniae* in 2000 (the same isolate that was sent in 1998), *Staphylococcus saprophyticus* (a CoNS), a group B beta-hemolytic streptococcus, and vancomycin-intermediate *S. epidermidis* in 2001 (the same isolate that was sent in 1998). Coordinating centers were established in Argentina, Bulgaria, China, Colombia, Croatia, the Czech Republic, Finland, Korea, Japan, and Saudi Arabia to facilitate the distribution of organisms and reporting of results.

**Testing.** Participants were instructed to test the organisms only once with their standard testing method. Laboratories were directed to process the isolate as if it had been obtained from a positive blood culture. For each organism, a data sheet was returned by the individual laboratories or the coordinating centers for distribution to their participants. In addition, we conducted an anonymous survey of participants to determine the usefulness of the EQAS-AST proficiency testing program.

**RESULTS**

**Reporting of challenges.** One hundred fifty participants reported results for an *S. enterica* subsp. *enterica* serovar Enteritidis isolate that was resistant to ampicillin and tetracycline but susceptible to chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole. Of the 150 laboratories, 26% used an MIC method and 74% used disk diffusion. Only one laboratory reported a susceptible result for ampicillin, and another reported a susceptible result for tetracycline. However, 136 laboratories (91%) tested and reported agents other than those recommended by the NCCLS for *Salmonella* infections, such as gentamicin, narrow- and improved-spectrum cephalosporins, and imipenem (Table 1). No laboratories reported false resistance to fluoroquinolones.

Of 150 laboratories, 139 (93%) were able to identify the *Staphylococcus* challenge organism as CoNS, 3 (2%) identi-
fied the organism as *S. aureus*, and 8 (5%) did not report an identification. Of the 139 that identified the organism as a CoNS, 122 (88%) correctly identified the organism as *S. saprophyticus*. Of 140 laboratories that tested and reported results for oxacillin, 97 (70%) obtained the correct MIC within 1 doubling dilution of the reference MIC or the correct diameter within 3 mm of the reference disk diffusion result. Of those that obtained the correct results, 61% of the MICs and 74% of the disk diffusion results, respectively, were interpreted with the appropriate interpretative criteria for oxacillin (Table 2), i.e., those for CoNS. Nineteen laboratories (20%) reported this organism as oxacillin susceptible, resulting in 19 very major errors (11 obtained by the BMD or Etest method and 8 obtained by disk diffusion). In addition, 10 laboratories (10%) reported this organism as intermediate to oxacillin, resulting in 10 minor errors (1 obtained by an MIC method and 9 obtained by disk diffusion) (Table 3).

**Testing challenges.** Laboratories were sent a *vanB*-containing *E. faecalis* isolate that was vancomycin resistant but penicillin and ampicillin susceptible. Five (3%) laboratories reported the organism as ampicillin resistant, and eight (6%) reported the organism as penicillin resistant; in addition, one laboratory reported a beta-lactamase-positive result, which was incorrect (Table 4). Seventy-four (89%) out of 83 laboratories correctly reported disk diffusion results within ±3 mm of the reference result. Forty-seven (71%) of 66 laboratories testing by an MIC method reported a result that was ±1 doubling dilution of the CDC result. Of the 13 laboratories that reported vancomycin-susceptible results for the disk diffusion method, only two reported an unacceptably large zone diameter.

The results for the *S. pneumoniae* challenge are shown in Table 5. This strain is susceptible to penicillin and cephalosporins but resistant to fluoroquinolones. Ninety-one of the 110 laboratories that tested a fluoroquinolone chose ciprofloxacin, for which there are no NCCLS interpretive criteria. The zone diameter results for ciprofloxacin ranged from no zone to 35 mm; with a mean of 15 mm. Ten of 12 laboratories that tested levofloxacin and all 4 of the laboratories testing ofloxacin reported resistant results consistent with the NCCLS interpretive criteria.

The group B beta-hemolytic streptococcus (*S. agalactiae*) challenge strain was resistant to erythromycin and tetracycline but remained susceptible to penicillin, extended-spectrum cephalosporins, and clindamycin. It contains the *mef(A)* macrolide efflux gene, which mediates resistance to erythromycin but not clindamycin. Sixty percent of disk diffusion users reported this strain as erythromycin intermediate rather than resistant, which is consistent with the relatively low erythromycin MIC of 8 μg/ml. The organism should be reported as clindamycin susceptible, although 10 laboratories, all disk diffusion users, reported intermediate or resistant results. Seven laboratories (7%) using disk diffusion reported this strain as penicillin resistant although the reference zone diameter result for penicillin was very large (30 mm). A total of 11 laboratories reported resistant disk diffusion results for cefotaxime and or ceftriaxone (Table 6).

**Repeat challenges to assess improvement in test selection.** A pneumococcal isolate with reduced susceptibility to penicillin was sent in 1998 and again in 2000 and was tested both times by a subset of 52 laboratories. This organism produces a zone size of 13 to 15 mm around a 1-μg oxacillin disk, indicating that a penicillin MIC test should be performed. In 1998, 32 (71%) out of 45 laboratories testing oxacillin reported a zone of ≤19 mm, while in 2000, 22 (69%) out of 32 testing oxacillin reported a zone diameter of ≤19 mm. Although the MIC of penicillin for this organism is typically in the susceptible range (modal MIC = 0.06 μg/ml), penicillin MICs as high as 0.5 μg/ml, interpreted as intermediate, were reported by two laboratories in 2000 (Table 7). MIC results of >0.125 μg/ml were

### Table 4. Results for *E. faecalis*

<table>
<thead>
<tr>
<th>Antimicrobial agent (no. of laboratories)</th>
<th>Reference result (category)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of laboratories reporting DD results (range in mm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of laboratories reporting MIC results (range in μg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD (μg/ml)</td>
<td>DD (mm)</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin (146)</td>
<td></td>
<td></td>
<td>1 (S)</td>
</tr>
<tr>
<td>Penicillin (131)</td>
<td>4 (S)</td>
<td>17 (S)</td>
<td>85 (15–34)</td>
</tr>
<tr>
<td>Cephalosporin (84)</td>
<td>0.5 (S)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 (S)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52 (14–24)</td>
</tr>
<tr>
<td>Vancomycin (149)</td>
<td>32 (R)</td>
<td>15 (I)</td>
<td>13 (17–23)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DD, disk diffusion.
<sup>b</sup> Consistent with a VanB phenotype.

### Table 5. Results for *S. pneumoniae*

<table>
<thead>
<tr>
<th>Antimicrobial agent (no. of laboratories)</th>
<th>Reference result (category)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of laboratories reporting DD results (range in mm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of laboratories reporting MIC results (range in μg/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD (μg/ml)</td>
<td>DD (mm)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin (12)</td>
<td>8 (R)</td>
<td>10 (R)</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin (107)</td>
<td>≤0.01 (S)</td>
<td>0</td>
<td>54 (52–45)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DD, disk diffusion.
<sup>b</sup> S, susceptible; I, intermediate; R, resistant.

Downloaded from http://jcm.asm.org/ on May 28, 2021 by guest
considered errors. Eight laboratories used penicillin disks in 2000, compared to 10 laboratories in 1998, with only 2 laboratories making the same error twice. In 1998, 18 laboratories tested cefotaxime and/or ceftiraxone by disk diffusion although there are no interpretive criteria for penicillin and cephalosporin disks in the NCCLS guidelines for this organism. In 2000, the number dropped to 13 laboratories. In 1998, 18 (35%) of 52 laboratories reported oxacillin screen test results of ≤19 mm without follow-up MIC testing whereas that number dropped to 7 (13%) of 52 in 2000. Thus, 16 (31%) of 52 laboratories improved their testing methods for penicillin, cephalosporins, or both drug classes in 2000. Finally, this isolate is also erythromycin resistant. In 2000, no laboratories reported erythromycin-susceptible results, which is an improvement over 1998, when 10% of the laboratories reported the isolate as erythromycin susceptible.

A glycopeptide-intermediate strain of *S. epidermidis*, previously sent in 1998, was sent to participants again in 2001. The vancomycin MIC for this organism was 8 μg/ml, and the teicoplanin MIC was 16 μg/ml (Table 8). Of the 52 laboratories that tested this organism in both 1998 and 2001, 12 changed their testing method for this isolate from vancomycin from disk diffusion to an MIC method in 2001, resulting in a 19% improvement in the results.

**Follow-up survey.** Of 115 laboratories returning the survey, 107 (93%) received feedback letters from the CDC or their coordinating centers critiquing their results. Ninety-nine of 107 (93%) received feedback letters from the CDC or their coordinating centers, recommen-
dations. Six of seven responding coordinating centers will continue to distribute proficiency test strains and feedback of results to laboratories after the EQAS-AST program is completed.

**TABLE 7. Results for *S. pneumoniae***

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Reference result (category)b</th>
<th>No. of laboratories reporting DD results (range in mm)c</th>
<th>No. of laboratories reporting MIC results (range in μg/ml)d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD (μg/ml)</td>
<td>DD (mm)c</td>
<td>S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.12 (S)</td>
<td>22 (S)</td>
<td>39</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>4 (R)</td>
<td>10 (R)</td>
<td>5</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.06 (S)</td>
<td>21 (S)</td>
<td>8</td>
</tr>
</tbody>
</table>

a DD, disk diffusion.
b S, susceptible; I, intermediate; R, resistant.

c Reference result (category).
d MIC, minimum inhibitory concentration.

**TABLE 8. Results for *S. epidermidis***

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Reference result (category)b</th>
<th>No. of laboratories reporting DD results (range in mm)c</th>
<th>No. of laboratories reporting MIC results (range in μg/ml)d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD (μg/ml)</td>
<td>DD (mm)c</td>
<td>S</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>&gt;16 (R)</td>
<td>6 (R)</td>
<td>7</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>16 (I)</td>
<td>14 (S)</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>8 (I)</td>
<td>16 (S)</td>
<td>46</td>
</tr>
</tbody>
</table>

a DD, disk diffusion.
b S, susceptible; I, intermediate; R, resistant.

c Reference result (category).
d MIC, minimum inhibitory concentration.

**DISCUSSION**

In the past, proficiency testing programs for antimicrobial susceptibility testing have focused primarily on testing accuracy. However, equally important is assessment of reporting accuracy. Although there were few testing errors with the *S. enterica* subsp. *enterica* serovar Enteritidis and *S. saprophyticus* isolates, there were multiple reporting errors with these organisms. NCCLS guidelines state that certain antimicrobial agents should not be tested or reported for *Salmonella* species because they may give false-susceptible results. Nonetheless, 136 laboratories tested and reported antimicrobial agents that are inappropriate for *Salmonella* infections (Table 1). Although salmonellae are tested with the same set of disks or MIC panels used for other gram-negative enteric organisms, laboratories must ensure that only the appropriate antimicrobial agents are reported. Some antimicrobial agents, such as the aminoglycosides, are always inappropriate; on the other hand, extended-spectrum cephalosporins, such as cefotaxime and ceftiraxone, are appropriate to report for extra-intestinal *Salmonella* infections. Thus, it is important to test and report these agents selectively.

In 2000, NCCLS changed the oxacillin breakpoints for CoNS to ≤0.25 μg/ml or ≥18 mm for susceptible and ≥0.5 μg/ml and ≤17 mm for resistant (no intermediate disk breakpoint). These breakpoints correlate much better with the results of mecA testing for most CoNS strains. The purpose of the *S. saprophyticus* challenge was to verify that laboratories are aware of this change. Since the *S. saprophyticus* challenge organism demonstrates an oxacillin MIC of 1 μg/ml and a zone diameter much smaller than the breakpoint of 17 mm (the mean was 12 mm), it would be classified as resistant by the new NCCLS criteria, presuming that the organism was not isolated from urine. In most instances, *S. saprophyticus* is recovered from the urinary tract.
tract and, according to the NCCLS, does not require routine testing because these infections respond to antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections (e.g., nitrofurantoin, trimethoprim-sulfamethoxazole, or a fluoroquinolone) (11). Because 29 laboratories reported this organism as susceptible or intermediate to oxacillin, resulting in 19 very major errors and 10 minor errors, respectively, it is clear that many laboratories used the S. aureus breakpoints even when 88% of the laboratories correctly identified this organism as S. suprophyliticus.

The E. faecalis challenge strain was V583, the first vanB-containing (low-level vancomycin resistance) strain reported from the United States. This strain typically yields vancomycin MIC and disk diffusion results in the intermediate range (13). Although the mean vancomycin MIC reported by participating laboratories was 64 µg/ml (resistant), the disk diffusion mean was 14 mm (resistant). The NCCLS recommends incubating both MIC and disk diffusion tests for a full 24 h before reading and interpreting results and also recommends retesting of isolates that yield disk diffusion results in the intermediate range by an MIC method (9–11). The vancomycin zones for enterococci should be read with transmitted light instead of reflected light, and any isolates with haze or growth within the zone of inhibition should be considered resistant. Disk diffusion results for vancomycin for enterococci are usually more difficult to interpret than MIC results. It is possible that the teicoplanin-susceptible results typical of vanB-containing strains caused some laboratories to assume that borderline vancomycin-resistant results were in error. In retrospect, this organism would not have been a good choice for proficiency testing if only qualitative results were considered because the accuracy of the disk diffusion tests spans the interpretative categories of susceptible, intermediate, and resistant. However, when the accuracy of the results is assessed independently of the interpretation, only 10% of laboratories reported vancomycin MICs more than 1 doubling dilution from the reference value or disk diffusion results greater than 3 mm from the reference disk result. These data are encouraging since previous studies have documented the difficulties in detecting low-level vancomycin resistance (1, 4, 16).

S. agalactiae is an unusual proficiency-testing organism, but given the increased emphasis on detecting this organism in pregnant women (2) and the concern over increasing macrolide resistance in group B streptococci, we decided to include it as one of our challenges. While the NCCLS has published disk diffusion breakpoints for beta-lactam drugs such as penicillin, cefotaxime, and ceftriaxone, these tests can be difficult to read. Seven laboratories reported false penicillin resistance obtained by disk diffusion, and one laboratory reported false-resistance obtained by an MIC method. To our surprise, 11 laboratories reported resistance to cefotaxime and/or ceftriaxone. Penicillin and cephalosporin resistance has not been reported in group B streptococci.

Fluoroquinolone resistance is emerging in pneumococci in several countries (3, 6). Although there are no NCCLS breakpoints for ciprofloxacin for S. pneumoniae, 91 (61%) laboratories tested this antimicrobial agent and 79 (87%) of these laboratories reported an interpretation for ciprofloxacin for our pneumococcal challenge organism in 2000. The mean disk diffusion result was 15 mm, which was reported by 15 laboratories, and 13 (87%) of these laboratories interpreted this result as indicating resistance. Nine laboratories tested ciprofloxacin by an MIC method, and six of these laboratories reported interpretations, all indicating resistance. Twelve laboratories (8%) tested the S. pneumoniae challenge against levofloxacin; four tested ofloxacin and one tested trovafloxacin. Breakpoints have been established for the latter three antimicrobial agents.

In 1998, participants were sent a pneumococcal isolate that was resistant to erythromycin but susceptible to chloramphenicol and beta-lactam drugs such as penicillin and cefotaxime. In addition, this pneumococcal isolate consistently gives an oxacillin zone diameter of <19 mm and so, according to NCCLS guidelines, the laboratory should perform an MIC test rather than report a result based on the oxacillin screen test alone. Participants were also challenged with an S. epidermidis isolate with reduced susceptibility to vancomycin. As noted previously, disk diffusion does not detect decreased susceptibility to glycopeptides in staphylococci (16); however, many laboratories continue to use disk diffusion to test staphylococci against glycopeptides. Although disk diffusion works well for most other drug classes, alternate testing methods for glycopeptides must be used. For laboratories that routinely use disk diffusion, the BHI vancomycin agar screen test, which was developed for enterococci, can be used to detect strains of staphylococci with reduced susceptibility to vancomycin and teicoplanin.

Feedback letters were sent to participants in 1998 including recommendations to improve testing methods for the pneumococci and staphylococci. The same laboratories were challenged again with the pneumococcal and staphylococcal isolates 2 years later to assess whether laboratory performance had improved. In 1998, only 27 (52%) out of 52 laboratories tested the S. pneumoniae isolate correctly with an MIC method for penicillin, although 32 (71%) out of 45 reported an oxacillin zone diameter of <19 mm. This improved to 73% in 2000 with 38 out of 52 laboratories performing an MIC test for penicillin although only 22 (68%) out of 32 reported a zone diameter of <19 mm for oxacillin. There were more laboratories correctly using an MIC method for penicillin in 2000, although the number of laboratories accurately testing oxacillin did not significantly change. Moreover, only 10% of the laboratories in 1998 were able to detect reduced susceptibility to vancomycin in staphylococci; this improved to 29% in 2001. Only two laboratories reported using the BHI vancomycin agar screen test in 1998 and 2000, and these laboratories were not the same in each year. Although many laboratories continue to use incorrect testing methods (e.g., disk diffusion for testing of pneumococci with penicillin and for detection of reduced susceptibility to vancomycin in staphylococci), our results indicate that laboratory performance can be improved by proficiency tests, followed by feedback of results. In some cases, laboratories were aware of the correct testing methods (and noted this on their data collection sheets) but, because of financial limitations, were not able to use the appropriate test methods. The improved performance of the laboratories and positive survey responses support the belief that the EQAS-AST program has been very useful in identifying areas for improvement in susceptibility testing methods and, in some cases, has led to better laboratory performance.
ACKNOWLEDGMENTS

We thank all WHO EQAS-AST participants for sending their results. We also thank the EQAS-AST coordinating centers, the WHO regional offices, and the CDC shipping department for distributing proficiency test organisms.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

REFERENCES