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## Original articles

# Susceptibility testing of *Klebsiella* spp.—an international collaborative study in quality assessment

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**In order to compare the prevalence of antibiotic resistance in different geographical areas, it is necessary to ensure agreement between laboratories on the assignment of strains to 'susceptible' and 'resistant' categories. An international quality assessment was performed to investigate the performance of susceptibility testing of *Klebsiella* spp. Ninety-five strains of klebsiellae were selected from clinical isolates at the London Hospital Medical College (LHMC). These included strains with a diversity of susceptibility profiles to amoxycillin/clavulanate, piperacillin, ceftazidime, cefuroxime, ciprofloxacin, gentamicin and trimethoprim. The strains were sent to 13 participating laboratories in Europe and the USA and laboratories were asked to test the susceptibility of these strains to these antibiotics by their usual methods. They were also asked to provide details of the method used to test susceptibility. Several different standard recommended testing methods were used. Reporting of susceptibilities was generally accurate, but a number of anomalies were noted. Discrepancies of reporting between the LHMC and the participating laboratories was more marked for resistant strains, particularly in the detection of resistance to cefuroxime and ciprofloxacin, as well as the assignment of susceptibility and resistance to piperacillin and amoxycillin/clavulanate. Some discrepancies could be attributed to the use of different breakpoints, leading to differing assignment of susceptibility. Methodological variations including disc content, inoculum and failure to measure and interpret zone sizes consistently also led to anomalies. This quality assessment programme has helped to identify problems in susceptibility testing which should be investigated further.**

## Introduction

Laboratory antimicrobial susceptibility testing of strains from individual patients is designed to predict responses to therapy and influence the clinician's choice of antibiotics when treating an infection. Comparisons of prevalence of resistant strains are frequently made in order to monitor the spread of antibiotic-resistant bacteria in different countries. The objectives of these two types of examination are different. The former aims to predict clinical

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response by confirming susceptibility and the guideline is frequently based on pharmacological data. The latter aims to detect changes from the normal pattern of susceptibility and the guidelines relate closely to the normal population of strains.

Despite attention to methods, it is recognized that inter-laboratory discrepancies in susceptibility testing exist and that consensus on performance and on interpretation could be improved. Quality control to monitor performance is currently provided in the UK by the National External Quality Assessment Scheme for Microbiology (NEQAS).[1] One problem with such programmes is that only a few strains are examined. The use of a larger number of reference organisms with different susceptibility profiles and resistance mechanisms would identify sources of discrepancy within individual laboratories for further examination.

Recently, such a quality assessment was carried out for the susceptibility testing of *Haemophilus influenzae* in a number of laboratories in Europe and the USA.[2] The study demonstrated problems in the testing of a number of antibiotics and identified confounding factors, both in the methods used and in choice of guidelines for resistance. The aim of this study was to assess the agreement between laboratories from different countries in defining susceptible and resistant strains of klebsiellae and to identify factors affecting discrepancy.

## Materials and methods

### *Bacterial strains*

One hundred *Klebsiella* spp. were collected over a period of 6 months in 1993 from clinical isolates at three local hospitals linked to the LHMC. They were identified by the API 20E system (bioMérieux, Basingstoke, UK) and forwarded to the participating laboratories on nutrient agar slopes.

### *Susceptibility testing by LHMC*

The antibiotics tested were amoxycillin/clavulanate, ampicillin, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, piperacillin and trimethoprim. The susceptibility of each isolate was determined by the LHMC by disc diffusion and the MIC was determined by agar dilution. The diffusion testing was carried out using a spectrophotometrically standardized inoculum which was subsequently used at a dilution known to give semi-confluent growth. The medium used was IsoSensitest agar (Unipath, Basingstoke, UK) with 3% lysed horse blood and plates were incubated at 37°C in air for 18 h. Zone diameters were read with electronic callipers and entered into a computer (BIOMIC system, Giles Scientific Inc., NY, USA). To determine the MIC, inoculates yielding $10^4$ cfu/spot were inoculated on to plates of IsoSensitest agar containing doubling dilutions of the antibiotic to be tested. The MIC was defined as the lowest concentration of antibiotic inhibiting growth when the plates were read after 18 h incubation in air at 37°C.

Histograms of zone diameters and MICs obtained by the LHMC and scattergrams of zone diameter against $\log_2$MIC were plotted to identify the sensitive and resistant populations for each antibiotic tested (data not shown).

### *β-Lactamase production*

The type of β-lactamase produced by each strain was inferred by categorization of isolates by antibiograms. Isoelectric focusing of extracts obtained by ultrasonication of overnight broth cultures of each strain was then performed on an ampholine-based gel for the range of pH 6.0–8.0. Extended-spectrum β-lactamase (ESBL) production was confirmed by demonstration of synergy between ceftazidime and clavulanic acid (4 mg/L). The correlation between antimicrobial resistance and the type of β-lactamase produced was determined; statistical significance was tested with the $\chi^2$ test.

### *Participating laboratories*

Of the 13 laboratories enrolled, six were from the UK, two from the USA and one each from Greece, Norway, Poland, Sweden and Turkey. Each participating laboratory was required to perform susceptibility testing using their routine method. A result sheet and a questionnaire on the methods (inoculum size, media, disc content of antimicrobial agent, interpretation and guidelines used) were sent with the strains. The zone diameters measured in the participating laboratories and their interpretation were recorded and forwarded to the LHMC for analysis.

### *Data analysis*

Data were entered into an Apple Macintosh Powerbook 5300cs computer and analysed with the computer package Statview II. The participating laboratories' results, recorded as 'resistant', 'intermediate' or 'susceptible', were regarded as 'in agreement' if the results were the same as those of the LHMC, and as 'in disagreement' if different.

## Results

### *Characterization of bacterial strains*

Of the 100 klebsiella isolates distributed, five were found to be either mixed or non-viable on subculture and these were excluded from the analysis (three further such isolates were also removed from the analysis of laboratory B). Of the remaining 95 strains, 83 were identified

as *Klebsiella pneumoniae* var. *aerogenes* and 12 as *K. pneumoniae* var. *oxytoca*. The results of susceptibility testing of the strains for each antibiotic by the LHMC are shown in Figures 1–8, with the LHMC represented as laboratory A. The correlation between the MIC and the zone diameter determined by the central laboratory was high (data not shown).

All strains produced at least one $\beta$-lactamase and all were resistant to ampicillin. The predominant enzyme produced was SHV1 (58 strains); six strains produced TEM and four produced both SHV1 and TEM enzymes. Nine strains had evidence of ESBL production, seven *K. oxytoca* strains hyperproduced the chromosomal enzyme K1 (KOXY) and two *K. aerogenes* strains were subsequently confirmed to produce an AmpC ('class I') enzyme.[3] Seven strains produced other enzymes which were not fully identified. Resistance to piperacillin and amoxycillin/clavulanate was associated with production of SHV1 and TEM together, K1, ESBL and AmpC $\beta$-lactamases ($P < 0.001$). All strains with no zone to piperacillin had associated co-resistance to amoxycillin/clavulanate, suggesting TEM-1 $\beta$-lactamase hyperproduction. Resistance to cefuroxime was associated with production of AmpC and K1 enzymes, but not ESBL ($P < 0.001$), while resistance to ceftazidime was associated with production of either AmpC or ESBL ($P < 0.001$).

### *Susceptibility testing methods used by participating laboratories*

The methods used by the 13 participating laboratories are shown in the Table, with the LHMC represented as laboratory A. One laboratory (laboratory E) routinely used a broth microdilution method, while all the others used disc diffusion tests. Three of the laboratories using disc diffusion tests (laboratories C, G and H) used Stokes' method with a $\beta$-lactamase-negative *Escherichia coli* control strain.

All the laboratories that followed USA National Committee for Clinical Laboratory Standards (NCCLS) methodology used Mueller–Hinton agar. Most European laboratories used diagnostic sensitivity or IsoSensitest agar. Supplements were used by three laboratories (laboratories B, E and H).

The majority of laboratories used discs containing 10 μg ampicillin, 20 μg amoxycillin/10 μg clavulanate, 30 μg cephalosporin and 10 μg gentamicin. Discs containing larger amounts of piperacillin, ciprofloxacin and trimethoprim discs were commonly used in the laboratories from outside Europe. With the exception of the 30 μg ceftazidime disc, the antibiotic content of the discs used by laboratory P differed from those used by the other laboratories.

Most participating laboratories used a standard inoculum equivalent to the McFarland 0.5 standard to achieve semi-confluent growth. However, the techniques used for the preparation and estimation of the inoculum were variable, with no two laboratories following identical procedures.

### *Susceptibility test results recorded at participating laboratories*

The majority of participating laboratories tested all of the

**Table.** Susceptibility testing methods used by participants to test *Klebsiella* isolates

| Laboratory | Medium | Supplement | Interpretative guidelines | Method of reading |
|---|---|---|---|---|
| A | ISA | 3% LHB | in-house | calliper/BIOMIC |
| B | DST | 5% LHB | in-house/BSAC | calliper/BIOMIC |
| C | ISA | – | Stokes' | calliper |
| D | DST | – | BSAC | ruler |
| E | DST | PNPG (50 mg/L) | BSAC/NCCLS combined[a] | – |
| G | DST | – | Stokes' | ruler |
| H | DST | 7% LHB | Stokes' | ruler |
| J | ISA | – | Swedish RGA | calliper |
| K | MHA | – | NCCLS | BIOMIC |
| L | MHA | – | NCCLS | ruler |
| M | MHA | – | NCCLS | ruler |
| N | MHA | – | NCCLS | calliper |
| O | MHA | – | NCCLS | BIOMIC |
| P | ISA | – | in-house | ruler |

Abbreviations: DST, diagnostic sensitivity agar; ISA, IsoSensitest agar; LHB, lysed horse blood; MHA, Mueller–Hinton agar; PNPG, *para*-nitrophenol glycerol.
[a]Dilution method used.

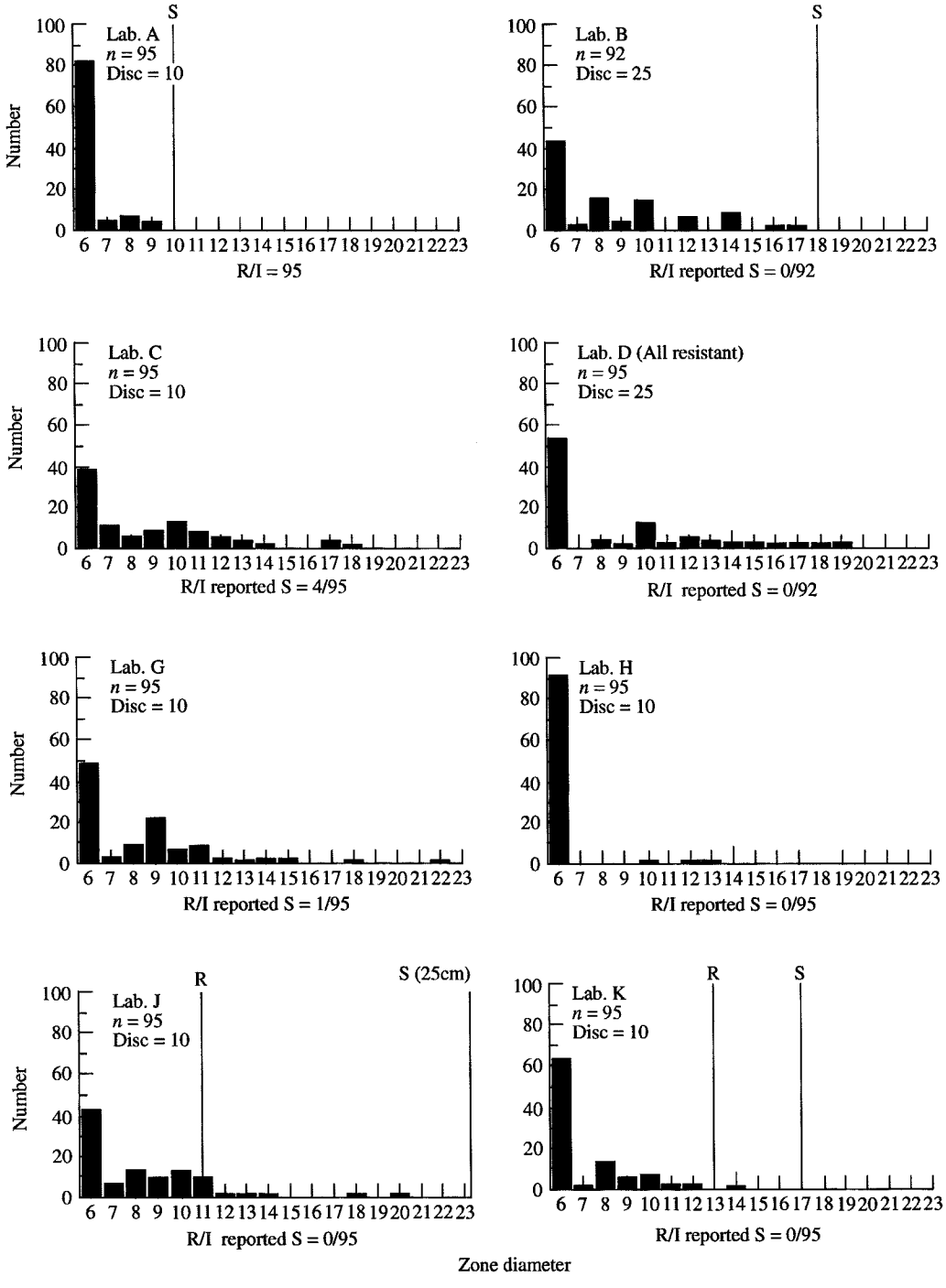


**Figure 1.** Inhibition zones (mm) of ampicillin for the LHMC (laboratory A) and each other laboratory. Black bars, ampicillin-resistant, white bars, ampicillin-sensitive; S, susceptible; R/I, either resistant or intermediate; *n*, number of strains tested; disc content in μg.

95 strains distributed, with the exception of laboratories B and D, which tested 92, and laboratory P, which tested 93. Laboratory P did not routinely test susceptibility to amoxycillin/clavulanate or piperacillin. Susceptibility to piperacillin was also not routinely tested by laboratories J and H, although laboratory H did test for piperacillin susceptibility for the purposes of this survey. Three laboratories (laboratories K, M and N) tested for trimethoprim/sulphamethoxazole rather than trimethoprim. Laboratory N routinely performed a screen for ESBL production and, if this was positive, the susceptibility results of the cephalosporins were not reported, even if susceptibility testing subsequently suggested them to be sensitive (i.e. were regarded as resistant).

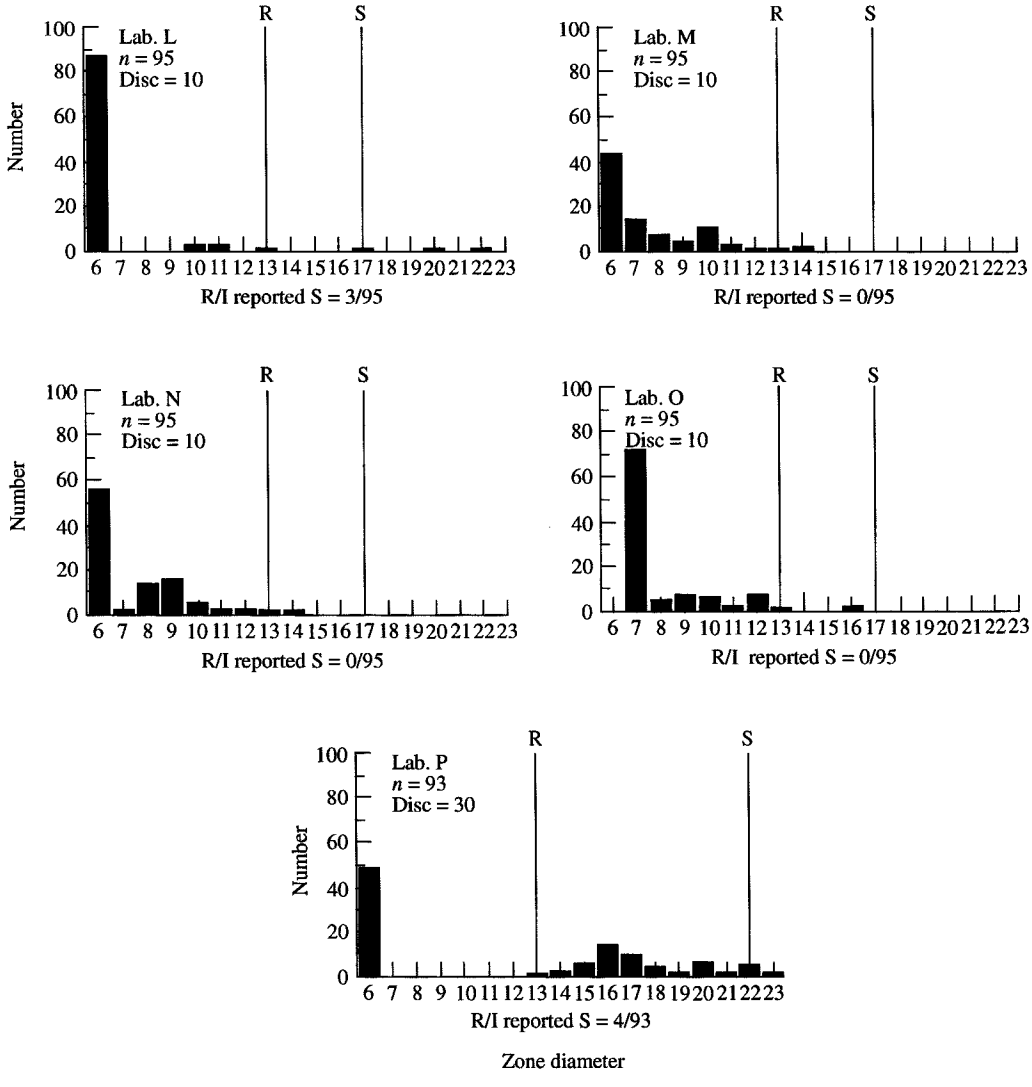


**Figure 1.** *Continued*

With the exception of laboratory E, which used the dilution method, inhibition zone diameters of each antibiotic recorded in each laboratory were plotted on separate histograms indicating the population distribution (Figures 1–8). The susceptibility guidelines used by the participating laboratories for each of the antimicrobial agents are shown by solid lines (with the exception of laboratories C, G and H which used Stokes' method). The central laboratory is represented as laboratory A.

The strains were most extensively studied at the LHMC and the designation of resistant by the LHMC (laboratory A) was used as the standard against which other laboratories were assessed. The LHMC result was taken as 'correct' in scoring the laboratory results. Although discrepant results were obtained by all laboratories, the discrepancies were with a varying number of variable strains and varying antibiotics. The consensus result for most strain/antibiotic combinations was similar to that of the LHMC.

Analysis of the data from the participating laboratories revealed a number of transcription errors and some of the participants failed to follow their own guidelines when interpreting zone diameters as susceptible or resistant. Such discrepancies between the recorded zone diameter and the interpretation of that result were recorded as 'discordant' results, although the histograms show the true zone sizes reported. Preferential reporting of even numbers was noted for the laboratories that used Stokes' method, particularly laboratory H.

## *Ampicillin*

Figure 1 shows histograms of the inhibition zones obtained with ampicillin discs from each reporting laboratory. The overall concordance of reporting of ampicillin resistance between the LHMC and the participating laboratories was high (99%). Most laboratories reported ampicillin resistance regardless of the zone size. Nine of the lab-

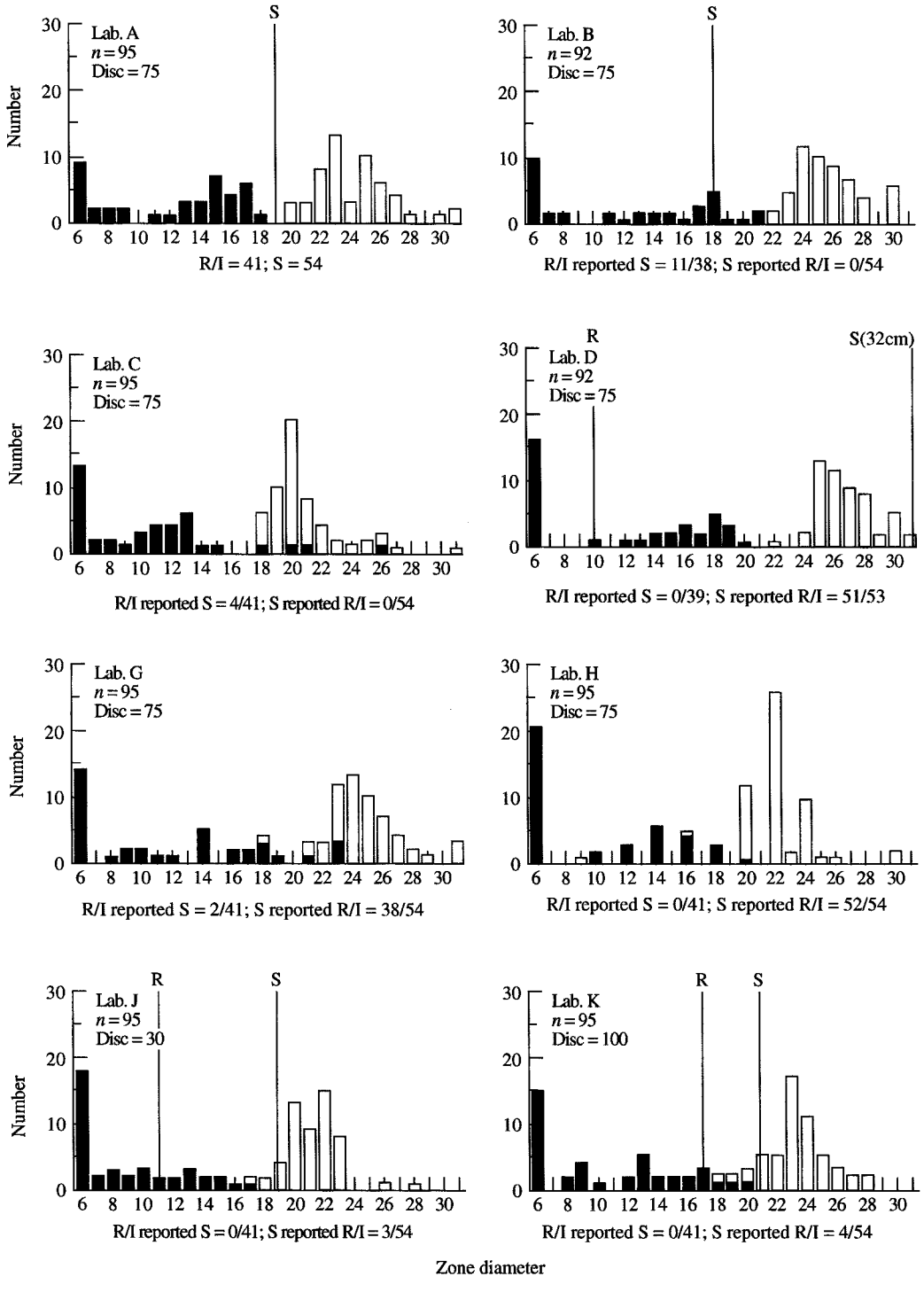


**Figure 2.** Inhibition zones (mm) of piperacillin for the LHMC (laboratory A) and each other laboratory. Black bars, piperacillin-resistant; white bars, piperacillin-sensitive; abbreviations as in Figure 1.

oratories (laboratories B, D, E, H, J, K, M, N and O) reported results in complete concordance with the LHMC and three others (laboratories C, G and L) had only minor differences of interpretative agreement. Laboratory L reported three strains with higher zone diameters than the other laboratories that used NCCLS guidelines. This resulted in these strains being classified as sensitive using the standard NCCLS breakpoints and may have been caused by the inoculum effect. Laboratory P, which used the highest disc content (30 μg), reported higher zones of inhibition and a bimodal distribution of susceptibilities. Although only four of the resistant strains were reported

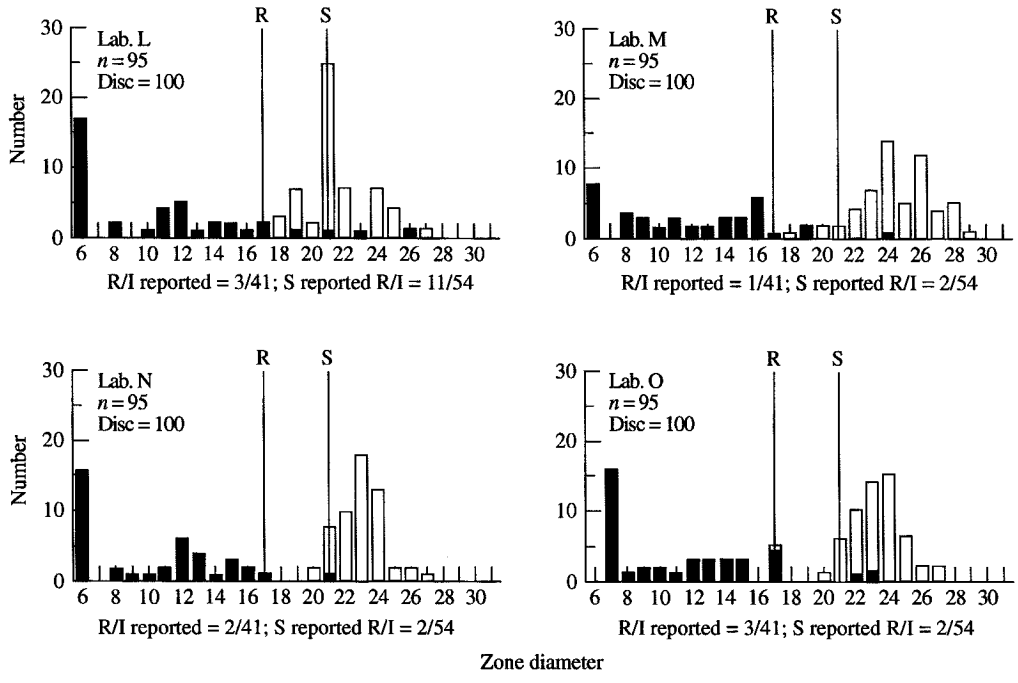


**Figure 2.** *Continued*

as sensitive by laboratory P; 43 strains were reported as intermediate as a result of the wide separation of resistant and susceptible breakpoints.

### *Piperacillin*

Forty-one of the 95 strains were resistant to piperacillin. The discrepancies in reporting piperacillin-susceptibility between the LHMC and the participating laboratories are shown in Figure 2. Four laboratories reported the same 41 resistant strains as the LHMC (laboratories D, H, J and K). The other participating laboratories reported a number of resistant strains with zone diameters within their sensitive population. This problem of distinguishing resistant and sensitive strains was found equally in laboratories that used 75 μg or 100 μg discs. However, laboratory J, which used discs containing 30 μg piperacillin, was the most accurate in the reporting of these two populations. In laboratory B, the adoption of inappropriate breakpoints was responsible for the failure to detect resistance. If the susceptibility guidelines had been adjusted to 22 mm rather than 18 mm, 95% of resistant strains would have been recognized.

Two of the participating laboratories (laboratories B and C) reported piperacillin-sensitive strains in complete concordance with the LHMC, while six reported minor discordant results only. Of the other participants, the concordance of the results for one of the laboratories (laboratory D) could have been improved to 96% (rather than 4%) by moving the susceptibility breakpoint to 21 mm. Laboratory G, which used Stokes' method, reported 38 sensitive strains as intermediate; variation in the inoculum size of either test or control strain may explain these results. The discrepancies in reporting by laboratory L result from a proportion of sensitive strains being reported as intermediate. This was not found by the other laboratories using the NCCLS guidelines, and may again be a result of the inoculum used. The high number of susceptible strains reported as resistant by laboratory H was a result of their policy of regarding ampicillin-resistant klebsiellae as also resistant to piperacillin.

### *Amoxycillin/clavulanate*

Results for amoxycillin/clavulanate susceptibility and zone distribution recorded in the participating laboratories are shown in Figure 3. All laboratories used a 30 μg disc containing 20 μg amoxycillin and 10 μg clavulanate. A number of laboratories (laboratories B, K, L and N) were not consistent in following their interpretative guidelines when reporting, with resultant errors for both sensitive and resistant strains.

The reporting of amoxycillin/clavulanate resistance was a particular problem and only one laboratory had full concordance with the LHMC (laboratory N). Laboratories using the NCCLS and Mueller–Hinton agar (laboratories K to O) had significantly fewer errors than laboratories using other guidelines ($P < 0.05$). There was a high level of discrepancies for the laboratory which used the dilution method (laboratory E), and two of the laboratories that used Stokes' method (laboratories C and G). All the participating laboratories experienced problems in the

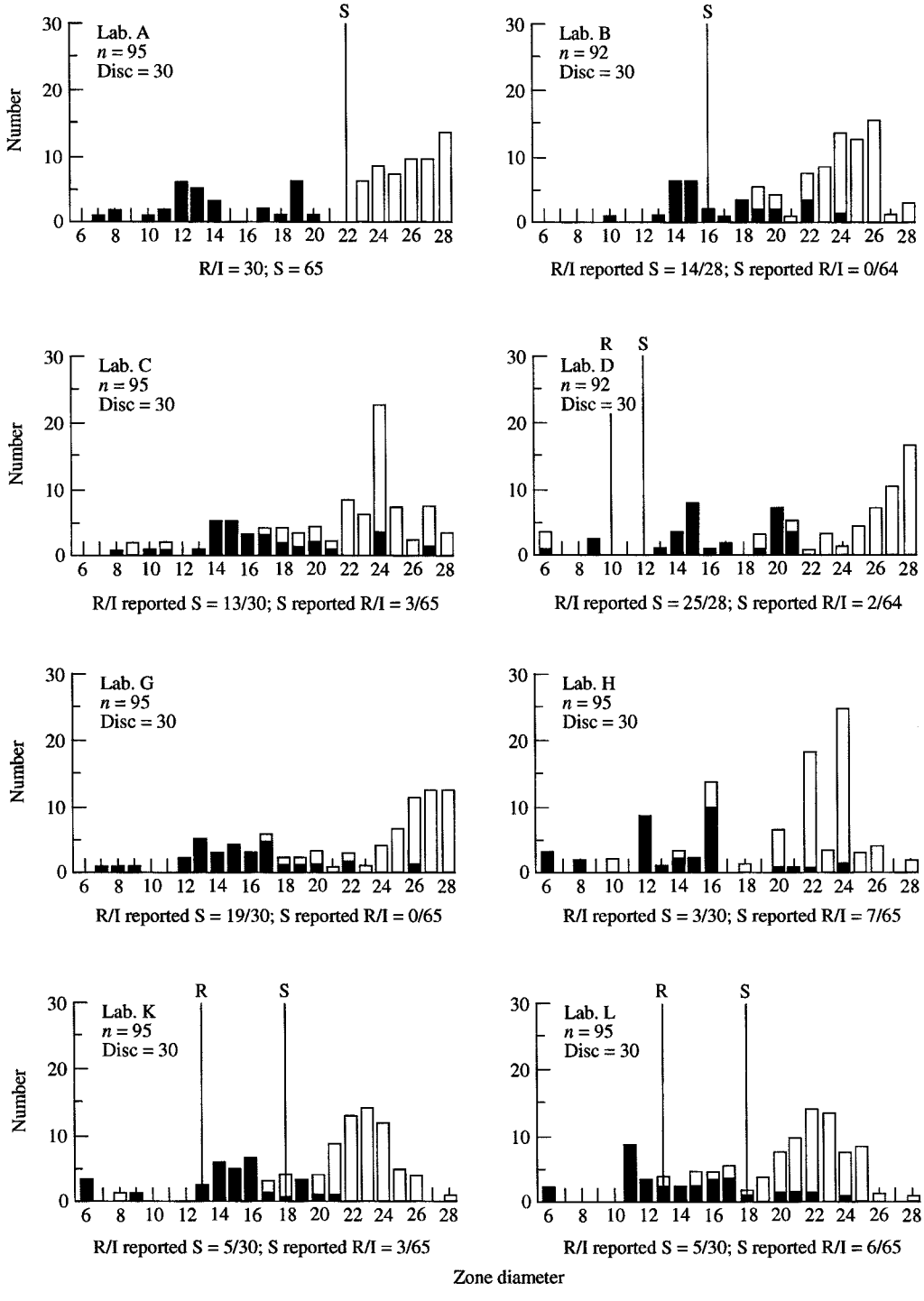


**Figure 3.** Inhibition zones (mm) of amoxycillin/clavulanate for the LHMC (laboratory A) and each other laboratory. Black bars, amoxycillin/clavulanate-resistant; white bars, amoxycillin/clavulanate-sensitive; abbreviations as in Figure 1.

allocation of strains with zone diameters close to the breakpoint to resistant or sensitive. This reflects the difficulty of any test to distinguish such strains, but is, in part, also likely to result from the inoculum effect. Despite this, the adoption of different interpretative guidelines would improve the agreement of detecting resistance with the LHMC for laboratories B, D and K. For example, for laboratory D, the use of a breakpoint of 22 mm would result in 100% concordance in the reporting of resistant strains.

All laboratories were >89% efficient in detecting amoxycillin/clavulanate-resistant strains. Slightly fewer strains were correctly characterized as sensitive by the laboratories using NCCLS methods, though this did not

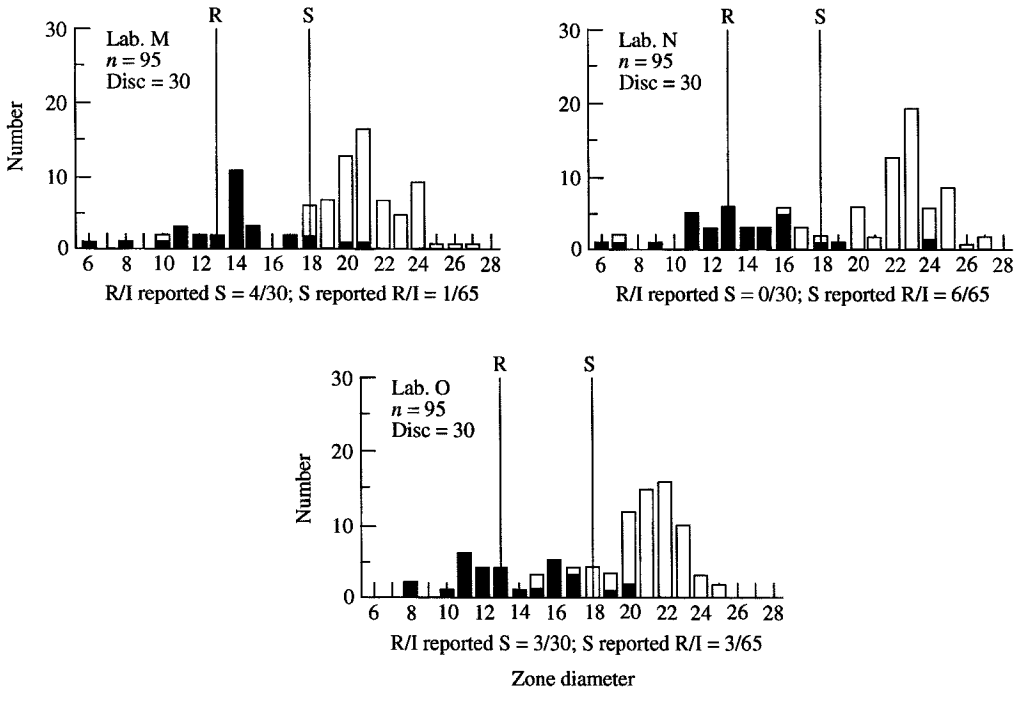


**Figure 3.** *Continued*

reach significance. This is partly a result of their selected breakpoint, which gave better overall identification of resistant strains, resulting in subsequent corresponding errors in the reporting of sensitive strains.

### *Cefuroxime*

Eleven strains were reported as resistant to cefuroxime by the LHMC. The discrepancies between the LHMC and the participants are shown in Figure 4. The reports returned from laboratories J, L and N contained inconsistencies between the zone diameter found and the allocation to resistant or sensitive by their guidelines.

The concordance between the LHMC and the participating laboratories in the reporting of the clearly cefuroxime-resistant strains was high. However, four of the resistant strains gave only a slightly reduced zone diameter with the 30 μg disc used by all the participants except laboratory P. The majority of reporting errors was a result of the incorrect identification of these strains, and was a particular problem if the strains' zone diameter was around the chosen breakpoint. Overlap between these and sensitive strains were reported around the breakpoint by a number of laboratories (laboratories B, C, D, K, M, N and O). Laboratory P had the most reporting errors and was the only laboratory to use a 60 μg disc. This resulted in the majority of resistant strains having larger zone diameters, with three strains reported well within the susceptible population.

The reporting of strains sensitive to cefuroxime was accurate for most participating laboratories. The allocation of 80% of the sensitive strains to an intermediate category by laboratory D was the result of the wide separation of resistant and sensitive breakpoints.

### *Ceftazidime*

Figure 5 shows histograms of the inhibition zones obtained with ceftazidime discs from each reporting laboratory. Failure to follow interpretative guidelines consistently was noted for laboratories H, J, K and P.

Only 11 strains were resistant to ceftazidime and, with a few exceptions, the methods adopted by most of the laboratories divided the resistant and sensitive strains into two populations; the reporting of these strains was largely in agreement with the LHMC. Three laboratories (laboratories B, M and N) reported all results in concordance with the LHMC, whereas six showed only one or two discrepant results. Complete concordance could have been achieved for laboratories D, J and K with a slight adjustment of their interpretative guidelines. Despite reporting the use of the same disc content (30 μg) as all the other participants, laboratory P reported all resistant strains as having higher zone diameters. This resulted in laboratory P having the highest number of reporting errors for resistant strains (36%). It was not possible from the data returned to identify the reason for this.

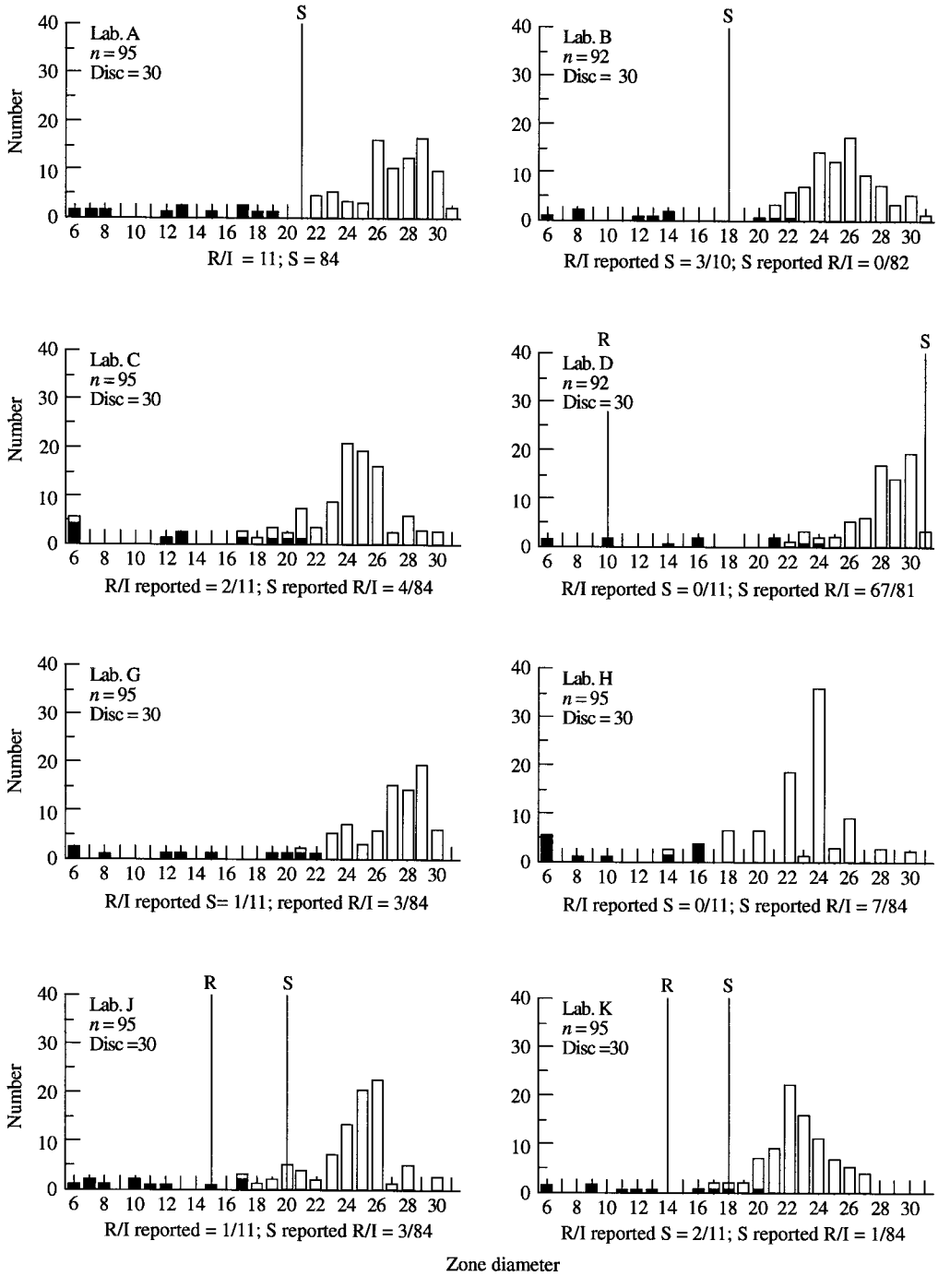


**Figure 4.** Inhibition zones (mm) of cefuroxime for the LHMC (laboratory A) and each other laboratory. Black bars, cefuroxime-resistant; white bars, cefuroxime-sensitive; abbreviations as in Figure 1.

### *Gentamicin*

Twelve of the strains were found to be gentamicin-resistant by the LHMC and the accuracy of reporting of susceptibilities to gentamicin by the participating laboratories was generally high (Figure 6). Five of the laboratories (laboratories B, E, H, K and M) were 100% accurate in the reporting of resistant and susceptible strains. Similar results could have been obtained by altering the guidelines of laboratories J (to 16 mm) and P (to 21 mm). For the remaining laboratories, the errors in the reporting of resistant strains resulted from the presence of a single outlying resistant strain within the susceptible population. However, in each case the strain concerned was different for each laboratory.

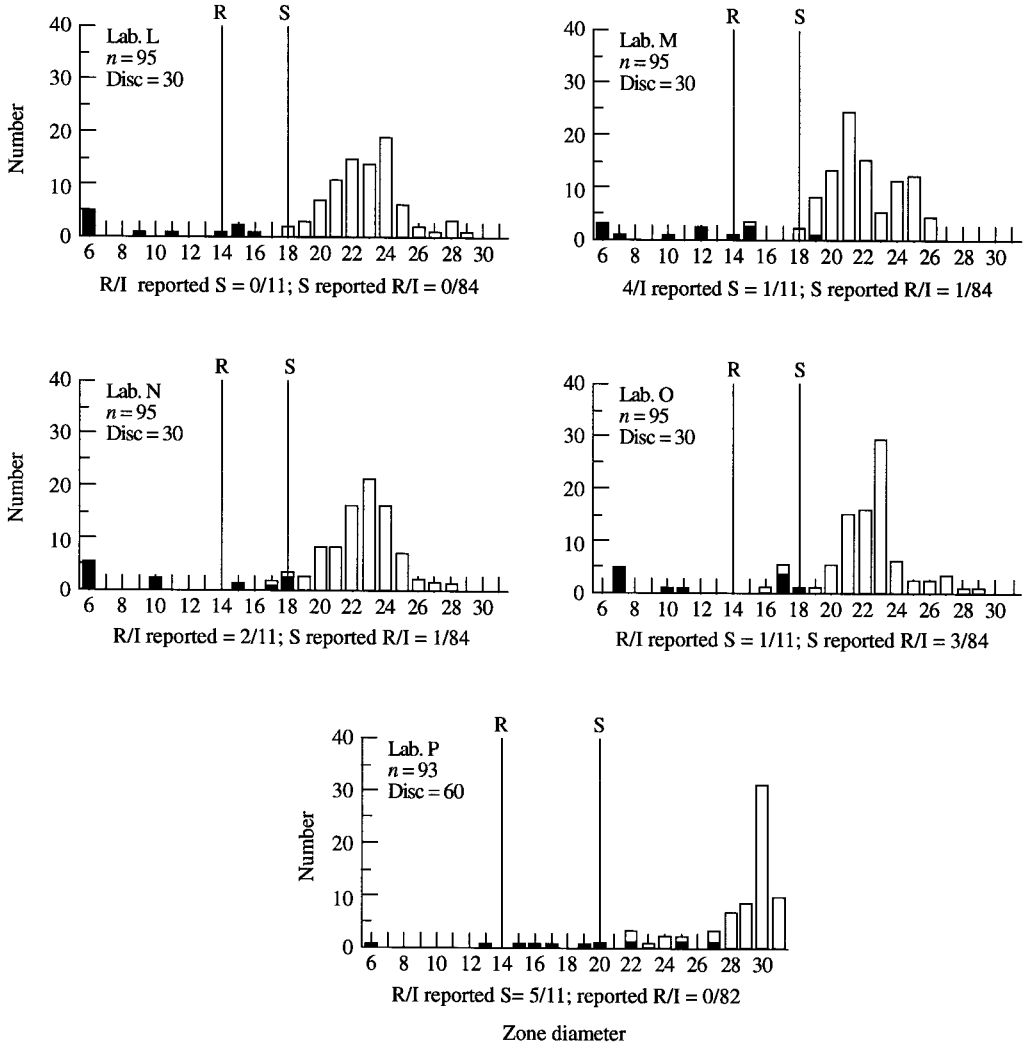


**Figure 4.** *Continued*

## *Ciprofloxacin*

There was full concordance in the reporting of the seven ciprofloxacin-resistant strains between the LHMC and the three participating laboratories that used Stokes' method (laboratories C, G and H). More errors in reporting resistant strains were recorded by the laboratories that used a 5 or 10 μg disc rather than a 1 μg disc. These errors were mainly associated with resistant strains that had slightly reduced zone diameters, as these were distributed around the breakpoint in laboratories using the higher disc content (Figure 7). For many laboratories the results are improved if the interpretative guidelines are reviewed. For example, if laboratory D had used a single breakpoint of 24 mm, all resistant strains would have been correctly identified. In laboratory B, a zone diameter of 18 mm was adopted as the breakpoint, despite the three 19 mm zones produced (of which two resulted from resistant organisms).

The reporting of the ciprofloxacin-sensitive strains was generally accurate, with eight laboratories (laboratories B, D, J, K, L, N, O and P) agreeing with the LHMC. The few errors that were reported all involved strains with zone diameters around the breakpoint.

## *Trimethoprim*

The discrepancies in reporting trimethoprim susceptibility between the LHMC and the participating laboratories are shown in Figure 8. Laboratories D, H, K, L and N all had discrepancies in following their interpretative guidelines when reporting. Laboratories K, N and O tested susceptibility to trimethoprim/sulphamethoxazole and, although the latter two laboratories were able to distinguish resistant from sensitive isolates as well as laboratories testing trimethoprim, laboratory K had the highest error rate for both susceptibility patterns. This laboratory reported nine resistant strains with zone diameters well

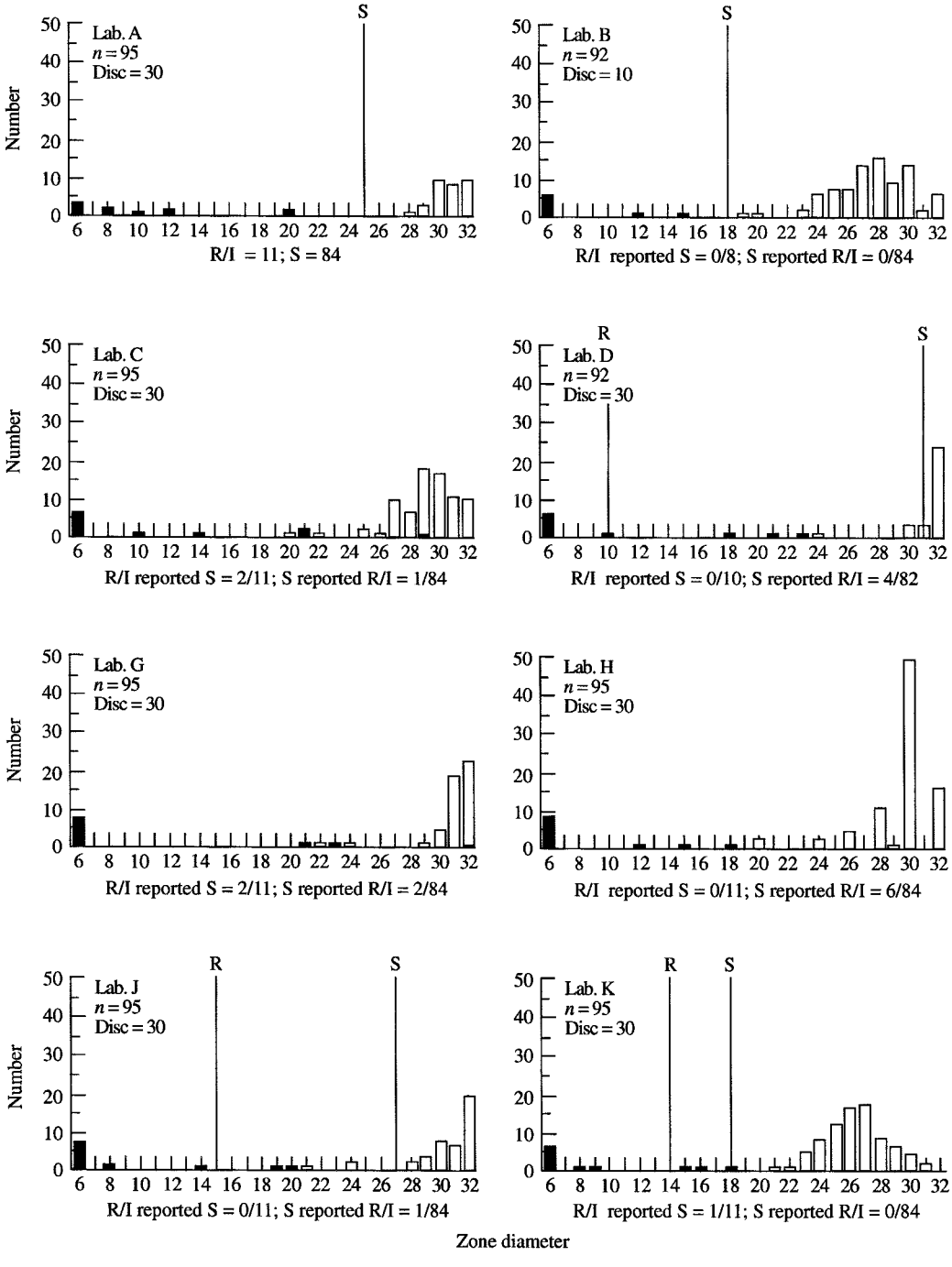


**Figure 5.** Inhibition zones (mm) of ceftazidime for the LHMC (laboratory A) and each other laboratory. Black bars, ceftazidime-resistant; white bars, ceftazidime-sensitive; abbreviations as in Figure 1.

within the sensitive population. It may be that the guidelines followed by the laboratory (NCCLS) are not suitable for testing this antibiotic combination. The other laboratories showed only one or two discordant results for the reporting of trimethoprim resistance; in each case the strains misreported were different.

The concordance in the reporting of trimethoprim-sensitive strains was >92% for nine of the laboratories. Two of the laboratories (G and H) with higher errors of reporting used Stokes' method. The use of widely separated resistant and sensitive breakpoints resulted in laboratory D reporting 69 sensitive strains as intermediate. If a single breakpoint of 21 mm is used, the accuracy of reporting of susceptible strains is improved to 96% (with one resistant strain misreported as sensitive). Inappropriate interpretative guidelines were also apparent in some other laboratories, where either resistance was reported as sensitive or vice versa (Figure 8).

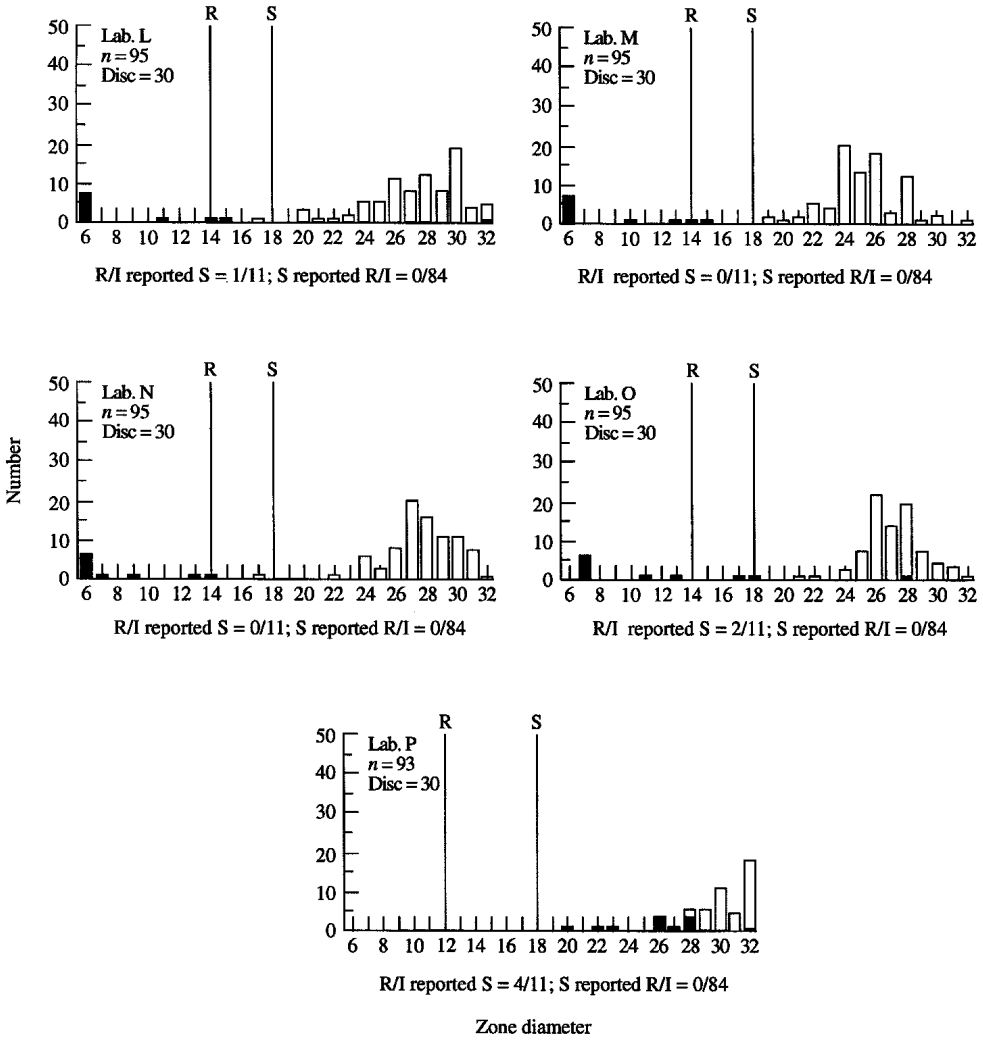


**Figure 5.** *Continued*

## Discussion

Reporting of antimicrobial susceptibility has a major influence in determining antibiotic choice. In an attempt to improve agreement in reporting, there has been a recent trend towards national and international standardization of susceptibility testing.[4–6] The most widely used methods are NCCLS ones.[7] Adoption of such methods should lead to the use of broadly appropriate methods and optimize the choice of medium, disc content and incubation conditions. However, some local variation in methodology, e.g. in inoculum size and the accuracy of measurement and interpretation of zone sizes, will still occur. The adoption of a standard set of interpretative breakpoints along with a standard method could lead to inaccuracies in reporting if these interlaboratory variations significantly affect the results of the test.

The aim of this study was to examine the accuracy of reporting of susceptibility of *Klebsiella* spp. and to identify factors affecting performance. Klebsiellae account for 10–20% of opportunistic Gram-negative infections from in-patients[8] and have a wide range of resistance profiles and mechanisms. The clinical strains selected for this study included those with a range of resistance to the antibiotics tested and also many of the resistance mechanisms recognized to exist in this species. The strains selected were representative of the range of klebsiellae likely to be encountered in a clinical laboratory, and were similar to other published collections of this species.[9]

Although it is possible that there was more attention to detail and meticulous performance during testing by the LHMC than is possible in routine daily busy practice, there was general agreement in the results obtained by the LHMC and the majority of participating laboratories. In a few cases a resistant strain was reported by a single laboratory as sensitive. These 'outlier' strains were assumed to be the result of the loss of a resistance determinant during storage or to errors of subculture or transcription. The study did allow the identification of some of the factors that gave rise to discrepancies in reporting. It also showed that discrepancies in susceptibility reporting can occur between laboratories that use

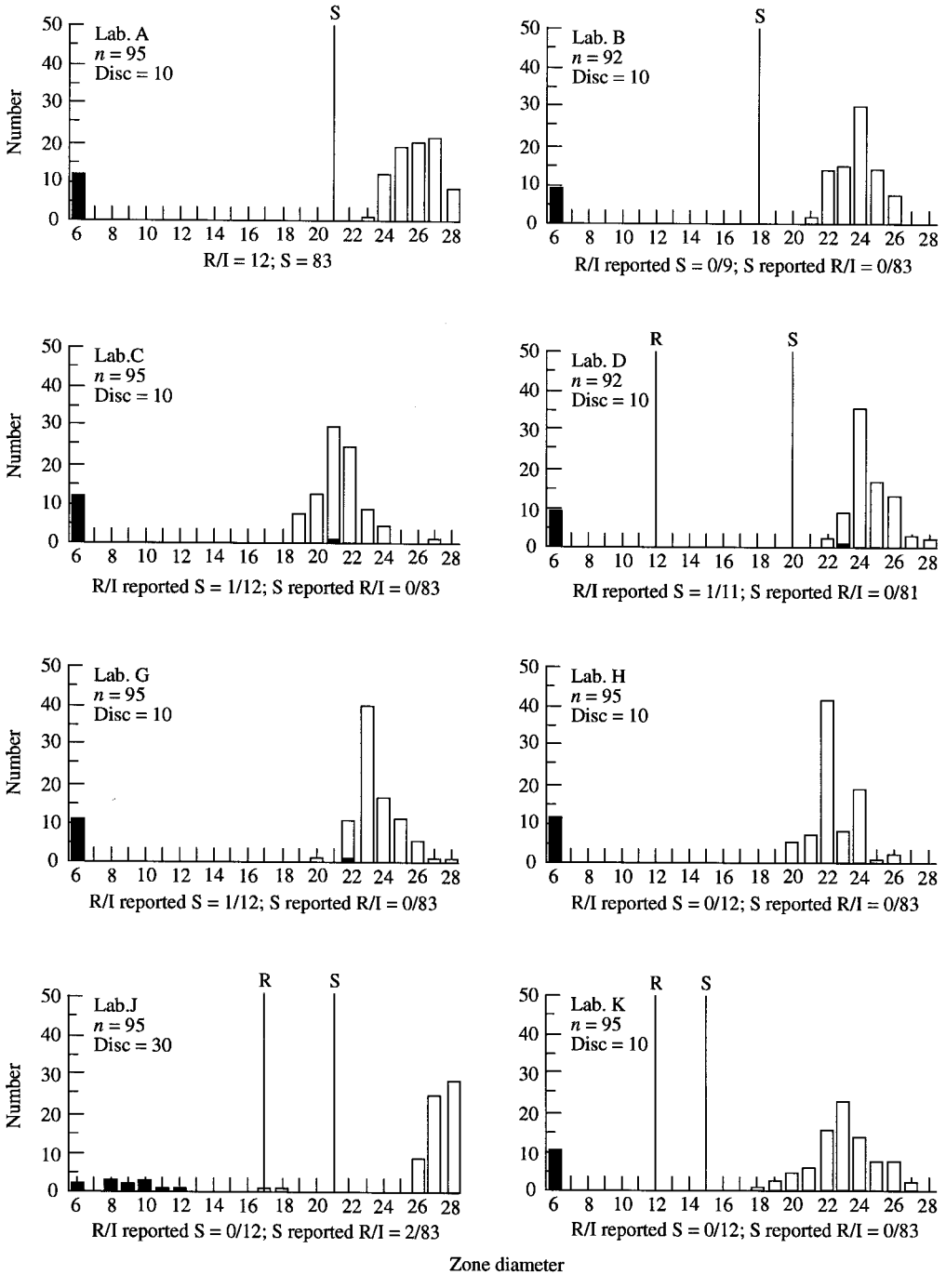


**Figure 6.** Inhibition zones (mm) of gentamicin for the LHMC (laboratory A) and each other laboratory. Black bars, gentamicin-resistant; white bars, gentamicin-sensitive; abbreviations as in Figure 1.

the same methods adopted from published standards. However, the advantage of using standard as opposed to non-standard methods was seen in the close agreement of the results between the NCCLS laboratories.

One important cause of inter-laboratory variation is inoculum size. There was considerable variation in the preparation, standardization and application of the inoculum, and no two laboratories used the same method. Variation in the inoculum used is known to have significant effects on susceptibility reporting and this has particularly been shown for *Klebsiella* spp. with the testing of piperacillin.[10] A wide range of colony counts has been reported when the turbidity of colony suspensions is adjusted to match a 0.5 McFarland turbidity standard.[11] Similar problems in standardizing the inoculum by visually adjusting the bacterial suspension to a McFarland standard has also been noted and has led to the recommendation of the use of a spectrophotometer for standard-

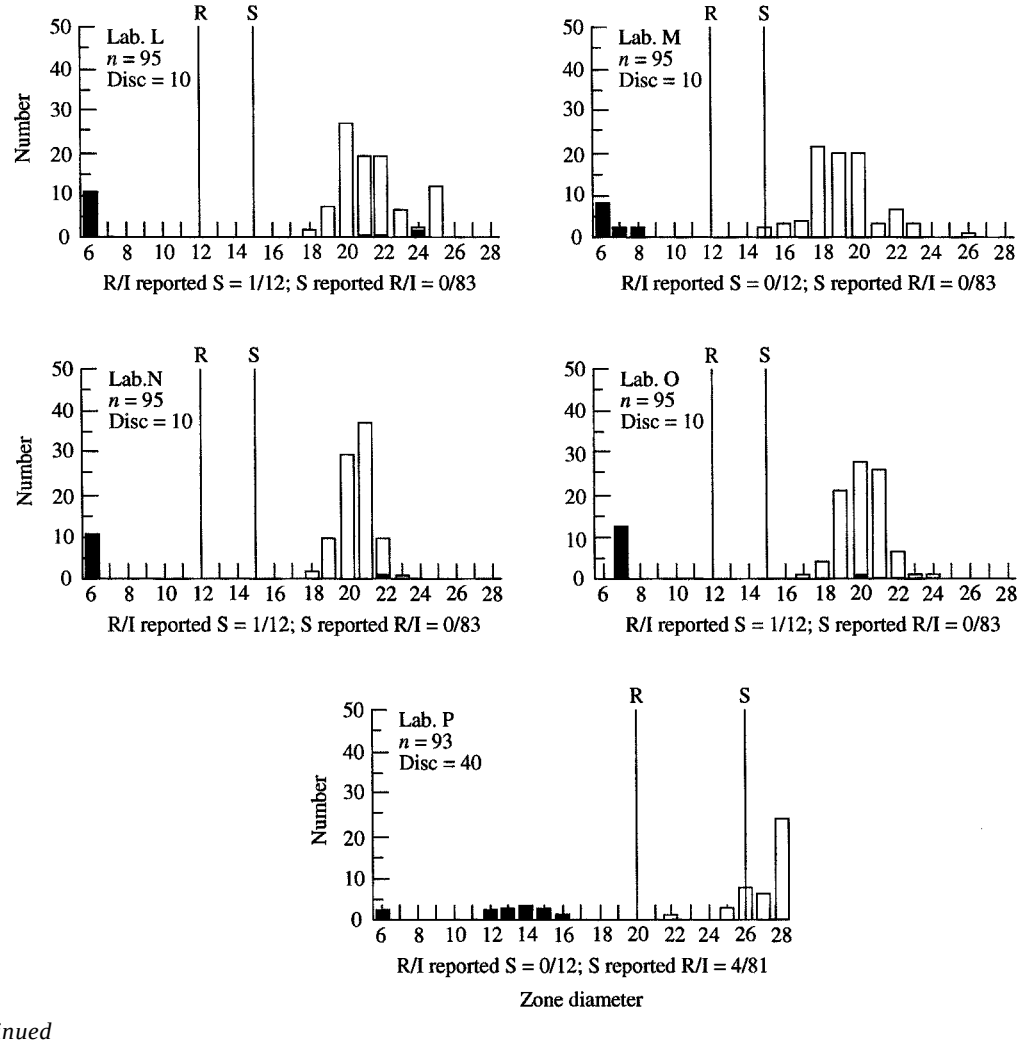


**Figure 6.** *Continued*

ization.[12] In routine daily practice, accurate and standardization of inoculum by such a method is difficult to ensure, although this is done routinely at the LHMC.

The measurement and interpretation of zone sizes was also identified as a potential source of error. Preferential reporting of even numbers by laboratories that used Stokes' method (particularly laboratory H) is a result of measuring the zone radius and doubling this figure. As well as being inherently inaccurate in itself, this also doubles any error of measurement. A number of interpretative and transcription errors were also identified. Such reporting inconsistencies could be reduced by the use of a calliper based or video reading system under computerized control.

Even when resistant and sensitive populations were clearly distinguished, misreporting still occurred as a result of the use of inappropriate guidelines. Although problems will occur with strains around the breakpoint, the chosen breakpoint should discriminate between clearly resistant and susceptible strains. In this study, misreporting of strains due to inappropriate guidelines was particularly noted for piperacillin, amoxycillin/clavulanate, ceftazidime and ciprofloxacin. Guidelines will generally have been adopted along with standardized methods, but inter-laboratory variations in methodology may result in the need for local adjustment of these guidelines. The results of histogram analysis showed that frequently results could be improved when laboratory-specific guidelines, based on the results reported by the individual laboratory, were used.

The finding in this study of uniform resistance to ampicillin and production of $\beta$-lactamases by all strains supports the view that $\beta$-lactamase production is now virtually universal in the genus, and is usually of SHV type.[13] Although the reporting of resistant strains was generally accurate, this study identified potential problems in the control of the inoculum and the use of high disc contents for susceptibility testing. The solution may be not to test ampicillin at all or, if it is tested, to record all the answers as resistant.

The association of piperacillin-resistance with high-level expression of TEM and/or SHV1 or with other broadly active $\beta$-lactamases confirms previous observations.[14] It has been recognized that the level of

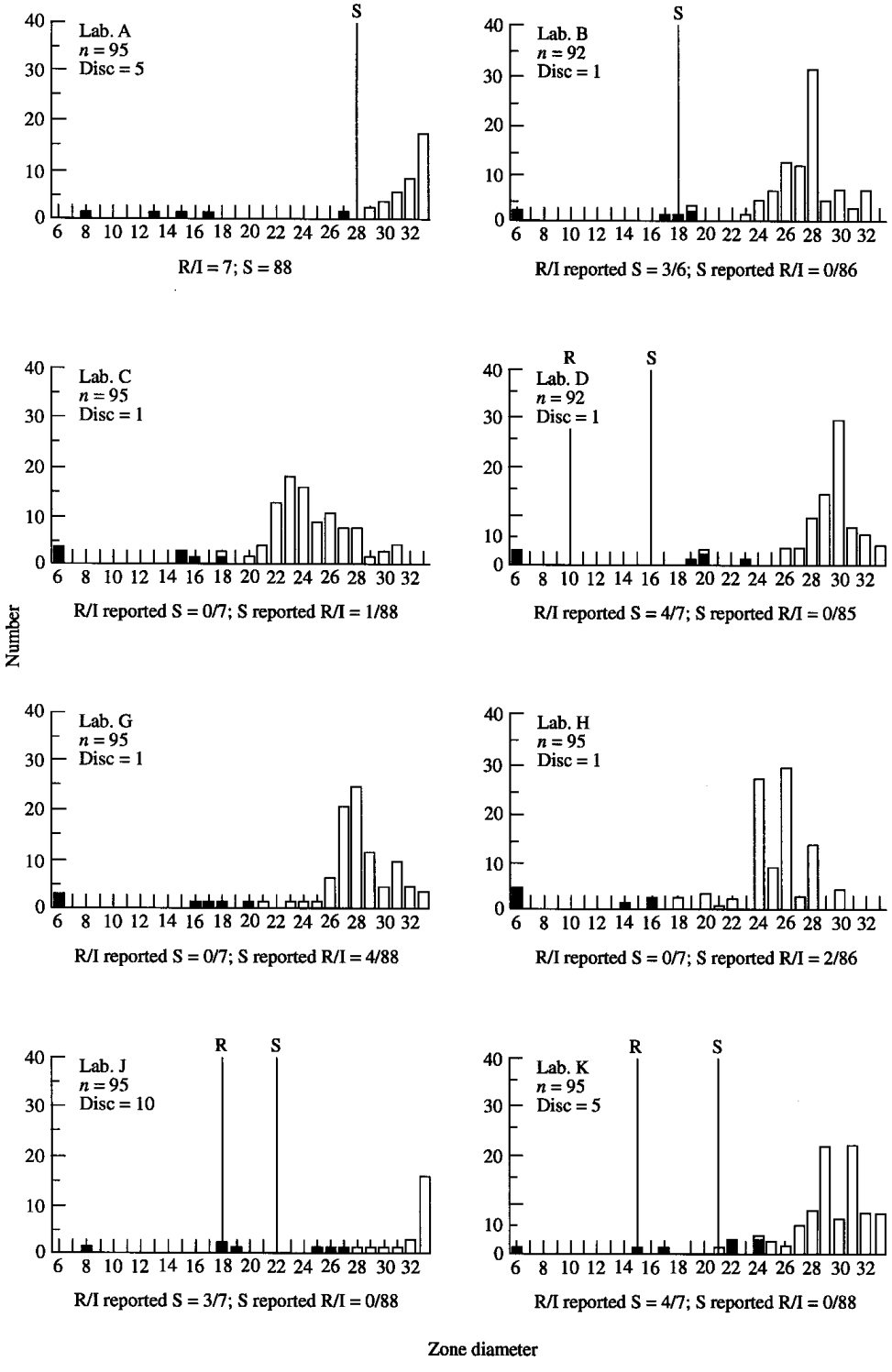


**Figure 7.** Inhibition zones (mm) of ciprofloxacin for the LHMC (laboratory A) and each other laboratory. Black bars, ciprofloxacin-resistant; white bars, ciprofloxacin-sensitive; abbreviations as in Figure 1.

$\beta$-lactamase expression in some strains of klebsiellae may be insufficient to form a substantial reduction in the zone of inhibition around a 75 μg disc.[8] This will result in some resistant strains being identified as susceptible, and may explain why laboratories that used a 75 or 100 μg disc reported resistant strains within their sensitive populations. In this study the laboratory that discriminated best between the two populations was the one that used a lower disc content, 30 μg (laboratory J).

Most of the errors in the reporting of susceptibility to amoxycillin/clavulanate involved strains around the breakpoint. It is likely that the inoculum effect was responsible

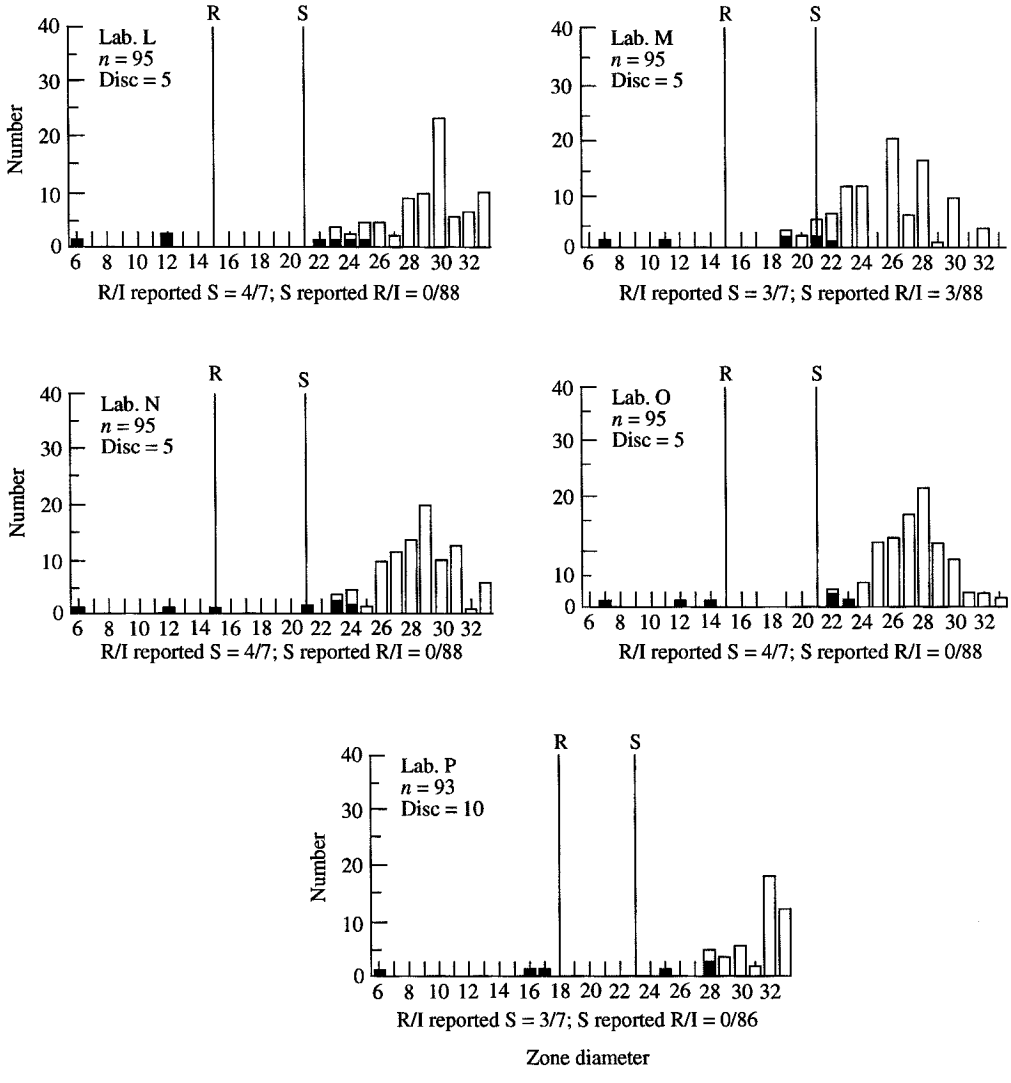


**Figure 7.** *Continued*

for some of these errors, but other factors that are known to affect performance, such as disc instability and the use of a non-$\beta$-lactamase-producing control organism (Stokes' method), could have been important. Further local information would be needed to identify which of other factors were influencing performance. In the present study, laboratories that used the NCCLS guidelines (and Mueller–Hinton agar) were significantly more accurate in reporting resistance ($P < 0.05$).

An important observation by both the central and participating laboratories was that strains producing ESBLs appeared sensitive to cefuroxime. Such producers of ESBLs are prone to being reported as sensitive to cephalosporins, despite clinical evidence that ESBL production is associated with clinical failure.[15] A recent study of klebsiellae on European intensive care units confirmed that up to 33% of ESBL-producers may be reported as susceptible to third-generation cephalosporins.[9] One suggested way to avoid such misreporting is to adopt a screening test for ESBL production and report all positive strains resistant to penicillins and cephalosporins regardless of the results of susceptibility testing.[16]

There were fewer errors in the reporting of resistance to ciprofloxacin for those laboratories that used a 1 μg disc. Only a few ciprofloxacin-resistant strains were included in the survey and although this difference did not reach significance, it is worthy of further study. For the remaining antibiotics tested, the majority of discrepancies resulted from the adoption of inappropriate interpretative guidelines, and results could have been improved by local review of these.

In conclusion, this study demonstrated that a number of problems exist in the performance testing of *Klebsiella* spp. It confirms that no one method is superior for susceptibility testing as long as the method used is able to separate resistant and sensitive strains, is reproducible and uses appropriate interpretative guidelines. In some cases improvement of individual performance could be achieved either by changes to methodology, such as disc content, preparation of inoculum and measurement and reporting

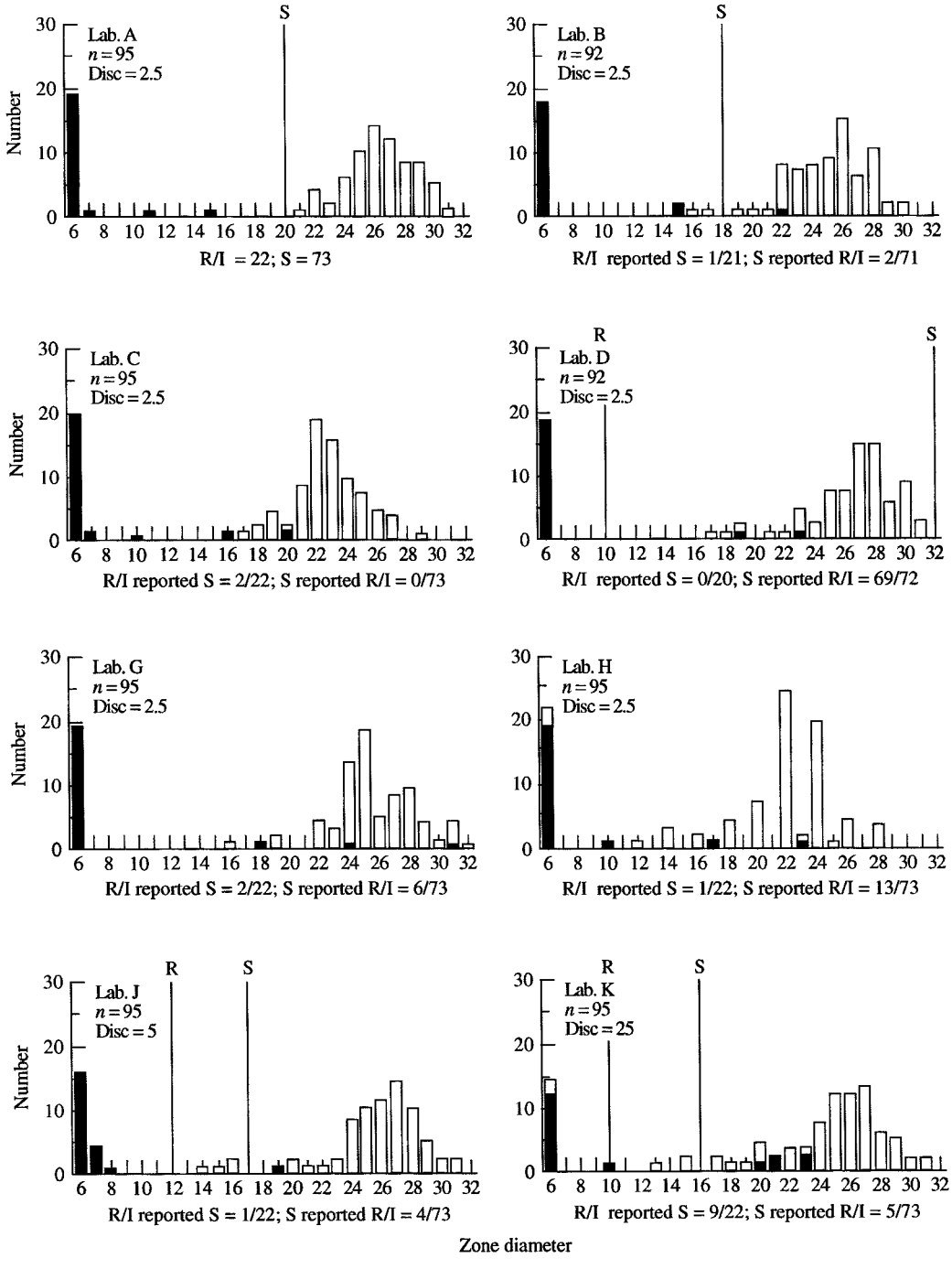


**Figure 8.** Inhibition zones (mm) of trimethoprim for the LHMC (laboratory A) and each other laboratory. Black bars, trimethoprim-resistant; white bars, trimethoprim-sensitive; abbreviations as in Figure 1.

of zone diameter, or by adjusting the interpretative guidelines. Recommendations, based upon this and our previous study[2] are as follows: (i) it is not necessary for all laboratories to perform antimicrobial susceptibility testing in the same way, but tests need to be performed in a reproducible manner; (ii) the methods selected should be able accurately to differentiate susceptible and resistant strains; (iii) discs containing lower amounts of antibiotic should be used; (iv) inocula should be prepared in a reproducible manner; (v) zone diameters should be measured precisely and care should be taken to avoid interpretative and transcription errors; (vi) if a particular system for susceptibility testing is adopted, this cannot be confined to interpretative breakpoints, but the prescribed methodology has to be precisely followed; (vii) interpretative breakpoints can be generated in an individual

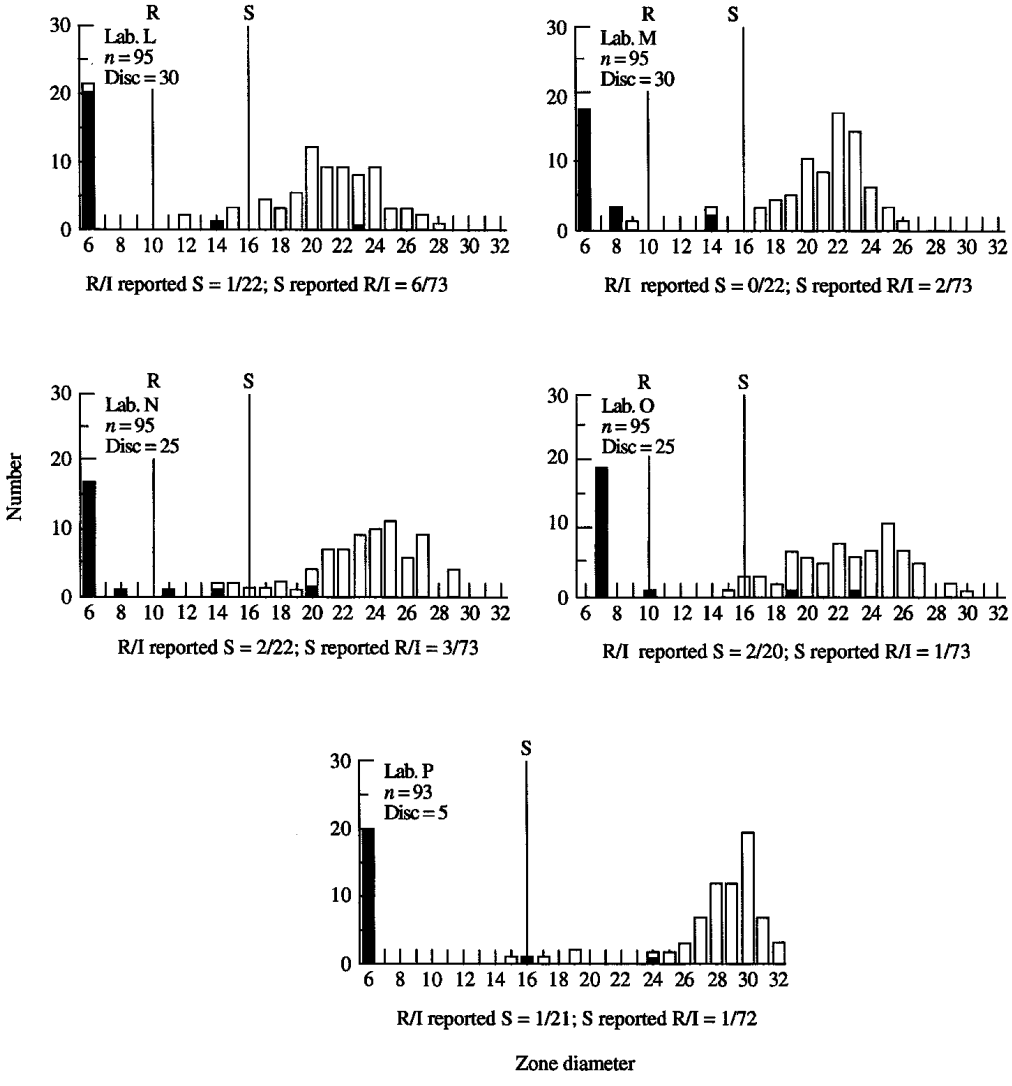


**Figure 8.** *Continued*

laboratory and should not be based solely upon tabulated guidelines since different methods are used in each laboratory.

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