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A new method for normalized interpretation of antimicrobial resistance from disk test results for comparative purposes

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Objective To evaluate a calibration method for disk diffusion antibiotic susceptibility tests, using zone diameter values generated in the individual laboratory as the internal calibrator for combinations of antibiotic and bacterial species.

Methods The high-zone side of zone histogram distributions was first analyzed by moving averages to determine the peak position of the susceptible population. The accumulated percentages of isolates for the high zone diameter values were calculated and converted into probit values. The normal distribution of the ideal population of susceptible strains was then determined by using the least-squares method for probit values against zone diameters, and the ideal population was thereby defined, including mean and standard deviation. Zone diameter values were obtained from laboratories at the Karolinska Hospital (KS) and Växjö Hospital (VX), and from two laboratories (LabA, LabB) in Argentina. The method relies on well standardized disk tests, but is independent of differences in MIC limits and zone breakpoints, and does not require the use of reference strains. Resistance was tentatively set at below 3 SD from the calculated, ideal mean zone diameter of the susceptible population.

Results The method, called normalized interpretation of antimicrobial resistance, was tested on results from the KS and VX clinical microbiology laboratories, using the disk diffusion method for antimicrobial susceptibility tests, and for two bacterial species, *Staphylococcus aureus* and *Escherichia coli*. In total, 114,217 test results were included for the clinical isolates, and 3,582 test results for control strains. The methodology at KS and VX followed the standard of the Swedish Reference Group for Antibiotics (SRGA). Zone diameter histograms for control strains were first analyzed to validate the procedure, and a comparison of actual means with the calculated means showed a correlation coefficient of \( r = 0.998 \). Results for clinical isolates at the two laboratories showed an excellent agreement for 54 of 57 combinations of antibiotic and bacterial species between normalized interpretations and the interpretations given by the laboratories. There were difficulties with *E. coli* and mecillinam, and *S. aureus* and tetracycline and rifampicin. The method was also tested on results from two laboratories using the NCCLS standard, and preliminary results showed very good agreement with quality-controlled laboratory interpretations.

Conclusions The normalized resistance interpretation offers a new approach to comparative surveillance studies whereby the inhibition zone diameter results from disk tests in clinical laboratories can be used for calibration of the test.

Keywords Antibiotic susceptibility testing, disk diffusion test, surveillance, antibiotic resistance, zone histogram

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INTRODUCTION

Antimicrobial resistance surveillance is becoming critical in a global situation of increased occurrence and spread of resistance genes among bacterial pathogens. Several surveillance programs have been launched to meet the need for antimicrobial resistance information [1–4]. Such studies are usually performed in one of two ways. One method is to send all clinical isolates from participating laboratories to a reference laboratory, where antimicrobial susceptibility testing is performed with a reference MIC method [1,2]. A similar centralized approach, but using standardized disk diffusion testing, has also been employed [5]. The TSN database [6,7], on the other hand, represents a decentralized project, with test results being produced in the individual laboratories, but compiled and analyzed centrally. Millions of susceptibility test results have thus been aggregated, which gives an idea of the enormous amount of antimicrobial susceptibility data available for surveillance, if these test results can be made comparable.

In earlier investigations, we have studied ways of calibrating the disk diffusion antimicrobial susceptibility test in order to improve accuracy and to make results comparable. Two methods were described [8,9]. The first method, called ‘peak-corrected breakpoints’ or ‘control strain peak correction’, compares the reference strain zone values from the reference authority issuing interpretive breakpoints with the zone values produced in the individual laboratory; a correction of the zone breakpoints can then be made. Such a semicalibration reduced false-resistant interpretations from 4.4% to 2.3% [8]. Another method, called single-strain regression analysis (SRA) [9,10], a true calibration method, reduced false-resistant results from 4.4% to 0.14% [8]. Neither of these methods, however, lends itself to more widespread use for surveillance. Recently, a method for normalized interpretation of antimicrobial resistance has been invented (G. Kronvall, Patent pending, Swedish Patent Office, 2001-04-10, Appl. no. 0101251-7). The method utilizes the fact that the normal, susceptible population of clinical isolates of any species forms an antimicrobial inhibition zone histogram which is not influenced on the high-zone side by an increase in the number of resistant isolates in the same histogram. The mathematical reconstruction of the normal, susceptible population, which has been shown to follow parametric criteria to a sufficient degree [11], permits the setting of a lower limit for the susceptible population. This normalized resistance interpretation requires a standardized disk test but is independent of MIC limits used for setting zone breakpoints, and other methodological variations, and is therefore especially suited for antimicrobial resistance surveillance. In the present study, we have tested this new principle on results from susceptibility tests of both control strains and clinical isolates, and in laboratories using the Swedish Reference Group for Antibiotics (SRGA) standard, or that of the National Committee for Clinical Laboratory Standards (NCCLS).

MATERIALS AND METHODS

Bacterial strains, culture media, and species identification

Staphylococcus aureus and Escherichia coli strains were isolated and identified in clinical specimens by the use of standard methods [12–14]. The majority of the consecutive specimens included comprised wound secretions and urine samples. Control strains S. aureus ATCC 29213 and E. coli ATCC 25922 were tested regularly in disk diffusion tests at the laboratories. Two Swedish clinical microbiology laboratories participated in the study, Karolinska Hospital (KS), Stockholm Sweden, and Växjö General Hospital (VX) Växjö, Sweden. WHONET-filed results were also available from two clinical microbiology laboratories in Argentina (LabA and LabB), and were used for comparisons with the Swedish results.

Disk diffusion antimicrobial susceptibility testing

The antimicrobial susceptibilities of clinical isolates were determined by use of the disk diffusion method according to the SRGA [15], with interpretations adjusted for species groups (http://www.srga.org) [9,16]. Bacterial strains were inoculated on Oxoid Iso-Sensitest Agar (Oxoid Ltd, Basingstoke, UK). Antibiotic disks were purchased from Oxoid AB (Oxoid AB, Sollentuna, Sweden). Antibiotic disks were placed on the inoculated surface, followed by pre-diffusion at room temperature for 30 min, and then by overnight
incubation at 36 °C ± 1 °C in air. Inhibition zone diameters were measured in millimeters with a pair of calipers. The interpretation of susceptibility followed species-related guidelines issued by the SRGA [16]. The total numbers of disk test results included in the present study were 114,217 for the clinical isolates (KS 74,969; VX 39,248) and 3582 for the ATCC strains (KS 840; VX 2742).

Antibiotic susceptibility testing in laboratories in Argentina followed the NCCLS standard and included regular testing of the control strains S. aureus ATCC 29213 and E. coli ATCC 25922. Results were entered into WHONET files with zone diameter recordings included, comprising a total of 8823 records.

Method for normalized resistance interpretation

The method is based on analysis of zone diameter histograms from individual laboratories for the antibiotic disks used and for different bacterial species separately (G. Kronvall, Patent pending, Swedish Patent Office, 2001-04-10, Appl. no. 0101251-7). Zone diameter histograms for combinations of antimicrobials and bacterial species were constructed with percentage strains or number of strains on the y-axis against the zone diameter values on the x-axis. Histograms for normally susceptible isolates or control strains show homogeneous populations that follow a normal Gaussian distribution well, but with a slightly peaked shape and a skew towards higher zone values [11]. Parametric statistical measures, the mean and standard deviation, have been shown to describe these histogram populations accurately [11]. When clinical isolates of any combination of antibiotic and individual bacterial species are analyzed similarly, the position of the normally susceptible wild-type strains is unchanged, whereas the resistant or intermediately resistant strains form more or less well-defined populations at the lower end of the zone diameter spectrum.

The basis for the present normalization procedure is the fact that resistant isolates give inhibition zone diameters which are below those of the susceptible population, often with no zone at all. The high-zone side of the normal population of susceptible strains therefore remains unchanged by the occurrence of resistant isolates. This provides the internal reference that makes histograms for the same combination of antimicrobial and bacterial species from any laboratory comparable.

The first step in the reconstruction of the susceptible population is detection of the peak of the susceptible strains. This is done by calculating moving weighted averages of numbers of strains for two or more zone diameter values, starting from the high-zone values. When the averages start to decrease, then the previous zone value is taken as the peak of the susceptible population (for averages of more than two zone diameter values, a correspondingly higher zone diameter can be chosen). The estimated total number of susceptible strains in the reconstructed susceptible population is then twice the number of strains down to the peak zone value.

The next step is expression of the true number of strains down to the peak value as the accumulated percentage values of the estimated susceptible population. These percentage values are then transformed to probit values [17], and the linear relationship between probit values and inhibition zone values is calculated from the highest zone down to the peak by use of the least-squares method, giving the regression coefficients and the product–moment correlation coefficient. The mean value of the estimated susceptible population and its standard deviation are thereby made known, and the whole curve can be constructed.

Several of the parameters can be varied in this procedure, if required. In preliminary experiments, a weighted average based on two zone diameter values was found to produce reliable estimations, and was therefore chosen as a starting parameter in the present investigations, but results for averages based on three and four values were also calculated. In the computer program, some other corrections have also been included. Single outliers (defined as single results with a gap of 3 mm to the next lower positive zone value) were excluded from the normalization calculations. For the straight-line determination of probit versus zone diameter, the uppermost 2% are omitted in the least-squares method, but included otherwise.

RESULTS

S. aureus ATCC 29213 and E. coli ATCC 25922 results

Reference strains S. aureus ATCC 29213 and E. coli ATCC 25922 were tested routinely in the participating laboratories for control purposes. The
antibiotics included for testing the control strains were identical in the two Swedish laboratories for 13 of 19 different combinations of antibiotic and bacterial species. For validation of the normalization procedure, 32 control strain histograms from KS and VX were analyzed, and the mean values and standard deviations were calculated. The histograms were then analyzed by use of the new normalized interpretation method, giving calculated mean values and standard deviations for the ideal distributions. Examples of histograms with true numbers of isolates as well as the corresponding normalized distribution of isolates are shown in Figure 1. On visual inspection, there was good agreement between true histograms and the normalized distributions.

A comparison between the true means and the means of the normalized distributions was made, and the results are shown in Figure 2. Since the control strain histograms represent homogeneous populations, the calculated ideal distributions should follow these populations quite closely, and these experiments therefore checked the validity of the normalization procedure. The results showed a very close correlation between the true means and the calculated ideal means, with a
correlation coefficient $r = 0.998$. The mean value of the absolute numbers for the differences was 0.4 mm, and that for the true differences was 0.08 mm, which means that the calculated ideal distributions followed the true histogram peaks of the control strains very closely. Only one of 32 control strain histograms had a difference between the true and calculated means of more than 1 mm.

A tentative limit for the susceptible population set to three standard deviations below the calculated means would theoretically include 99.86% of the susceptible population above this limit. The calculated lower 3 SD limits for the individual histograms were adjusted to nearest-integer values, and the true populations of zone diameter values for the control strains were then checked by using these limits. Out of 3582 control strain results, there were eight zone diameter recordings below the calculated limits, i.e. 0.22% of the results. This is a little higher than the theoretical value of 0.14%. An examination of the individual histograms revealed that six of the eight deviations came from one single histogram, the case already noted above because of the difference between the means. The two other random deviations accounted for 0.06%, which is below the expected theoretical 0.14%.

Analysis of clinical isolates of *S. aureus* and *E. coli*

In total, 57 histograms from KS and VX with 114 217 test results were then analyzed for the presence of inhibition zone values below a 3SD limit for the calculated normalized population of susceptible isolates in the individual histograms. The ideal distributions were determined by the normalized resistance interpretation method with a weighted average based on isolates for two zone diameters, and also with elimination of outliers in the calculations. Results for netilmicin, trimethoprim, co-trimoxazole and ciprofloxacin were available at both laboratories and for both species. Ciprofloxacin 10-μg disks were used at KS, whereas VX used 5-μg disks. Histograms for *E. coli* and ciprofloxacin for the two laboratories are shown in Figure 3. Normalized means for the calculated ideal wild-type distributions were 39.3 mm at KS (2081 isolates, Figure 3a) and 35.4 mm at VX (4718 isolates, Figure 3b), respectively, for *E. coli* and ciprofloxacin at the two laboratories. The resistance levels among *E. coli* strains (defined as ‘R +I’ combined) reported by the laboratories were 25.8% and 2.5% at KS and VX, respectively, and by the normalized interpretation the figures obtained were 27% and 3.2%, respectively.

Among antimicrobials used for both *S. aureus* and *E. coli* in both laboratories, trimethoprim gave histogram distributions which indicated many resistant isolates. The results from trimethoprim tests and normalized calculations are shown in Figure 4. The calculated means for the normalized distributions were, for *S. aureus*, 25.6 and 26.5 mm for KS (Figure 4a) and VX (Figure 4b), respectively, and for *E. coli*, 29.5 and 30.6 mm, respectively.
Regular interpretations of resistance (R+I categories combined) showed 4.2% and 7.7% for *S. aureus* and the respective laboratories, and 42.1% and 13.0% for *E. coli*. The figures obtained using the automatic normalized resistance calculation were 4.2%, 8.3%, 42.4%, and 13.2%, respectively. These figures corresponded well with the figures generated in the laboratories using species-related interpretations according to the SRGA.

The results from disk tests of *S. aureus* and *E. coli* with the other antibiotics at KS and VX were also analyzed, and a summary of the results is shown in Tables 1 and 2. For the majority of combinations analyzed, there was almost complete agreement between the resistance rates reported (percentage R+I) when using species-related interpretations according to the SRGA and the calculated normalized resistance interpretations (percentage n−R) when using the new method (Tables 1 and 2).

**Figure 3** Inhibition zone diameter histograms for clinical isolates of *E. coli* from disk diffusion tests with ciprofloxacin at KS (10-μg disk) (a) and at VX (5-μg disk) (b). The calculated distributions using the normalization procedure are shown in the histogram plots.
For *E. coli* and mecillinam at KS, the histogram showed isolates with an unusually wide distribution of zone diameter values, without a distinct separation of resistant isolates from susceptible ones. The normalized resistance interpretation produced a result, 13.7%, which was between the 8.3% R and the 26.8% R + I values according to the interpretations by the laboratories. Therefore, the lack of separation of susceptible strains from strains with resistance mechanisms makes it difficult to apply the present method for mecillinam. This is not so apparent in the VX histogram, since the number of resistant isolates was much lower. For two *S. aureus* histograms at KS, those for tetracycline and rifampicin, there were also differences which showed up as inconsistent results when the procedure for normalized interpretation was carried out with averages based on two, three and four zone diameter values, and the results were therefore excluded. Our data suggested that the normalized resistance interpretation method produced results which were
consistent and reliable for comparative purposes for 54 of the included 57 histograms of disk diffusion test results for clinical isolates with different combinations of bacterial species and antibiotics.

The method of normalized resistance interpretation was also tested in preliminary investigations of histograms from two laboratories in Argentina (LabA and LabB) that were using the NCCLS standards for disk diffusion tests. The calculated ideal distributions of susceptible strains showed lower mean values in these laboratories than at KS and VX, reflecting differences between the two standards, such as differences in inoculum size. In general, there were few resistant isolates in the two laboratories in Argentina, and two representative examples are shown in Figure 5b,d in comparison with results from KS (Figure 5a,c). For *E. coli* and imipenem, the resistance figure was 0% in both laboratories according to their own
### Table 1: Antimicrobial resistance rates of *S. aureus* isolates according to SRGA interpretations (percentage $R + I$) and to automated, normalized resistance interpretation (percentage $n-R$)

<table>
<thead>
<tr>
<th></th>
<th>KS</th>
<th></th>
<th>VX</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>$R + I$</td>
<td>$n-R$</td>
<td>No.</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>4398</td>
<td>5.6</td>
<td>5.6</td>
<td>1967</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3608</td>
<td>0.8</td>
<td>0.9</td>
<td>109</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>821</td>
<td>1.2</td>
<td>1.3</td>
<td>1813</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>155</td>
<td>67.7</td>
<td>67.7</td>
<td>1804</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>3570</td>
<td>4.3</td>
<td>4.3</td>
<td>1800</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>3605</td>
<td>3.8</td>
<td>5.9</td>
<td>1800</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>278</td>
<td>3.2</td>
<td>2.5</td>
<td>109</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>268</td>
<td>13.1</td>
<td>–</td>
<td>107</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>814</td>
<td>2</td>
<td>–</td>
<td>109</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>862</td>
<td>0</td>
<td>0</td>
<td>107</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>503</td>
<td>23.1</td>
<td>22.3</td>
<td>326</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>314</td>
<td>38.2</td>
<td>38.2</td>
<td>157</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>502</td>
<td>0.6</td>
<td>0.2</td>
<td>157</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>502</td>
<td>4.2</td>
<td>4.2</td>
<td>157</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>3565</td>
<td>0.8</td>
<td>1.2</td>
<td>1803</td>
</tr>
</tbody>
</table>

*No susceptible category for this combination. The wild-type population is designated intermediately susceptible. Only percentages of resistant (R) isolates are included.*

### Table 2: Antimicrobial resistance rates of *E. coli* isolates according to SRGA interpretations (percentage $R + I$) and to automated, normalized resistance interpretation (percentage $n-R$)

<table>
<thead>
<tr>
<th></th>
<th>KS</th>
<th></th>
<th>VX</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>$R + I$</td>
<td>$n-R$</td>
<td>No.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>7207</td>
<td>56.1</td>
<td>55.4</td>
<td>4732</td>
</tr>
<tr>
<td>Mecillinam</td>
<td>5425</td>
<td>26.8</td>
<td>13.7</td>
<td>4449</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>2176</td>
<td>37</td>
<td>36.5</td>
<td>4733</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>4928</td>
<td>4</td>
<td>3.6</td>
<td>4733</td>
</tr>
<tr>
<td>Loracarbef</td>
<td>1684</td>
<td>7.9</td>
<td>7.3</td>
<td>85</td>
</tr>
<tr>
<td>Cepirome</td>
<td></td>
<td></td>
<td></td>
<td>204</td>
</tr>
<tr>
<td>Cefbuten</td>
<td></td>
<td></td>
<td></td>
<td>187</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2182</td>
<td>9.9</td>
<td>8.3</td>
<td>224</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>2214</td>
<td>4.1</td>
<td>5.9</td>
<td>224</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>1368</td>
<td>6</td>
<td>5.1</td>
<td>155</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1348</td>
<td>0.1</td>
<td>0.2</td>
<td>155</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2172</td>
<td>6.2</td>
<td>6.2</td>
<td>155</td>
</tr>
<tr>
<td>Amikacin</td>
<td>408</td>
<td>0.3</td>
<td>0.3</td>
<td>155</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>410</td>
<td>1.2</td>
<td>1.4</td>
<td>290</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td></td>
<td></td>
<td></td>
<td>117</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>4919</td>
<td>14.7</td>
<td>12</td>
<td>4718</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2081</td>
<td>25.8</td>
<td>27</td>
<td>4450</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>4895</td>
<td>8</td>
<td>1.2</td>
<td>4437</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>6190</td>
<td>42.1</td>
<td>42.4</td>
<td>315</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>1597</td>
<td>15.5</td>
<td>16.3</td>
<td>315</td>
</tr>
</tbody>
</table>

*No susceptible category for this combination. The wild-type population is designated intermediately susceptible. Only percentages of resistant (R) isolates are included.*

For information on SRGA guidelines, see [http://www.srga.org](http://www.srga.org)
Figure 5  *E. coli* clinical isolates tested for susceptibility to imipenem at KS (1348 isolates) and at LabB (256 isolates), and to gentamicin at KS (2172 isolates) and at LabA (272 isolates). Interpretive zone breakpoints were $S \geq 23$ mm, $R \leq 16$ mm (a), $S \geq 16$ mm, $R \leq 13$ mm (b), $S \geq 21$ mm, $R \leq 17$ mm (c), and $S \geq 15$ mm, $R \leq 12$ mm (c), respectively, with calculated 3SD limits of $n$–$R \leq 27$ mm, $n$–$R \leq 21$ mm, $n$–$R \leq 21$ mm, and $n$–$R \leq 14$ mm, respectively. Bars denote clinical isolates, and calculated distributions are shown as lines.
interpretations, and at KS and LabB, the figures were 0.7% and 1.6% at KS and LabB, respectively, according to normalized interpretations. For *E. coli* and gentamicin, the figures were 5.6% and 8.8% according to the laboratories, and 6.8% and 9.9% according to the normalized interpretation method.

**DISCUSSION**

The need for global surveillance of antimicrobial resistance has been recognized [18]. So far, however, this has not been realized to any appreciable extent, mainly because the most commonly used method for antimicrobial susceptibility testing, the disk diffusion method, produces results in individual microbiology laboratories that are not directly comparable. When the methodologies can be evaluated and are found to be similar, then the results can be used for comparisons between hospitals and countries [19]. This is, however, not always the case. When some US laboratories were studied recently for compliance with the NCCLS standard, as few as 14.3% for *Neisseria gonorrhoeae*, 30.1% for *Haemophilus influenzae* and 41.6% for *Streptococcus pneumoniae* followed the standard [20]. Among Canadian laboratories, only 23 of 66 laboratories followed the NCCLS standard as claimed [21]. In another investigation of 130 laboratories, all except one from outside of the USA, the results from testing six distributed organisms indicated problems [22]. It is therefore interesting to note that a distinguished WHO expert group, in its guidelines for surveillance, has advocated standardization in order to make disk diffusion test results comparable [23]. This would actually mean that, for many years to come, it would not be possible to obtain a full global picture of antimicrobial resistance.

In the present study, a new method has been tested for making disk diffusion test results comparable regardless of the standard used and independent of differences in MIC limits or zone breakpoints, and without any extra requirements for the methodology other than measuring the inhibition zones and recording the results. This method is called normalized resistance interpretation, and it distinguishes the deduced population of normal isolates from those with an increase in resistance. The results of this approach are in some ways similar to those of species-related interpretation, which was introduced in Sweden in the 1970s and is now fully established, resulting in improved accuracy of disk test results [16,24]. It was therefore appropriate to test the present method on results from two Swedish laboratories (KS and VX) which, in addition, differ in their rates of antimicrobial resistance. The results showed very good agreement between the reported levels of resistance and the results from normalized resistance interpretation (Tables 1 and 2). This method therefore has the potential for more general use in surveillance of antimicrobial resistance.

An irregularity of histograms frequently encountered is the well known fact that results for even zone values are often higher than those for odd zone values. This is illustrated in Figure 1b. If the zone diameters for a given antibiotic disk are
quite large, then the zones of inhibition are often incomplete, and the diameter has to be calculated by doubling the radius, which, by necessity, gives an even number. In such cases, values for odd zone diameters are often absent. However, in the present case (Figure 1b), the inhibition zones are comparatively small, and the histogram might therefore represent the fact that observers often prefer even numbers if the measuring device allows some degree of interpretation. This possible explanation for uneven histograms, as well as other subjective modifications of results, would be avoided if some electronic reading device was employed. Image processing of disk test results has been possible for many years [25], but recent improvements in technology have made such equipment more suitable for laboratory use on a wider scale [26,27].

For two of the 57 clinical isolate histograms of antibiotic disk zone diameters and bacterial species, the computer-generated results indicated that the distribution of isolates was irregular and therefore did not permit a reliable calculation of the normalized resistance rate (Tables 1 and 2). This was evident from the divergent results based on averages of two, three and four histogram bars for the moving average. The discrepancy offers the possibility, in future applications of the method, of automatically excluding histograms that will not provide reliable information.

There is a large and untapped source of information regarding antimicrobial resistance rates in disk diffusion test results available at local microbiology laboratories. Studies of centralized versus local testing have indicated that routine disk diffusion test results might be used for surveillance under certain conditions [28]. It was once concluded that disk tests are ‘deceptively easy to perform’, and also that histograms collected in different laboratories might be used for comparative purposes, but no objective method for such an analysis was presented [29]. The possibility of using disk data for surveillance has been suggested by O’Brien, who is the original inventor of the WHONET computer program for recording and analyzing disk diffusion test results [19,30,31]. We suggest that normalized resistance interpretation according to the present study and generated by computer calculations should be tested on this and other rich sources of information accumulated through decades of meticulous recording in laboratory databases [25].

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