The development of the BSAC standardized method of disc diffusion testing

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The BSAC Working Party on Susceptibility Testing has developed a standardized method of disc susceptibility testing which has been `field tested' in 19 diagnostic laboratories in the UK and Ireland. The method employs semi-defined media, a semi-confluent inoculum and relates zones of inhibition with BSAC-specified MIC breakpoints to interpret susceptibility. The bacteria selected for the trial included clinical isolates and control strains from the ATCC and NCTC national collections. Organisms were chosen because they had known attributes, such as being fully susceptible or having a demonstrated mechanism of resistance. The results from this survey are very encouraging. With the commonly isolated Enterobacteriaceae, specifically *Escherichia coli*, *Proteus mirabilis* and *Klebsiella* spp., no major problems were observed except with gentamicin and cefuroxime. In the case of gentamicin, problems were associated with resistant strains of *P. mirabilis* with MICs of 2 mg/L, being falsely reported susceptible. For cefuroxime, it is not unexpected that results were unreliable, as the MIC distribution straddles the *in vitro* breakpoint concentration (following the results of this study the MIC and zone diameter breakpoints have been amended to improve reporting). No major problems were encountered for *Pseudomonas aeruginosa* with the agents studied. The `field survey' has shown that disc testing is unreliable for determining the susceptibility of coagulase-negative staphylococci to teicoplanin and that the detection of glycopeptide resistance in enterococci is improved by incubation for a full 24 h. Inconsistencies observed with fastidious organisms were associated with incorrect inocula. Zone diameter data for the control strains studied provide information that can be utilised by diagnostic laboratories to monitor the daily performance of testing.

Introduction

In-vitro susceptibility testing is one of the most important functions of a diagnostic laboratory. The importance of getting the answer `right' is obvious because the results have clinical significance in the treatment of individual patients. In a broader context, combined data from these tests are used to monitor levels of resistance to antibiotics, which in turn influence prescribing policies and the future development of new agents.

The task of the Working Party was to develop a method of testing, appropriate for use in the UK and Ireland, that would fulfill the following requirements: (i) provide a robust method of testing where interpretation of susceptibility was equated with the BSAC MIC breakpoints; (ii) be a viable alternative to methods already in place; (iii) provide standardized methodology and defined interpretative criteria for clinically relevant bacteria; (iv) provide advice notes to aid interpretation; (v) give results in concordance with expected results for the existing National External Quality Assurance Programmes (NEQAS); and (vi) provide a system for diagnostic laboratories to monitor the performance of diagnostic susceptibility testing as part of a quality control programme.

The adoption of any new method into a diagnostic laboratory can be traumatic and, given the current workload pressures, can be impossible if changes are too extensive. With this in mind, when the Working Party began developing a standardized method in 1997, it took into consideration current methods of susceptibility testing[1] so that its adoption by clinical laboratories would be as painless as possible. Sources of information that were utilized by the Working Party in the development of the standardized method included published literature[2-8] and unpublished data provided by members of the Working Party.

In this paper, each stage of the development of the method is discussed (except for the detection of methicillin/oxacillin resistance in staphylococci)[9] rather than the detail of the method, which appears elsewhere in this Supplement.[10]

Methodology

Medium

IsoSensitest agar (ISA; Oxoid, Basingstoke, UK) was chosen as the medium for the standardized disc method for several reasons. First, it was the medium `preferred by the Working Party' and used to acquire MIC distribution data when BSAC MIC breakpoint concentrations were formulated.[11,12] Secondly, in 1997, when the method was developed, 50% of diagnostic laboratories in the UK and Ireland used ISA.[1] Thirdly, ISA has the added advantage of being a semi-defined medium, (although every batch should be demonstrated to be suitable)[13] that does not require the addition of lysed blood to neutralise thymidine, which affects the activity of folate inhibitors and is present in other culture media such as Diagnostic Susceptibility Test agar. Finally, the concentrations of calcium and magnesium in ISA are not detrimental to the activity of aminoglycosides and quinolones when testing pseudomonads.

In order to simplify media preparation, it was the intention of the Working Party to have one basal medium to which as few supplements as possible were needed to support the growth of both fastidious and less nutritionally demanding organisms.

ß-Nicotinamide adenine dinucleotide (NAD; Sigma Diagnostics, Poole, UK or Merck BDH, Poole, UK) was chosen as a suitable supplement for ISA because it supports the growth of haemophili and has been shown not to be inhibitory to any antibiotics.[14] Results when testing organisms not requiring NAD for growth (moraxellae, neisseriae and streptococci) are not affected by its presence. This means that, if necessary, only one medium need be prepared by a diagnostic laboratory for testing all fastidious organisms. Although only 5 mg/L NAD is required for haemophili, a concentration of 20 mg/L was chosen so that plates could be stored at 4-8^{0}C for extended periods before use (7-10 days depending on the storage facilities of individual laboratories).

Whole defibrinated horse blood, which has a specified shelf-life, was chosen rather than lysed blood because the performance of media containing lysed blood can vary depending on how the lysed blood is prepared (freezing and thawing, treatment with saponin, ultrasonication) and it is unnecessary to add lysed blood to ISA for tests on folate inhibitors. The Working Party considered that using defibrinated horse blood rather than lysed blood would reduce inter-laboratory variation in performance of media. However, it must be stressed that defibrinated blood may vary between suppliers and therefore the nutritional properties of every batch should be checked as part of a laboratory's quality control programme.

For the detection of methicillin/oxacillin resistance in staphylococci, Mueller-Hinton Agar (with the addition of 5% sodium chloride when testing *Staphylococcus aureus*) was originally chosen. Since the 'Field Survey',

however, this method has been superseded,[9] and a summary of methods for testing methicillin-resistant staphylococci is shown elsewhere in this Supplement (Andrews *et al*.[10]).

Inoculum

The inoculum density is one of the most important factors in the disc diffusion test. In studies undertaken by the Swedish Reference Group for Antibiotics, intra- and inter-laboratory reproducibility studies were affected most by an inoculum which was considered `too heavy', whereas a lighter inoculum gave less variation.[15]

The inoculum preferred in the UK and Ireland has historically been one that yields a lawn of semi-confluent growth. The data from a questionnaire in 1997, revealed that this was still true for >90% of diagnostic laboratories.[1] With this approach it can be seen if the inoculum is correct, whereas incorrect inoculum cannot be seen so reliably when confluent inocula are sought.

Although most laboratories are familiar with the preparation of an inoculum equivalent to semi-confluent growth, it was considered important to develop a reference method that contained a detailed procedure for obtaining the correct inoculum. Though not `fool-proof', comparison of the suspension density with a 0.5 McFarland standard has proved to be reliable in other methods.[15]

For all of the bacteria for which the standardized method was developed, the desired inoculum was therefore prepared by making suspensions of organism equivalent to a McFarland 0.5 standard in broth or distilled water. These suspensions were diluted further, where necessary, before inoculation on to the surface of the agar.[10] An illustration showing an acceptable range of inocula for a Gram-negative bacilli is shown in Figure 1. For the detection of methicillin/oxacillin resistance in staphylococci, suspensions of organisms equivalent to a 0.5 McFarland standard were prepared and inoculated on to the surface of the agar without further dilution.

Disc selection and disc contents

Practical constraints prevented recommendations being given for every possible organism/antimicrobial combination. Antibiotics were therefore chosen for inclusion in the development of the method on the basis of availability and frequency of testing in the UK and Ireland, reference to the BSAC guidelines[11] and inherent problem with testing, e.g. cefuroxime, with which the MIC breakpoint sits in the middle of the normal MIC distribution.

Disc contents were chosen on the basis of current practice and, where possible, to allow six agents to be tested on a 90 mm petri dish without undue overlapping of zones. However, it was recognised by the Working Party that for some groups of antimicrobials, particularly third generation cephalosporins, the disc contents currently available are too high, producing unacceptably large zones of inhibition when a semi-confluent inoculum is used.

Incubation

There is much debate about the atmospheric conditions necessary for adequate growth of newly isolated clinical strains of *Haemophilus influenzae* and *Streptococcus pneumoniae,* particularly as the presence of CO_2 reduces the activity of macrolide and azalide antibiotics, which are used to treat respiratory infections. There are two diametrically opposed opinions, one stating that for optimum growth to be achieved an atmosphere enriched with 4-6% CO_2 is needed, the other considering the addition of CO_2 unnecessary. The Working Party has developed the standardized method with incubation in CO_2 for the following reasons. First, BSAC MIC breakpoint concentrations were calculated using MIC data for *S. pneumoniae* and *H. influenzae* where test plates were incubated in 4-6% CO_2. Secondly, members of the Working Party have observed that 20% of *H. influenzae* and 40% of *S. pneumoniae* isolates from clinical samples require an atmosphere enriched with CO_2 to give semi-confluent growth (unpublished data). Thirdly, a recent publication has proposed that *in vivo* the acid within the respiratory tree may reduce the efficacy of the macrolide/azalide antibiotics.[16] Fourthly, the SRGA recommend incubation in 5% CO_2 when using supplemented ISA for testing *S. pneumoniae* and *H. influenzae.*[15]

The detection of glycopeptide resistance in enterococci has proved unreliable by disc testing methods.[17] However, detection is improved if the plates are incubated for at least 24 h before examination, which allows time for the resistant micro-colonies to be visibly detectable.

Incubation conditions for all of the genera studied are given elsewhere in this Supplement (Andrews *et al*.[10]).

MIC determinations

MICs were determined following BSAC recommendations[11] with the media and incubation conditions detailed above and an inoculum of 10^4 cfu/spot.

Antibiotic powders of known potency were obtained from the following sources: gentamicin, erythromycin, cefotaxime, teicoplanin, rifampicin (Aventis Pharma, Uxbridge, UK); penicillin, methicillin, ampicillin (GlaxoSmithKline, Worthing, UK); vancomycin (Lilly, Basingstoke, UK); chloramphenicol (Sigma); ciprofloxacin (Bayer, Wuppertal, Germany); cefuroxime, ceftazidime, trimethoprim (GlaxoSmithKline, Stevenage, UK); imipenem (Merck, Sharpe & Dohme, Hoddesdon, UK); amikacin (Bristol Myers Squibb, NJ, USA) and piperacillin, tazobactam, tetracycline (Wyeth Ayerst, Pearl River, NY, USA).

The MIC was defined as the lowest concentration of drug at which there was no visible growth of organism after incubation for 18-20h. The susceptibility of each strain was categorized according to BSAC MIC breakpoints concentrations.[11]

Scattergram analysis

In order to obtain a relationship between MICs and zones of inhibition, scattergram plots were generated for each of the antimicrobial/organism combinations studied (see, for example, Figure 2). Zone diameter breakpoints to separate the susceptible, and in some instances intermediate and resistant, populations were determined from these.

Field testing study

Following development of the tentative standardized method of disc diffusion testing, a field study was undertaken to test the performance of the method and to obtain data for control strains that could be used to establish acceptable limits of zone diameters. These limits could be used to monitor the performance of the test by diagnostic laboratories and the device manufacturers.

The method was evaluated initially by five members of the Working Party and subsequently by 14 diagnostic laboratories in the UK and Ireland.

Bacterial strains

Where possible, organisms from the `wild sensitive' population, those with marginal susceptibility and those clearly resistant to each of the drugs studied were selected. The following strains from national collections were included as appropriate, *Escherichia coli* NCTC 10418 and ATCC 25922, *Pseudomonas aeruginosa* NCTC 10662 and ATCC 27853, *S. aureus* NCTC 6571 and ATCC 25923, *S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and *H. influenzae* NCTC 11931 and ATCC 49247.

Two hundred and fifty organisms were studied, comprising 140 Enterobacteriaceae and *Acinetobacter* spp., 20 *Pseudomonas* spp., 20 enterococci, 10 haemophili, 10 haemolytic streptococci, 10 pneumococci, 30 staphylococci and the 10 control strains. The organisms were chosen because they had known attributes, such as being fully susceptible or having a demonstrated mechanism of resistance. The selection was, however, biased towards strains known to be difficult to test. For example, of the 15 strains of coagulase-negative staphylococci, seven were *Staphylococcus haemolyticus*, and of the 10 strains of *H. influenzae,* five had non-β-lactamase-mediated resistance to ampicillin. Consequently, it was anticipated that discrepancy rates would be high and not indicative of routine performance with typical clinical isolates. Rather, they would provide an effective challenge of the zone diameter breakpoints.

Selection of antimicrobial agents

The antimicrobial agents tested were as in Table I (discs were supplied by Oxoid).

Selection of media

All of the media used in this study were provided to each of the centres from single batches. For detecting methicillin/oxacillin resistance in staphylococci, Mueller-Hinton Agar from two sources (Oxoid, and Difco Laboratories, Oxford, UK) were tested, because published data had shown batch variation with Oxoid Mueller-Hinton agar.[18]

Zone measurement and interpretation

Participating laboratories were asked to measure zones of inhibition (mm) with a ruler, callipers or an automated zone measuring device.

Analysis of data

Zone diameter data from each of the centres was analysed as rates of false-resistant and false-susceptible interpretation. Inter- and intra-laboratory variation in zones of inhibition for the control strains were determined from the data provided by the five members of the Working Party, who tested strains on six separate occasions. Differences were tested by one-way analysis of variance, calculated with Graphpad Instat (San Diego, CA, USA). Cumulative distributions of the zones of inhibition were obtained by combining the data from all 19 centres. From these distributions, the 16^{th} and 84^{th} percentiles were read off empirically, to obtain a 'middle-range' that would contain 68% of the observations. A similar process was used, with 2.5 and 97.5 percentile points, to obtain a range encompassing 95% of the observations. If any distribution were normal, the above ranges corresponded to the mean $\pm$1.96 S.D., respectively. The percentile ranges obtained by this method are, however, still valid even if the data are statistically 'non-normal'.

Results

Some data were excluded from the analysis because of results wildly discrepant from the mean zone diameter of the other centres, either because measurement of zone diameters was incorrectly performed (one centre used digital callipers which were not calibrated in mm on some occasions), or because the wrong strain was tested.

False-resistant and false-susceptible interpretation

A summary of rates of false interpretation for the combined data from the 19 centres is shown in Table I.

Enterobacteriaceae and *Acinetobacter* spp.

For ampicillin, the rates of false-susceptible and -resistant interpretation were acceptable (1.1 and 6.5% respectively);[19] however, false interpretation was most frequent for those organisms with MICs close to the breakpoint concentration (8 mg/L). With individual bacterial genera, high false-susceptible rates were observed with *Citrobacter* and *Serratia* spp. (4.3 and 6.7%, respectively) and high false-resistant rates (16.0 and 31.6%, respectively) were observed for *Acinetobacter* and for an unusual ampicillin-susceptible *Enterobacter* sp.

It was anticipated when the study was designed that there would be difficulties with cefuroxime because the MIC distribution for Enterobacteriaceae and *Acinetobacter* spp. straddles the MIC breakpoint concentrations. For those strains with MICs $\leq$1 mg/L or $\geq$32 mg/L there were no major problems with reporting. However, for those organisms with MICs between 2 and 16 mg/L there was a high rate of false susceptible reporting (25.6%). Unfortunately, the species with MICs of 2-4 mg/L, i.e. *E. coli*, *Klebsiella* spp., *Proteus Mirabilis* and *Citrobacter* spp., are those most commonly encountered in diagnostic laboratories.

The false-susceptible and -resistant interpretation rates for ceftazidime were 1.6 and 8.8%, respectively. The instances of false-resistance were seen mainly for strains of *E. coli* and *Klebsiella* spp., with MICs of ceftazidime of 1 and 2 mg/L, respectively. With *E. coli* such MIC values are much higher than the `wild susceptible' population (MICs c. 0.12 mg/L). Some of these strains of *E. coli* and *Klebsiella* spp. had extended-spectrum β-lactamases and it was therefore appropriate that they were identified as having a possible mechanism of resistance to ceftazidime. Among the other bacterial species investigated, high rates of false interpretation to ceftazidine were observed with *Citrobacter* spp. (False- susceptible 5.2%, false-resistant 0.5%), *Serratia* spp. (false-susceptible 4.8%, false- resistant 6.7%) and *Acinetobacter* spp. (false-susceptible 1.0%, false-resistant 30.7%).

For ciprofloxacin, the overall false-susceptible and -resistant interpretation rates were acceptable at 0 and 5.94% respectively. Of the common bacterial pathogens, false- resistant interpretation was observed with strains of *E. coli* and *Klebsiella* spp. with ciprofloxacin MICs of 0.25 and 0.5 mg/L, respectively. These MICs are higher than those seen for the `wild susceptible' populations of these genera (MICs c. 0.015-0.03 mg/L) and probably represent strains with a mechanism of resistance to quinolones. High false- resistant reporting was also observed for *Serratia* and *Acinetobacter* spp., which are naturally less susceptible to ciprofloxacin than the other genera and have MICs closer to the breakpoint concentration.

Overall, the false-susceptible and -resistant rates of interpretation were acceptable for gentamicin (1.9 and 0.2% respectively). Moreover, false-susceptible reporting was confined to a few strains of *P. mirabilis*, *Citrobacter* spp. and *Serratia* spp., with MICs of gentamicin of 2-8 mg/L.

Pseudomonas species.

Unlike the Enterobacteriaceae and *Acinetobacter* spp., an intermediate category of susceptibility was defined for pseudomonads for some antimicrobials (gentamicin, amikacin, ciprofloxacin). In these instances, analysis

of false interpretation was calculated for the individual groups of susceptibility, i.e. susceptible, intermediate and resistant.

For gentamicin, separation of the resistant strains from the intermediate/susceptible population was very clear. However, strains with intermediate susceptibility to gentamicin (MICs 2-4 mg/L), were not clearly differentiated from susceptible strains (MICs ≤1 mg/L). For organisms with intermediate susceptibility to gentamicin the rate of false-susceptible interpretation was 14.1% and for the gentamicin-susceptible strains the rate of false- intermediate interpretation was 5.2%. All strains resistant to gentamicin were correctly interpreted.

No problems with interpretation were observed for amikacin-susceptible strains (MICs ≤4 mg/L). However, false interpretation was observed for two strains with MICs close to the MIC breakpoint of 16 mg/L (one strain with an MIC of 8 mg/L was interpreted as susceptible to amikacin by only 50% of the centres, the other with an MIC of 32 mg/L was mainly interpreted as having intermediate susceptibility).

For this study, only a 1 μg ciprofloxacin disc strength was studied, which did not allow differentiation of the organisms with intermediate susceptibility to ciprofloxacin (MICs 2-4 mg/L) from those with full resistance (MICs ≥8 mg/L).

For ceftazidime, no problems with interpretation were seen for *P. aeruginosa* with MICs of ≤4 or ≥2 mg/L. However, for isolates with intermediate MICs (8 or 16 mg/L), false-resistant and -susceptible interpretation of 3.2 and 4.1% respectively, was observed.

For Imipenem false rates of interpretation were acceptable (false-susceptible 1.4% and false-resistant 0.5%).

The addition of the β-lactamase inhibitor tazobactam to piperacillin reduced the reliability of the disc diffusion method, with an increase in false-susceptible interpretation from 0.3% when piperacillin was tested alone to 4.6% when combined with tazobactam (observed with two strains of *Pseudomonas* spp. with piperacillin MICs of 32 mg/L).

H. influenzae.

The difficulty in detecting non-β-lactamase-mediated resistance to ampicillin has been reported previously.[20] In this study, six centres failed to detect resistance in a non-β-lactamase-producing strain of *H. influenzae* with an ampicillin MIC of 32 mg/L.

The majority of β-lactamase producing strains of *H. influenzae* are susceptible to cefuroxime, although resistant strains have been reported.[21] No problems with interpretation of cefuroxime results were seen here with the β-lactamase negative strains, but one β-lactamase-positive strain with an ampicillin MIC of 128 mg/L and a cefuroxime MIC of 8 mg/L was falsely interpreted as susceptible to cefuroxime by four centres (false-susceptible rate 3.1%). At one of the centres, false interpretation may be attributed to an incorrect inoculum as the zones of inhibition were much larger than the centres correctly detecting resistance. For the other three centres there was no obvious reason for the failure to detect resistance.

Only cefotaxime-susceptible *H. influenzae* strains were used in this study and no false interpretation was observed. For tetracycline and chloramphenicol there was a clear differentiation between the resistant and susceptible populations by disc testing.

β-haemolytic streptococci

One of the major problems associated with testing β-haemolytic streptococci is that of obtaining a semi-confluent inoculum. It was clear from the field study that, in spite of having a detailed method for producing the correct inoculum, one centre obtained inocula that were `too heavy' and one 'too light' (zones of inhibition much smaller and larger respectively than those obtained by the other centres). Although the heavy inocula did not affect the interpretation of susceptibility, the light inoculum resulted in an erythromycin- resistant strain being interpreted as susceptible. False interpretation rates erythromycin and tetracycline were acceptable (Table I). All the β-haemolytic streptococci tested were susceptible to penicillin.

S. pneumoniae

The categorization of the penicillin susceptibility of the strains studied was based on penicillin MICs interpreted according to the penicillin MIC breakpoint concentration. However, disc testing was by screening with oxacillin discs. Although the method readily separated the susceptible and resistant populations, with no false interpretation observed, it did not differentiate between low- and high-level resistance to penicillin as noted previously.[22] No oxacillin- resistant, penicillin-susceptible strains were included in the study.

For erythromycin, tetracycline and chloramphenicol, acceptable rates of false interpretation were observed (Table I). With ciprofloxacin the Working Party has revised its recommendations for interpretation of susceptibility so that strains of pneumococci with MICs ≤2 mg/L are classed as having intermediate susceptibility and those with MICs ≥ 4 mg/L are classed as resistant. There is no susceptible category (based on clinical observations). False interpretation rates were acceptable (false-intermediate 0%, false- resistant 3.2%).

Enterococci

Detection of glycopeptide resistance in enterococci has proved difficult for diagnostic laboratories.[17] For vancomycin, false rates of interpretation were acceptable (false- susceptible 1.44%, false-resistant 4.02%). False-resistant interpretation was observed for a strain of *E. faecalis* with a vancomycin MIC of 4 mg/L and false-susceptible interpretation for a strain of *E. faecium* with a vancomycin MIC of 8 mg/L. VanA or vanB resistance phenotypes in other strains studied was detected by all of the centres. In the case of teicoplanin, determination of susceptibility was less reliable than with vancomycin, with many strains with MICs of teicoplanin between 1 and 4 mg/L being interpreted as resistant (false-susceptible 1.4%, false-resistant 10.9%).

No false interpretation was observed with ampicillin and with gentamicin, and high- level resistance was detected using 200 μg discs by all centres.

S. aureus

The detection of penicillin resistance depends not only on the measurement of a zone of inhibition, but also examination of the zone for the presence of a `heaped edge', and participants were therefore instructed to report as resistant any strain exhibiting this type of zone formation. Following these instructions, few problems were encountered (false- susceptible 0%, false-resistant 1.3%).

For the detection of methicillin/oxacillin resistance, two sources of Mueller-Hinton agar were tested (Difco and Oxoid). From these limited data on single batches of media, no difference in the rates of false interpretation were observed (Difco: false-susceptible 0%, false-resistant 0.8%; Oxoid: false-susceptible 0% and false-resistant 0.8%).

No false interpretation was observed with vancomycin; however, no strains with resistance to vancomycin were included. In the case of teicoplanin, one strain with an MIC of 4 mg/L was interpreted incorrectly by one centre as resistant to teicoplanin, but the overall false interpretation rates were acceptable (false-susceptible 0%, false-resistant 0.5%).

No false interpretation was observed for erythromycin, rifampicin and gentamicin.

Coagulase-negative staphylococci

As with *S. aureus,* few problems were encountered when testing penicillin (false-susceptible rate 0%, false-resistant rate 0.4%).

The results of methicillin testing on Difco Mueller-Hinton agar were acceptable, with rates of false-resistant and -susceptible interpretation being 3.7 and 0% respectively. Of interest were the results for one strain apparently falsely reported methicillin resistant by eight centres. When MICs were repeated using a commercial method (Etest; Cambridge Diagnostic Services, Cambridge, UK) on Mueller-Hinton agar, with and without 2% NaCl, the MICs of methicillin were 12 and 3 mg/L, respectively. These data highlight the difficulties when testing the methicillin susceptibility of staphylococci, whose tolerance of salt is variable. With Oxoid Mueller-Hinton agar, 1.9% false-resistant and 3.9% false- susceptible interpretation was seen (mainly with a strain with a methicillin MIC of 16 mg/L, interpreted correctly on Difco media).

As with the *S. aureus,* no false interpretation was observed with vancomycin. However, high rates of false susceptible interpretation were seen with teicoplanin (4.0%). False interpretation was not confined to strains with MICs close to the MIC breakpoint, but was randomly distributed over the range of MIC values.

As with *S. aureus*, no false interpretation was observed with erythromycin, rifampicin and gentamicin.

Determination of acceptable zone ranges for control ATCC and NCTC strains

A summary of the ranges for 68 and 95% of observations are shown in Table II. These were similar for both *E. coli* controls, NCTC 10418 and ATCC 25922, although the ranges were generally 1-2 mm larger for strain NCTC 10418. With the two control strains of *P. aeruginosa* (NCTC 10662 and ATCC 27853), ranges were similar, but again generally narrower for the ATCC control.

H. influenzae ATCC 49247 and NCTC 11931 have different susceptibilities to ampicillin and cefuroxime. ATCC 49247 has non-β-lactamase-mediated resistance to β-lactam antibiotics and is used to ensure that the method can detect such resistance, NCTC 11931 is a fully sensitive strain. Only one *S. pneumoniae* control was studied, ATCC 49619, which has reduced susceptibility to penicillin and gives small zones of inhibition around oxacillin discs.

With the *S. aureus* controls NCTC 6571 and ATCC 25923, slightly narrower ranges were obtained with ATCC 25923 for all of the antimicrobials studied except gentamicin.

Intra-laboratory variation

Individual laboratory performance (coefficients of variation, CVs) was calculated from the data provided by the five centres that tested the control strains with all of the antibiotics on five separate occasions. Data were then pooled for individual control strains and antibiotics to obtain a range and median CV (Table III). Irrespective of organism or antimicrobial, median CVs did not exceed 6.6% (range 0.9- 6.6%).

Inter-laboratory variation

Although, the combined ranges of zone diameters observed by the five centres were relatively narrow, when the individual data for each of the centres were challenged, statistically significant differences were seen (Table IV). Significant differences were generally restricted to one or two centres for each of the control strains. The largest number of differences (three centres) was seen with the NCTC 10662 *P. aeruginosa* control strain.

Discussion

With a few exceptions, the results from this survey are very encouraging and have demonstrated that the method of testing developed is suitable for use in diagnostic laboratories. In the case of *H. influenzae* and β-haemolytic streptococci, these data illustrate that individuals may have problems preparing the correct inoculum. It is hoped that the detailed methodology and the photographs illustrating acceptable limits for semi-confluent growth[10] will assist laboratories to prepare the correct inoculum for fastidious organisms and 'difficult' genera such as *Acinetobacter* and *Serratia* spp. Automated zone readers and callipers can assist the rapid measurement of zone diameter. However, as demonstrated by one of the centres taking part in the survey, if callipers are not calibrated correctly, erroneous results will be obtained.

With the commonly isolated Enterobacteriaceae*, E. coli, P. mirabilis* and *Klebsiella* spp., no major problems were observed, except with cefuroxime and gentamicin. It was anticipated when the study was designed that there would be problems with cefuroxime as the MIC distribution straddles the *in vitro* breakpoint concentration. This breakpoint concentration could be raised, but only if the necessary clinical response data were available. The unreliability of testing cefuroxime by disc diffusion raises the question of whether cefuroxime testing by this method should be recommended, although the extensive clinical use of cefuroxime creates a demand for testing. The problems seen with gentamicin were associated with strains of *P. mirabilis*, *Citrobacter* spp. and *Serratia* spp., with MICs between 2 and 8 mg/L being falsely categorized as susceptible. All of these strains had a mechanism of resistance to gentamicin, either comprising reduced permeability or aminoglycoside-modifying enzymes (Miss A King, personal communication), but the resistance was not detected by the disc method. The disc method of testing ceftazidime and ciprofloxacin detected those strains with possible extended- spectrum β-lactamases and possible quinolone resistance, respectively, whereas these appeared susceptible on the basis of MIC tests and thus warrant further investigation. Most diagnostic laboratories do not fully identify Gram-negative aerobic bacilli. This may present problems in reporting, if strains of *Acinetobacter* of *Serratia* spp. are isolated, as obtaining semi-confluent growth may prove difficult.

For *P. aeruginosa* no major problems were seen for the agents studied; however, it would seem necessary to use a 5 µg ciprofloxacin disc to differentiate the three populations of susceptible, low-level resistant and high-level resistant strains.

It was clear from the comments made in the replies to a questionnaire,[1] that many diagnostic laboratories experience problems testing *H. influenzae*. The major problem is that of consistently obtaining an inoculum yielding semi-confluent growth. It would appear from these data that the majority of centres taking part in the survey obtained the correct inoculum. However, five out of the 19 centres taking part had difficulty with the method and were unable to detect non-β-lactamase-mediated resistance to ampicillin and resistance to cefuroxime in strains of β-lactamase-producing *H. influenzae*. One of these strains had only one colonial appearance on a purity plate, yet disc testing differentiated two populations, of which one appeared resistant to

ampicillin whereas the other was more susceptible. The failure to detect resistance could therefore be attributed to incorrect inoculum or failure to examine the susceptibility plates closely, with smaller resistant colonies not being detected. The need to include strains with these mechanisms of resistance in the BSAC control strain collection is recognised. No problems with reporting were observed when testing cefotaxime, tetracycline and chloramphenicol against *H. influenzae.*

Among Gram-positive cocci, no problems were seen with the pneumococci. For the β-haemolytic streptococci, results were favourable, except for two centres that experienced problems obtaining semi-confluent growth, one with inocula that were too light and the other with inocular that were too heavy. For the enterococci the only problems encountered were with teicoplanin (raised false-susceptible and -resistant rates). No problems with reporting *S. aureus* were observed. However, for the coagulase-negative staphylococci, results with methicillin were more reliable on Difco Mueller-Hinton agar than on Oxoid media. These limited data suggest that there are differences between Difco and Oxoid Mueller-Hinton agars, but further studies are required with more strains and different batches of media. Unlike *S. aureus*, coagulase-negative staphylococci gave unreliable results with teicoplanin. The unreliability of these results suggests that teicoplanin susceptibility should not be determined by a disc diffusion method these organisms. It must also be stressed that the disc method developed for determining susceptibility of *S. aureus* to vancomycin will not detect the glycopeptide-intermediate strains of the kind isolated in Japan and North America. Further work on this problem is currently being undertaken.

With regard to limits of acceptability for zones of inhibition of control strains, very little difference was observed between the ATCC and NCTC strains. However, it is suggested that *H. influenzae* NCTC 11931 is a better control of routine testing than ATCC 49247, which is resistant to some of the β-lactam antibiotics. Despite the fact that the five centres taking part in the study of intra- and inter-laboratory variation have a special interest in susceptibility testing, the results show intra- and inter-laboratory variation. Intra-laboratory variation was generally within acceptable limits and, although many of the inter-laboratory differences were statistically significant, the actual zone diameters were mostly within acceptable ranges.

A standardized method of disc testing appropriate for use in the UK and Ireland has been long awaited. Although innovative when introduced, the Stokes' comparative method has not been developed and amended in a regulated fashion, and *ad hoc* changes made by laboratories have generally not been substantiated by experimental study. The introduction of new potent antibiotics has also presented problems with respect to interpretation. The results of this survey have shown that the BSAC method is suitable for use by diagnostic laboratories. Continued support by the BSAC will ensure that the method is developed and amended in response to new mechanisms of resistance and the introduction of new antimicrobials.

Acknowledgements

The Working Party would like to thank Dr T Marshall for assistance with statistical analysis and Mr N Lines for assistance with data presentation.

Centres taking part in `field testing' survey
Derriford Hospital, Plymouth: Hammersmith Hospital, London: St. Mary's Hospital, Isle of Wight: North Devon District Hospital, Barnstaple: New Cross Hospital, Wolverhampton: Trafford General Hospital, Manchester: Lewisham Hospital, London: City Hospital Belfast: University of Edinburgh Medical School: Northern General Hospital, Sheffield: St. Vincent's Hospital, Dublin: St. James' Hospital, Dublin: Public Health Laboratory, Exeter: Stobhill NHS Trust, Glasgow: Public Health Laboratory, Addenbrookes' Hospital, Cambridge: St. Thomas' Hospital, London: Southmead Hospital, Bristol: G.R. Micro Ltd., London: City Hospital, Birmingham.

References

1. Andrews, J.M., Brown, D.F.J., Wise, R. (1996). A survey of antimicrobial susceptibility testing in the United Kingdom. *Journal of Antimicrobial Chemotherapy* **37,** 187-204.
2. Andrews, J.M., Jin, Y-F., Wise, R. (1990). Disc sensitivity testing to ofloxacin. *Journal of Antimicrobial Chemotherapy* **26,** 453-6.
3. Andrews, J.M., Wise, R. (1993). Disk susceptibility testing for clarithromycin. *European Journal of Clinical Microbiology and Infectious Diseases* **12**, 972-4.
4. Andrews, J.M., Wise, R. (1993). Disk susceptibility testing for rufloxacin. *European Journal of Clinical Microbiology and Infectious Diseases* **12,** 974-6.
5. Andrews, J.M., Wise, R. (1991). Susceptibility testing of urinary pathogens to norfloxacin. *Journal of Antimicrobial Chemotherapy* **28**, 309-11.
6. Andrews, J.M., Wise, R. (1991). Susceptibility testing of cefpodoxime. *Journal of Antimicrobial Chemotherapy* **28**, 605-7
7. Andrews, J.M., Wise, R. (1992). Susceptibility testing of cefpirome. *Journal of Antimicrobial Chemotherapy* **29,** 81-4.
8. Andrews, J.M., Wise, R. (1989). Susceptibility testing of ciprofloxacin. *Journal of Antimicrobial Chemotherapy* **23**, 156-8.
9. Brown, D.F.J. (2001). Detection of methicillin/oxacillin resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* **48,** *Suppl. 1. 65-70.*
10. Andrews, J. M. for the BSAC working jparty on Susceptibility Testing. (2001). BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy* **48**, *Suppl. 1,* 43-57.
11. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. (1991). A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy* **27**, Suppl. D., 1-50.
12. Supplementary report by the Working Party on antibiotic sensitivity testing of the British Society for Antimicrobial Chemotherapy (1996). *Journal of Antimicrobial Chemotherapy* **38**, 1103-5.
13. Andrews, J.M., Ashby, J.P., Wise, R. (1990). Problems with Iso-Sensitest Agar. *Journal of Antimicrobial Chemotherapy* **26**, 596-7.
14. Andrews, J.M., Hadley, N., Brenwald, N.P., Wise, R. (1997). Susceptibility testing of fastidious organisms. *Journal of Antimicrobial Chemotherapy* **39**, 436.
15. Kahlmeter, G., Olsson-Liljequist, B. & Ringertz, S. (1997). Antimicrobial sensitivity testing in Sweden. IV. Quality assurance. *Scandinavian Journal of Infectious Diseases* Supplement **105,** 24-31.
16. Johnson, M.M., Hill, S.L., Piddock, L.J.V. (1999). Effect of carbon dioxide on testing of susceptibilities of respiratory tract pathogens to macrolide and azalide antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **43**, 1862-5.
17. Snell, J.J.S., Brown, D.F.J., Perry, S.F. & George, R. (1993). Antimicrobial susceptibility testing of enterococci: results of a survey conducted by the United Kingdom National External Quality Assessment Scheme for Microbiology. *Journal of Antimicrobial Chemotherapy* **32**, 401-12.
18. Hindler, J.A. & Inderlied, C.B. (1985). Effect of source of Mueller-Hinton agar and resistance frequency on the detection of methicillin-*resistant Staphylococcus aureus*. *Journal of Clinical Microbiology* **21**, 205-10.
19. Metzler, C.M. & DeHaan, R.M. (1974). Susceptibility tests of anaerobic bacteria: statistical and clinical considerations. *Journal of Infectious Diseases* **130,** 588-94.
20. Snell, J.J.S., Brown, D.F.J., Phua, T.J. (1986). Antimicrobial susceptibility testing *of Haemophilus influenzae*: trial organised as part of the United Kingdom National External Quality Assessment Scheme for microbiology. *Journal of Clinical Pathology* **39**, 1006-1012.
21. Brown, N.M., Bedford, K.A., Holt, H.A., McCulloch, S.Y., MacGowan, A.P., Reeves, D.S. (1992). Cefuroxime resistance in *Haemophilus influenzae*. *Lancet* **340**, 552.
22. Baquero, F. (1995). Pneumococcal resistance to β-lactam antibiotics: a global geographic overview. *Microbial Drug Resistance* **1**, 115-20.

Table I. Summary of rates (%) of false-susceptible and false-resistant interpretation for the 19 centres participating in the BSAC `field testing' study.

Organism	Antibiotic	Breakpoint[a]			Rate of false interpretation (%)		
		MIC (mg/L) ≤	Zone diameter (mm) ≥	Disc content μg (unless stated)	S	I	R
S. aureus	Erythromycin	0.5	20	5	0	-	0
(15 strains; 285 tests)	Rifampicin	0.06	30	2	0	-	0
	Gentamicin	1	20	10	0	-	0
	Vancomycin	4	12	5	0	-	0
	Teicoplanin	4	15	30	0	-	0.4
	Methicillin (Difco media)	4	14	5	0	-	0.8
	Methicillin (Oxoid media)	4	14	5	0	-	0.8
	Penicillin	0.12	25	1 unit	0	-	1.3
Coagulase-negative staphylococci	Erythromycin	0.5	20	5	0.4	-	0
(15 strains; 285 tests)	Rifampicin	0.06	30	2	0	-	0
	Gentamicin	1	20	10	0	-	0
	Vancomycin	4	12	5	0	-	0
	Teicoplanin	4	15	30	4.0	-	0.8
	Methicillin (Difco media)	4	14	5	0	-	3.7
	Methicillin (Oxoid media)	4	14	5	3.9	-	1.9
	Penicillin	0.12	25	1 unit	0	-	0.4
Enterobacteriaceae & *Acinetobacter* spp.	Ampicillin	8	18	10	1.1	-	6.5
(140 strains; 2660 tests)	Cefuroxime	≤ 1	25 = S	30	0	0	0
	"	2-16	18-24 = I	30	25.6	-	5.0
	"	≥ 32	≤17 = R	30	0	1.7	0
	Ciprofloxacin	1	18	1	0	-	5.9
	Ceftazidime	2	28	30	1.6	-	8.8
	Gentamicin	1	20	10	1.9	-	0.2
Pseudomonas spp.	Piperacillin	16	15	75	0.3	-	0
(20 strains; 380 tests)	Piperacillin/tazobactam	16	15	75/10	4.6	-	0
	Imipenem	4	22	10	1.4	-	0.5
	Ceftazidime	8	24	30	4.1	-	3.2
	Gentamicin[b]	≤ 1	22 = S	10	0	5.2	0
		2-4	15-21 = I	10	14.1	-	0

Organism	Antibiotic	Breakpoint[a] MIC (mg/L) ≤	Breakpoint[a] Zone diameter (mm) ≥	Disc content μg (unless stated)	Rate of false interpretation (%) S	I	R
		≥ 8	≤14 = R	10	0	0	-
	Amikacin[b]	≤ 4	22 = S	30	-	0	0
		8-16	18-21 = I	30	2.8	-	0
		≥ 32	≤17 = R	30	1.3	3.0	-
	Ciprofloxacin[b]	≤ 1	20 = S	1	-	0	0
		2-4	10-19 = I	1	0	-	4.2
		≥ 8	≤9 = R	1	0	0	-
H. influenzae	Tetracycline	1	22	10	0	-	0
(10 strains; 190 tests)	Chloramphenicol	2	25	10	0	-	0
	Ampicillin	1	20	2	3.9	-	0
	Cefuroxime	1	20	5	3.1	-	0
	Cefotaxime	1	25	5	0	-	0
S. pneumoniae	Penicillin	0.12	20[b]	Oxacillin 1[c]	0	-	0
(10 strains; 190 tests)	Tetracycline	1	20	10	0.5	-	0
	Erythromycin	0.5	20	5	0	-	0
	Chloramphenicol	8	18	10	0	-	0
	Ciprofloxacin	0.5-2[d]	10	1	-	0	3.2
β haemolytic streptococci	Penicillin	0.12	20	1 unit	0	-	0
(10 strains; 190 tests)	Tetracycline	1	20	10	0	-	0
	Erythromycin	0.5	20	5	1.7	-	1.7
Enterococci	Ampicillin	8	20	10	0	-	0
(20 strains; 380 tests)	Vancomycin	4	13	5	1.3	-	3.6
	Teicoplanin	4	20	30	1.4	-	10.9

[a] MICs or zones less than or equal to the indicated values were taken to denote susceptibility except where otherwise indicated for breakpoints denoting resistance (R) or intermediate status (I).
[b] Susceptible
[c] Oxacillin discs used for testing.
[d] Intermediate and resistant categories only

Table II. Control zone ranges for control ATCC and NCTC strains

Control strain	Antibiotic	95% of observations	68% of observations
E. Coli NCTC 10418	Ampicillin	21-26	22-25
	Cefuroxime	25-32	27-30
	Ceftazidime	32-40	34-39
	Trimethoprim	29-36	30-35
	Gentamicin	21-27	24-26
	Ciprofloxacin	31-40	32-38
E. Coli ATCC 25922	Ampicillin	16-22	17-21
	Cefuroxime	24-29	25-28
	Ceftazidime	31-39	32-37
	Trimethoprim	26-32	27-31
	Gentamicin	21-27	22-26
	Ciprofloxacin	31-37	32-36
P. aeruginosa NCTC 10662	Gentamicin	20-26	21-25
	Amikacin	21-30	23-28
	Ceftazidime	29-37	31-36
	Imipenem	20-27	21-26
	Piperacillin	27-35	28-32
	Piperacillin/Tazobactam	28-35	29-34
	Ciprofloxacin	21-28	23-26
P. aeruginosa ATCC 27853	Gentamicin	22-28	23-26
	Amikacin	26-32	27-30
	Ceftazidime	27-35	29-34
	Imipenem	23-28	24-27
	Piperacillin	27-34	28-33
	Piperacillin/Tazobactam	29-37	30-35
	Ciprofloxacin	24-30	26-29
H. influenzae ATCC 49247	Ampicillin	6-13	6-11
	Cefuroxime	6-16	6-14
	Cefotaxime	27-38	29-35
	Chloramphenicol	30-38	31-36
	Tetracycline	9-14	10-14
H. influenzae NCTC 11931	Ampicillin	22-30	24-28
	Cefuroxime	22-28	23-27
	Cefotaxime	33-45	35-42
	Chloramphenicol	30-40	31-36
	Tetracycline	27-35	28-33
S. pneumoniae ATCC 49619	Ciprofloxacin	14-21	15-18
	Erythromycin	23-36	25-30
	Oxacillin	8-16	10-15
	Chloramphenicol	21-29	22-26
	Tetracycline	26-36	28-34
E. faecalis ATCC 29212	Vancomycin	13-19	14-16
	Teicoplanin	19-25	20-33
	Ampicillin	26-35	28-33
	Gentamicin	22-27	22-27
S. aureus NCTC 6571	Vancomycin	14-20	15-18
	Teicoplanin	17-23	18-21
	Methicillin	18-30	22-28

Control strain	Antibiotic	95% of observations	68% of observations
S. aureus ATCC 25923	Gentamicin	24-30	25-29
	Penicillin	32-40	34-38
	Erythromycin	22-31	24-30
	Rifampicin	27-39	30-37
	Vancomycin	13-17	14-16
	Teicoplanin	16-20	17-19
	Methicillin	18-27	20-24
	Gentamicin	20-26	22-24
	Penicillin	29-36	29-34
	Erythromycin	22-29	23-27
	Rifampicin	29-36	30-34

Table III. Intra-laboratory variation in zone diameters for control strains tested on five separate occasions by five laboratories.

			CV %			
			ATCC control		NCTC control	
Organism	Control Number	Antibiotic	Range	Median	Range	Median
E. coli	ATCC 25922	Ciprofloxacin	1.5 - 4.1	3.0	1.4 - 6.5	2.8
	NCTC 10418	Gentamicin	2.0 - 8.2	2.1	1.6 - 3.2	2.5
		Ceftazidime	1.7 - 4.2	2.9	2.1 - 7.5	3.3
		Cefuroxime	1.5 - 3.9	3.1	1.5 - 3.6	2.7
		Ampicillin	1.9 - 7.0	5.2	2.0 - 4.6	4.1
P. aeruginosa	ATCC 27853	Gentamicin	1.6 - 7.3	3.4	2.1 - 7.0	3.7
	NCTC 10662	Amikacin	1.7 - 3.9	3.1	1.9 - 8.3	5.4
		Ceftazidime	1.2 - 3.3	2.6	1.5 - 3.6	2.7
		Imipenem	1.9 - 5.0	4.0	1.8 - 4.3	3.6
		Piperacillin	1.3 - 6.3	2.5	1.6 - 7.4	1.9
		Piperacillin/tazobactam	1.5 - 6.5	3.1	1.5 - 7.0	3.1
		Ciprofloxacin	1.5 - 8.0	3.5	2.1 - 6.3	3.1
S. aureus	ATCC 25923	Gentamicin	1.7 - 4.6	3.9	3.0 - 7.0	5.1
	NCTC 6571	Rifampicin	1.6 - 3.9	2.5	1.3 - 6.9	5.2
		Erythromycin	2.0 - 7.4	3.3	1.3 - 8.7	5.6
		Teicoplanin	2.1 - 4.6	2.7	0.0 - 7.3	6.2
		Vancomycin	3.0 - 6.9	3.8	2.9 - 8.2	5.4
		Penicillin	3.8 - 6.2	4.0	1.1 - 4.9	3.4
		Methicillin (Oxoid media)	1.5 - 2.4	1.9	3.3 - 10.7	5.1
		Methicillin (Difco media)	2.3 - 5.7	4.7	1.7 - 6.3	5.4
E. faecalis	ATCC 29212	Vancomycin	0.0 - 6.9	3.4	-	-
		Teicoplanin	1.8 - 8.3	2.3	-	-
		Ampicillin	1.5 - 6.1	3.9	-	-
		Gentamicin	0.0 - 2.3	1.8	-	-
S. pneumoniae	ATCC 49619	Oxacillin	0.0 - 9.4	6.6	-	-
		Erythromycin	1.4 - 8.6	4.5	-	-
		Chloramphenicol	1.7 - 8.1	5.9	-	-
		Tetracycline	1.6 - 12.1	4.5	-	-
		Ciprofloxacin	4.8 - 8.3	6.0	-	-
H. influenzae	ATCC 49247	Tetracycline	3.8 - 12.2	6.0	1.6 - 10.2	2.6
	NCTC 11931	Chloramphenicol	2.4 - 5.6	4.1	1.5 - 5.7	3.9
		Cefotaxime	0.6 - 3.7	1.8	1.3 - 6.0	4.0
		Cefuroxime	0.7 - 3.6	0.9	-	-
		Ampicillin	1.6 - 8.3	5.2	-	-

Table IV. Inter-laboratory variation in zones of inhibition for control strains

		ATCC control zone data for five centres		NCTC control zone data for five centres		No. of centres disagreeing out of the five centres	
Strain	Antibiotic	Range mm	*P*	Range mm	*P*	ATCC	NCTC
E. coli	Ciprofloxacin	32-36	0.5	32-38	0.3	-	-
ATCC 25922	Gentamicin	22-27	0.01	23-28	<.0001	1	1
NCTC 10418	Ceftazidime	32-37	0.2	33-40	0.1	-	-
	Cefuroxime	24-29	0.02	26-30	0.0001	1	1
	Ampicillin	16-23	<0.0001	21-27	<0.0001	2	2
P. aeruginosa	Gentamicin	22-29	.003	20-29	<0.0001	2	3
ATCC 27853	Amikacin	27-33	<0.0001	22-33	<0.0001	2	1
NCTC 10662	Ceftazidime	29-32	<0.0001	29-34	<0.0001	2	2
	Imipenem	23-27	.003	21-25	<0.0001	1	2
	Piperacillin	26-34	<0.0001	28-35	0.05	1	2
	Piperacillin/tazobactam	28-35	0.03	28-36	0.4	1	-
	Ciprofloxacin	22-30	0.002	22-29	0.006	2	1
H. influenzae	Tetracycline	10-14	0.0001	28-41	0.05	2	1
ATCC 49247	Chloramphenicol	31-38	0.06	31-39	0.003	-	1
NCTC 11931	Cefotaxime	27-38	0.4	34-42	0.8	-	-
	Cefuroxime	6-16	<0.0001	22-29	0.003	2	1
	Ampicillin	6-11	<0.0001	21-30	<0.0001	2	1
E. faecalis	Vancomycin	13-16	0.002	-	-	1	-
ATCC 29212	Teicoplanin	16-24	0.0008	-	-	1	-
	Ampicillin	26-31	0.005	-	-	1	-
S. pneumoniae	Oxacillin	9-15	<0.0001	-	-	2	-
ATCC 49619	Erythromycin	24-35	0.0003	-	-	1	-
	Chloramphenicol	21-30	<0.0001	-	-	2	-
	Tetracycline	24-34	0.02	-	-	1	-
	Ciprofloxacin	15-21	0.0003	-	-	1	-
S. aureus	Gentamicin	22-26	0.02	24-30	0.9	1	-
ATCC 25925	Rifampicin	31-34	0.01	32-40	0.15	1	-
NCTC 6571	Erythromycin	23-29	0.1	25-32	0.9	-	-
	Teicoplanin	16-19	0.0003	17-23	0.04	2	1
	Vancomycin	13-17	0.0001	15-20	0.2	2	-
	Penicillin	17-22	0.1	24-40	0.007	-	1
	Methicillin (Oxoid media)	18-26	<0.0001	23-34	<0.0001	2	1
	Methicillin (Difco media)	19-26	<0.0001	22-30	<0.0001	2	2

Figure 1. Acceptable range of inoculum for a Gram-negative rod.

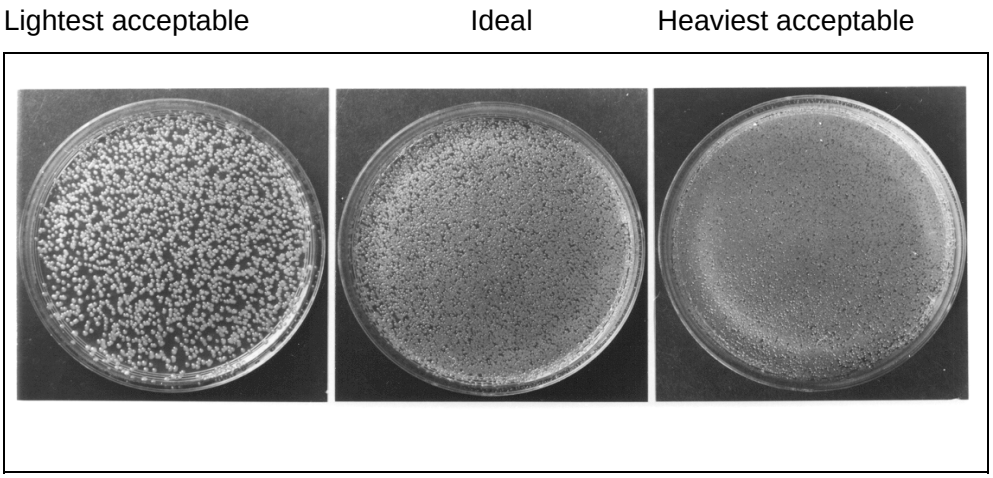

Figure 2