
The British Society for
Antimicrobial Chemotherapy

BSAC Methods for Antimicrobial Susceptibility Testing

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## Preface

Since the *Journal of Antimicrobial Chemotherapy* Supplement containing the BSAC standardized disc susceptibility testing method was published in 2001, there have been various changes to the recommendations and these have been posted on the BSAC website (http://www.bsac.org.uk). One major organizational change has been the harmonisation of MIC breakpoints in Europe.

In 2002 the BSAC agreed to participate with several other European national susceptibility testing committees, namely CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie, France), the CRG (Commissie Richtlijnen Gevoeligheidsbepalingen (The Netherlands), DIN (Deutsches Institut für Normung, Germany), NWGA (Norwegian Working Group on Antimicrobials, Norway) and the SRGA (Swedish Reference Group of Antibiotics, Sweden), in a project to harmonize antimicrobial breakpoints, including previously established values that varied among countries. This work is being undertaken by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) with the support and collaboration of the national committees, and is funded by the European Union, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the national committees, including the BSAC. The review process includes application of more recent techniques, such as pharmacodynamic analysis, and current data, where available, on susceptibility distributions, resistance mechanisms and clinical outcomes as related to *in vitro* tests. There is extensive discussion between EUCAST and the national committees, including the BSAC Working Party on antimicrobial susceptibility testing, and wide consultation on proposals. In the interest of international standardization of susceptibility testing, and the need to update older breakpoints, these developments are welcomed by the BSAC.

The implication of such harmonization is that over time some MIC breakpoints will change slightly and these changes will be reflected, where necessary, in corresponding changes to zone diameter breakpoints in the BSAC disc diffusion method. It is appreciated that changes in the method require additional work for laboratories in changing templates and laboratory information systems, and that the wider use of `intermediate' categories will add complexity. Nevertheless the benefits of international standardization are considerable, and review of some older breakpoints is undoubtedly warranted.

In line with the European consensus EUCAST MIC breakpoints are defined as follows:

- Clinically resistant: level of antimicrobial susceptibility which results in a high likelihood of therapeutic failure
- Clinically susceptible: level of antimicrobial susceptibility associated with a high likelihood of therapeutic success
- Clinically intermediate: a level of antimicrobial susceptibility associated with uncertain therapeutic effect. It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used; it also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation.

The presentation of MIC breakpoints (mg/L) has also been amended to avoid the theoretical 'gap' inherent in the previous system as follows:
MIC $\leq$ (as previously) MIC breakpoint concentration = organism is susceptible
MIC > (previously$\geq$) MIC breakpoint concentration = organism is resistant

In practice, this does result in changes to breakpoint systems based on two-fold dilutions. However, the appearance of the tables will change, e.g. R $\geq$16, S $\leq$8 will change to R>8, S $\leq$8.

## Disc Diffusion Method for Antimicrobial Susceptibility Testing

### 1. Preparation of plates

1.1 Prepare Iso-Sensitest agar (ISA) (see list of suppliers) or media shown to have the same performance as ISA, according to the manufacturer's instructions. Supplement media for fastidious organisms with 5% defibrinated horse blood or 5% defibrinated horse blood and 20 mg/L β-nicotinamide adenine dinucleotide (NAD) as indicated in Table 1. Use Columbia agar with 2% NaCl for methicillin/oxacillin susceptibility testing of staphylococci.

Table 1: Media and supplementation for antimicrobial susceptibility testing of different groups of organisms

| Organisms | Medium |
|---|---|
| Enterobacteriaceae | ISA |
| *Pseudomonas* spp. | ISA |
| *Stenotrophomonas maltophilia* | ISA |
| Staphylococci (tests other than methicillin/oxacillin) | ISA |
| *Staphylococcus aureus* (tests using cefoxitin to detect methicillin/oxacillin/cefoxitin resistance) | ISA |
| Staphylococci (tests using methicillin or oxacillin for the detection of methicillin/oxacillin/cefoxitin resistance) | Columbia agar (*see suppliers*) with 2% NaCl[1] |
| Enterococci | ISA |
| *Streptococcus pneumoniae* | ISA + 5% defibrinated horse blood[2] |
| α-Haemolytic streptococci | ISA + 5% defibrinated horse blood + 20 mg/L NAD |
| β-Haemolytic streptococci | ISA + 5% defibrinated horse blood[2] |
| *Moraxella catarrhalis* | ISA + 5% defibrinated horse blood[2] |
| *Haemophilus* spp. | ISA + 5% defibrinated horse blood + 20 mg/L NAD |
| *Neisseria gonorrhoeae* | ISA + 5% defibrinated horse blood[2] |
| *Neisseria meningitidis* | ISA + 5% defibrinated horse blood[2] |
| *Pasteurella multocida* | ISA + 5% defibrinated horse blood + 20 mg/L NAD |
| *Bacteroides fragilis, Bacteroides thetaiotaomicron, Clostridium perfringens* | ISA + 5% defibrinated horse blood + 20 mg/L NAD |
| *Campylobacter* spp. | ISA + 5% defibrinated horse blood[2] |
| Coryneform organisms | ISA + 5% defibrinated horse blood + 20 mg/L NAD |

[1] See Section 8.

[2] ISA supplemented with 5% defibrinated horse blood + 20mg/L NAD may be used.

1.2 Pour sufficient molten agar into sterile Petri dishes to give a depth of 4 mm ± 0.5 mm (25 mL in 90 mm diameter Petri dishes).

1.3 Dry the surface of the agar to remove excess moisture before use. The length of time needed to dry the surface of the agar depends on the drying conditions, e.g. whether a fan-assisted drying cabinet or 'still air' incubator is used, whether plates are dried before storage and storage conditions. **It is important that plates are not over dried.**

1.4 Store the plates in vented plastic boxes at 8-10°C prior to use. Alternatively the plates may be stored at 4-8°C in sealed plastic bags. Plate drying, method of storage and storage time should be determined by individual laboratories as part of their quality assurance programme. In particular, quality control tests should confirm that excess surface moisture is not produced and that plates are not over-dried.

## 2. Selection of control organisms

2.1 The performance of the tests should be monitored by the use of appropriate control strains (see section on control of antimicrobial susceptibility testing). The control strains listed (Tables 2a, 2b) include susceptible strains that have been chosen to monitor test performance and resistant strains that can be used to confirm that the method will detect a mechanism of resistance.

2.2 Store control strains at –70°C on beads in glycerol broth. Non-fastidious organisms may be stored at –20°C. Two vials of each control strain should be stored, one for an 'in-use' supply, the other for archiving.

2.3 Every week subculture a bead from the 'in-use' vial on to appropriate non-selective media and check for purity. From this pure culture, prepare one subculture on each of the following 5 days. For fastidious organisms that will not survive on plates for 5/6 days, subculture the strain daily for no more than 6 days.

Table 2a: Susceptible control strains or control strains with low-level resistance that have been chosen to monitor test performance of antimicrobial susceptibility testing

| Organism | Strain | | Characteristics |
|---|---|---|---|
| | Either | Or | |
| *Escherichia coli* | NCTC 12241 (ATCC 25922) | NCTC 10418 | Susceptible |
| *Staphylococcus aureus* | NCTC 12981 (ATCC 25923) | NCTC 6571 | Susceptible |
| *Pseudomonas aeruginosa* | NCTC 12903 (ATCC 27853) | NCTC 10662 | Susceptible |
| *Enterococcus faecalis* | NCTC 12697 (ATCC 29212) | | Susceptible |
| *Haemophilus influenzae* | NCTC 11931 | | Susceptible |
| *Streptococcus pneumoniae* | NCTC 12977 (ATCC 49619) | | Low-level resistant to penicillin |
| *Neisseria gonorrhoeae* | NCTC 12700 (ATCC 49226) | | Low-level resistant to penicillin |
| *Pasteurella multocida* | NCTC 8489 | | Susceptible |
| *Bacteroides fragilis* | NCTC 9343 (ATCC 25285) | | Susceptible |
| *Bacteroides thetaiotaomicron* | ATCC 29741 | | Susceptible |
| *Clostridium perfringens* | NCTC 8359 (ATCC 12915) | | Susceptible |

Table 2b: Control strains with a resistance mechanism that can be used to confirm that the method will detect resistance.

| Organism | Strain | Characteristics |
|---|---|---|
| *Escherichia coli* | NCTC 11560 | TEM-1 ß-lactamase-producer |
| *Staphylococcus aureus* | NCTC 12493 | *MecA* positive, methicillin resistant |
| *Haemophilus influenzae* | NCTC 12699 (ATCC 49247) | Resistant to ß-lactams (ß-lactamase-negative) |

## 3. Preparation of inoculum

The inoculum should give semi-confluent growth of colonies after overnight incubation. Use of an inoculum that yields semi-confluent growth has the advantage that an incorrect inoculum can easily be observed. A denser inoculum will result in reduced zones of inhibition and a lighter inoculum will have the opposite effect. The following methods reliably give semi-confluent growth with most isolates.

**NB.** Other methods of obtaining semi-confluent growth may be used if they are shown to be equivalent to the following.

3.1 Comparison with a 0.5 McFarland standard

3.1.1 *Preparation of the 0.5 McFarland standard*
Add 0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2$. $2H_2O$) to 99.5 mL of 0.18 M $H_2SO_4$ (1% w/v) with constant stirring. Thoroughly mix the suspension to ensure that it is even. Using matched cuvettes with a 1 cm light path and water as a blank standard, measure the absorbance in a spectrophotometer at a wavelength of 625 nm. The acceptable absorbance range for the standard is 0.08-0.13. Distribute the standard into screw-cap tubes of the same size and volume as those used in growing the broth cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light at room temperature. Vigorously agitate the turbidity standard on a vortex mixer before use. Standards may be stored for up to six months, after which time they should be discarded. Prepared standards can be purchased (See list of suppliers), but commercial standards should be checked to ensure that absorbance is within the acceptable range as indicated above.

3.1.2 *Inoculum preparation by the growth method* (for non-fastidious organisms, e.g. Enterobacteriaceae, *Pseudomonas* spp. and staphylococci)
Touch at least four morphologically similar colonies (when possible) with a sterile loop. Transfer the growth into Iso-Sensitest broth or an equivalent that has been shown not to interfere with the test. Incubate the broth, with shaking at 35-37°C, until the visible turbidity is equal to or greater than that of a 0.5 McFarland standard.

3.1.3 *Inoculum preparation by the direct colony suspension method* (the method of choice for fastidious organisms, i.e. *Haemophilus* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis, Streptococcus pneumoniae*, $\alpha$ and $\beta$-haemolytic streptococci, *Clostridium perfringens, Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Campylobacter* spp., *Pasteurella multocida* and Coryneform organisms).
Colonies are taken directly from the plate into Iso-Sensitest broth (or equivalent) or sterile distilled water. The density of the suspension should match or exceed that of a 0.5 McFarland standard.

**NB**. With some organisms production of an even suspension of the required turbidity is difficult and growth in broth, if possible, is a more satisfactory option.

3.1.4 *Adjustment of the organism suspension to the density of a 0.5 McFarland standard*
Adjust the density of the organism suspension to equal that of a 0.5 McFarland standard by adding sterile distilled water. To aid comparison, compare the test and standard suspensions against a white background with a contrasting black line.

**NB**. Suspension should be used within 15 min.

3.1.5 *Dilution of suspension in distilled water before inoculation*

Dilute the suspension (density adjusted to that of a 0.5 McFarland standard) in distilled water as indicated in Table 3.

Table 3: Dilution of the suspension (density adjusted to that of a 0.5 McFarland standard) in distilled water

| Dilute 1:100 | Dilute 1:10 | No dilution |
|---|---|---|
| β-Haemolytic streptococci | Staphylococci | *Neisseria gonorrhoeae* |
| Enterococci | *Serratia* spp. | *Campylobacter* spp. |
| Enterobacteriaceae | *Streptococcus pneumoniae* | |
| *Pseudomonas* spp. | *Neisseria meningitidis* | |
| *Stenotrophomonas maltophilia* | *Moraxella catarrhalis* | |
| *Acinetobacter* spp. | α-haemolytic streptococci | |
| *Haemophilus* spp. | *Clostridium perfringens* | |
| *Pasteurella multocida* | Coryneform organisms | |
| *Bacteroides fragilis* | | |
| *Bacteroides thetaiotaomicron* | | |

**NB.** These suspensions should be used within 15 min of preparation.

3.2 Photometric standardization of turbidity of suspensions

A photometric method of preparing inocula was described by Moosdeen *et al* (1988)[1] and from this the following simplified procedure has been developed. The spectrophotometer must have a cell holder for 100 x 12 mm test tubes. A much simpler photometer would also probably be acceptable. The 100 x 12 mm test tubes could also be replaced with another tube/cuvette system if required, but the dilutions would need to be recalibrated.

3.2.1 Suspend colonies (touch 4-5 when possible) in 3 mL distilled water or broth in a 100 x 12 mm glass tube (note that tubes are not reused) to give just visible turbidity. It is essential to get an even suspension.

**NB.** These suspensions should be used within 15 min of preparation.

3.2.2 Zero the spectrophotometer with a sterile water or broth blank (as appropriate) at a wavelength of 500 nm and measure the absorbance of the bacterial suspension.

3.2.3 From table 4 select the volume to transfer (with the appropriate fixed volume micropipette) to 5 mL sterile distilled water.

3.2.4 Mix the diluted suspension to ensure that it is even

**NB**. Suspension should be used within 15 min. of preparation

Table 4: Dilution of suspensions of test organisms according to absorbance reading

| Organisms | Absorbance reading at 500 nm | Volume (μL) to transfer to 5 mL sterile distilled water |
|---|---|---|
| Enterobacteriaceae<br>Enterococci<br>*Pseudomonas* spp.<br>Staphylococci | 0.01 - 0.05 | 250 |
| | >0.05 - 0.1 | 125 |
| | >0.1 - 0.3 | 40 |
| | >0.3 - 0.6 | 20 |
| | >0.6 - 1.0 | 10 |
| *Haemophilus* spp.<br>Streptococci<br>Miscellaneous fastidious Organisms | 0.01 - 0.05 | 500 |
| | >0.05 - 0.1 | 250 |
| | >0.1 - 0.3 | 125 |
| | >0.3 - 0.6 | 80 |
| | >0.6 - 1.0 | 40 |

**NB**. As spectrophotometers may differ, it may be necessary to adjust the dilutions slightly to achieve semi-confluent growth with any individual set of laboratory conditions.

## 3.3 Direct antimicrobial susceptibility testing of urine specimens and blood cultures

Direct susceptibility testing is not advocated as the control of inoculum is very difficult. Direct testing is, however, undertaken in many laboratories in order to provide more rapid test results. The following methods have been recommended by laboratories that use the BSAC method and. will achieve the correct inoculum size for a reasonable proportion of infected urines and blood cultures If the inoculum is not correct (i.e. growth is not semi-confluent) or the culture is mixed, the test must be repeated.

### 3.3.1 Urine specimens

#### 3.3.1.1 Method 1

Thoroughly mix the urine specimen, then place a 10 μL loop of urine in the centre of the susceptibility plate and spread evenly with a dry swab.

#### 3.3.1.2 Method 2

Thoroughly mix the urine specimen, then dip a sterile cotton-wool swab in the urine and remove excess by turning the swab against the inside of the container. Use the swab to make a cross in the centre of the susceptibility plate and spread evenly with another sterile dry swab. If only small numbers of organisms are seen in microscopy, the initial cotton-wool swab may be used to inoculate and spread the susceptibility plate.

### 3.3.2 Positive blood cultures

The method depends on the Gram reaction of the infecting organism.

#### 3.3.2.1 Gram-negative bacilli.

Using a venting needle, place one drop of the blood culture in 5 mL of sterile water, then dip a sterile cotton-wool swab in the suspension and remove excess by turning the swab against the inside of the container. Use the swab to spread the inoculum evenly over the surface of the susceptibility plate.

3.3.2.2 Gram-positive organisms.

It is not always possible accurately to predict the genera of Gram-positive organisms from the Gram's stain. However, careful observation of the morphology, coupled with clinical information, should make an "educated guess" correct most of the time.

Staphylococci and enterococci.
Using a venting needle, place three drops of the blood culture in 5 mL of sterile water, then dip a sterile cotton-wool swab in the suspension and remove excess by turning the swab against the inside of the container. Use the swab to spread the inoculum evenly over the surface of the susceptibility plate.

Pneumococci, "viridans" streptococci and diptheroids.
Using a venting needle, place one drop of the blood culture in the centre of a susceptibility plate, and spread the inoculum evenly over the surface of the plate.

**4. Inoculation of agar plate**

Use the adjusted suspension within 15 min to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess liquid by turning the swab against the side of the container. Spread the inoculum evenly over the entire surface of the plate by swabbing in three directions. Allow the plate to dry before applying discs.

**NB.** If inoculated plates are left at room temperature for extended times before the discs are applied, the organism may begin to grow, resulting in reduced zones of inhibition. Discs should therefore be applied to the surface of the agar within 15 min of inoculation.

**5. Antimicrobial discs**

Refer to interpretation tables 6-23 for the appropriate disc contents for the organisms tested.

5.1 Storage and handling of discs.

Loss of potency of agents in discs will result in reduced zones of inhibition. To avoid loss of potency due to inadequate handling of discs the following are recommended:

5.1.1 Store discs in sealed containers with a desiccant and protected from light (this is particularly important for some light-susceptible agents such as metronidazole, chloramphenicol and the quinolones).

5.1.2 Store stocks at -20°C except for drugs known to be unstable at this temperature. If this is not possible, store discs at <8°C.

5.1.3 Store working supplies of discs at <8°C.

5.1.4 To prevent condensation, allow discs to warm to room temperature before opening containers.

5.1.5 Store disc dispensers in sealed containers with an indicating desiccant.

5.1.6 Discard discs on the expiry date shown on the side of the container.

5.2 Application of discs

Discs should be firmly applied to the dry surface of the inoculated susceptibility plate. The contact with the agar should be even. A 90 mm plate will accommodate six discs without unacceptable overlapping of zones.

## 6. Incubation

If the plates are left for extended times at room temperature after discs are applied, larger zones of inhibition may be obtained compared with zones produced when plates are incubated immediately. Plates should therefore be incubated within 15 min of disc application.

6.1 Conditions of incubation
Incubate plates under conditions listed in table 5.

Table 5: Incubation conditions for antimicrobial susceptibility tests on various organisms

| Organisms | Incubation conditions |
|---|---|
| Enterobacteriaceae | 35-37°C in air for 18-20 h |
| *Acinetobacter* spp. | 35-37°C in air for 18-20 h |
| *Pseudomonas* spp. | 35-37°C in air for 18-20 h |
| *Stenotrophomonas maltophilia* | 30°C in air for 18-20 h |
| Staphylococci (other than methicillin/oxacillin/cefoxitin) | 35-37°C in air for 18-20 h |
| *Staphylococcus aureus* using cefoxitin for the detection of methicillin/oxacillin/cefoxitin resistance | 35°C in air for 18-20 h |
| Staphylococci using methicillin or oxacillin to detect resistance | 30°C in air for 24 h |
| *Moraxella catarrhalis* | 35-37°C in air for 18-20 h |
| $\alpha$-Haemolytic streptococci | 35-37°C in 4-6% $CO_2$ in air for 18-20 h |
| $\beta$-Haemolytic streptococci | 35-37°C in air for 18-20 h |
| Enterococci | 35-37°C in air for 24 h[1] |
| *Neisseria meningitidis* | 35-37°C in 4-6 % $CO_2$ in air for 18-20 h |
| *Streptococcus pneumoniae* | 35-37°C in 4-6 % $CO_2$ in air for 18-20 h |
| *Haemophilus* spp. | 35-37°C in 4-6 % $CO_2$ in air for 18-20 h |
| *Neisseria gonorrhoeae* | 35-37°C in 4-6 % $CO_2$ in air for 18-20 h |
| *Pasteurella multocida* | 35-37°C in 4- 6% $CO_2$ in air for 18-20 h |
| Coryneform organisms | 35-37°C in 4-6% $CO_2$ in air for 18-20 h |
| *Campylobacter* spp. | 42°C in microaerophilic conditions for 24 h |
| *Bacteroides fragilis, Bacteroides thetaiotaomicron, Clostridium perfringens* | 35-37°C in 10% $CO_2$/10% $H_2$/80% $N_2$ for 18-20 h (anaerobic cabinet or jar) |

[1]It is essential that plates are incubated for at least 24 h before reporting a strain as susceptible to vancomycin or teicoplanin.

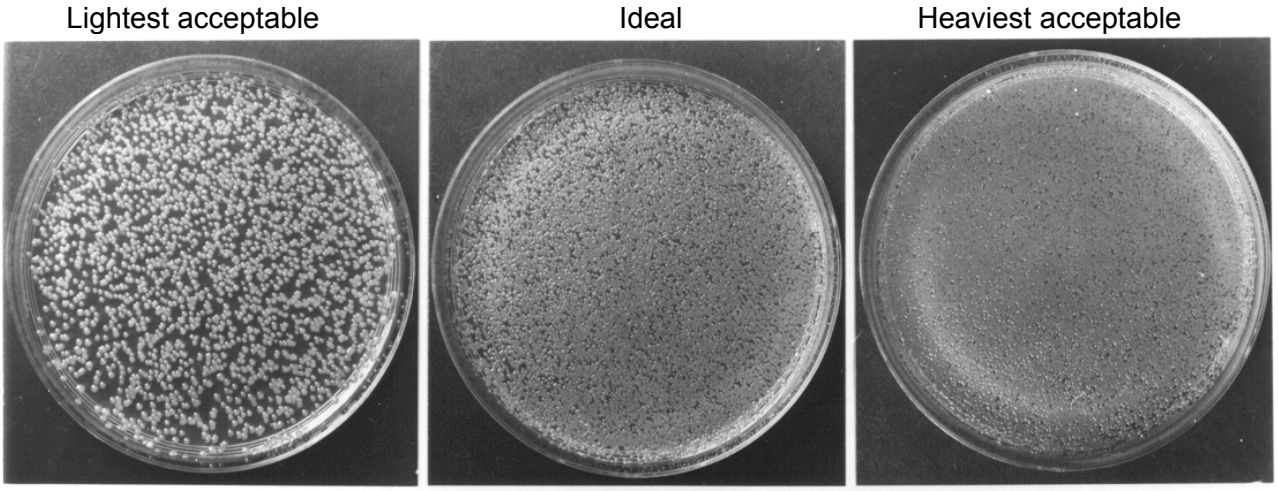
**NB.** Stacking plates too high in the incubator may affect results owing to uneven heating of plates. The efficiency of heating of plates depends on the incubator and the racking system used. Control of incubation, including height of plate stacking, should therefore be part of the laboratory's Quality Assurance programme.

## 7. Measuring zones and interpretation of susceptibility

### 7.1 Acceptable inoculum density

The inoculum should give semi-confluent growth of colonies on the susceptibility plate, within the range illustrated in Figure 1.

Figure 1: Acceptable inoculum density range for a Gram-negative rod




### 7.2 Measuring zones

7.2.1 Measure the diameters of zones of inhibition to the nearest millimetre (zone edge should be taken as the point of inhibition as judged by the naked eye) with a ruler, callipers or an automated zone reader.

7.2.2 Tiny colonies at the edge of the zone, films of growth as a result of the swarming of *Proteus* spp. and slight growth within sulphonamide or trimethoprim zones should be ignored.

7.2.3 Colonies growing within the zone of inhibition should be subcultured and identified and the test repeated if necessary.

7.2.4 When using cefoxitin for the detection of methicillin/oxacillin/cefoxitin resistance in *S. aureus,* measure the obvious zone, taking care to examine zones carefully in good light to detect minute colonies that may be present within the zone of inhibition (see Figure 3)

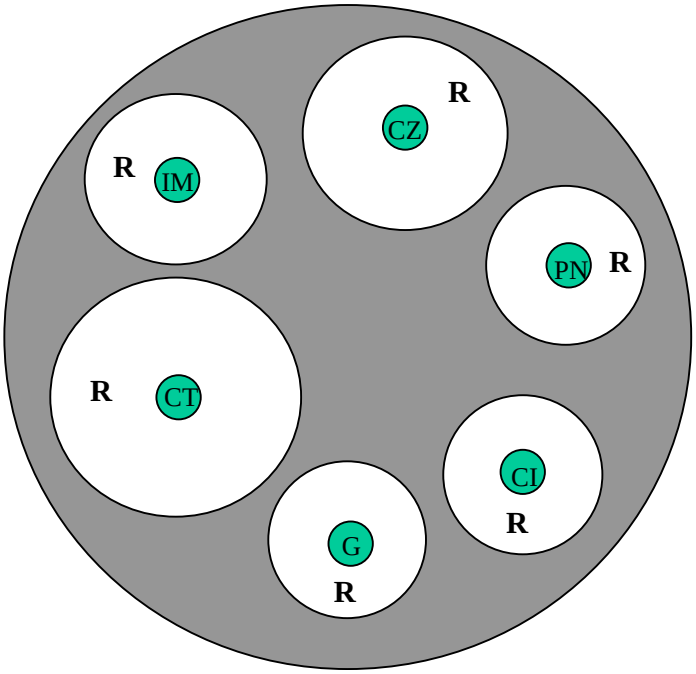
7.2.5 Confirm that the zone of inhibition for the control strain falls within the acceptable ranges in Tables 20-23 before interpreting the test (see section on control of the disc diffusion method).

### 7.3 Use of templates for interpreting zone diameters

A template may be used for interpreting zone diameters (see Figure 2). A program for preparing templates is available from the BSAC (http://www.bsac.org.uk).

The test plate is placed over the template and the zones of inhibition are examined in relationship to the template zones. If the zone of inhibition of the test strain is within the area marked with an 'R', the organism is resistant. If the zone of inhibition is equal to or larger than the marked area, the organism is susceptible.

Figure 2: Template for interpreting zone diameters




## 8. Oxacillin/cefoxitin testing of staphylococci

Methicillin susceptibility testing is difficult with some strains. Expression of resistance is affected by test conditions and resistance is often heterogeneous, with only a proportion of cells showing resistance. Adding NaCl or lowering incubation temperatures increases the proportion of cells showing resistance. Methicillin susceptibility testing of coagulase-negative staphylococci is further complicated as some strains do not grow well on media containing NaCl and are often slower-growing than *Staphylococcus aureus*. Detection of methicillin resistance in coagulase-negative staphylococci may require incubation for 48 h.

### 8.1 Method for detection of oxacillin resistance in *S. aureus* and coagulase-negative staphylococci

#### 8.1.1 Medium

Prepare Columbia (See list of suppliers) or Mueller-Hinton agar (See list of suppliers) following the manufacturer's instructions and add 2% NaCl. After autoclaving, mix well to distribute the sodium chloride. Pour plates to give a depth of 4 mm (± 0.5 mm) in a 90 mm sterile Petri dish (25 ml). Dry and store plates as previously described (section 1).

#### 8.1.2 Inoculum

Prepare inoculum as previously described (section 3).

8.1.3 Control

Susceptible control strains (*Staphylococcus aureus* ATCC 25923 or NCTC 6571) test the reliability of disc content.
*Staphylococcus aureus* NCTC 12493 is a methicillin resistant strain and is used to check that the test will detect resistant organisms (although no strain can be representative of all the MRSA types in terms of their response to changes in test conditions).

8.1.4 Discs

Place a oxacillin 1 μg disc on to the surface of inoculated agar.
Discs should be stored and handled as previously described (section 5).

8.1.5 Incubation

Incubate plates for 24 h at 30°C.

8.1.6 Zone measurement

Measure zone diameters (mm) as previously described (section 7).
Examine zones carefully in good light to detect colonies, which may be minute, in zones. If there is suspicion that the colonies growing within zones are contaminants they should be identified and the isolate re-tested for resistance to methicillin/oxacillin if necessary.

8.1.7 Interpretation

For oxacillin interpretation is as follows:
Susceptible = ≥ 15 mm diameter, resistant = ≤ 14 mm diameter.

**NB. Hyper-production of β-lactamase does not confer clinical resistance to penicillinase-resistant penicillins and such isolates should be reported susceptible to oxacillin.** Some hyper-producers of β-lactamase give zones within the range of 7-14 mm and, if possible, such isolates should be checked by a PCR method for *mec*A or by a latex agglutination test for PBP2a. Increase in oxacillin zone size in the presence of clavulanic acid is not a reliable test for hyper-producers of β-lactamase as zones of inhibition with some MRSA also increase in the presence of clavulanic acid. Rarely, hyper-producers of β-lactamase give no zone in this test and would therefore not be distinguished from MRSA.

8.2 Detection of methicillin/oxacillin/cefoxitin resistance in staphylococci by use of cefoxitin as the test agent

8.2.1 Medium

Prepare Iso-Sensitest agar as previously described (section 1).

8.2.2 Inoculum

Prepare inoculum as previously described (section 3).

8.2.3 Control

Use control strains as previously described (section 8.1.3).

8.2.4 Discs

Place a 10 μg cefoxitin disc on the surface of inoculated agar.
Discs should be stored and handled as previously described (section 5).

8.2.5 Incubation

Incubate plates at 35°C for 18-20 h.

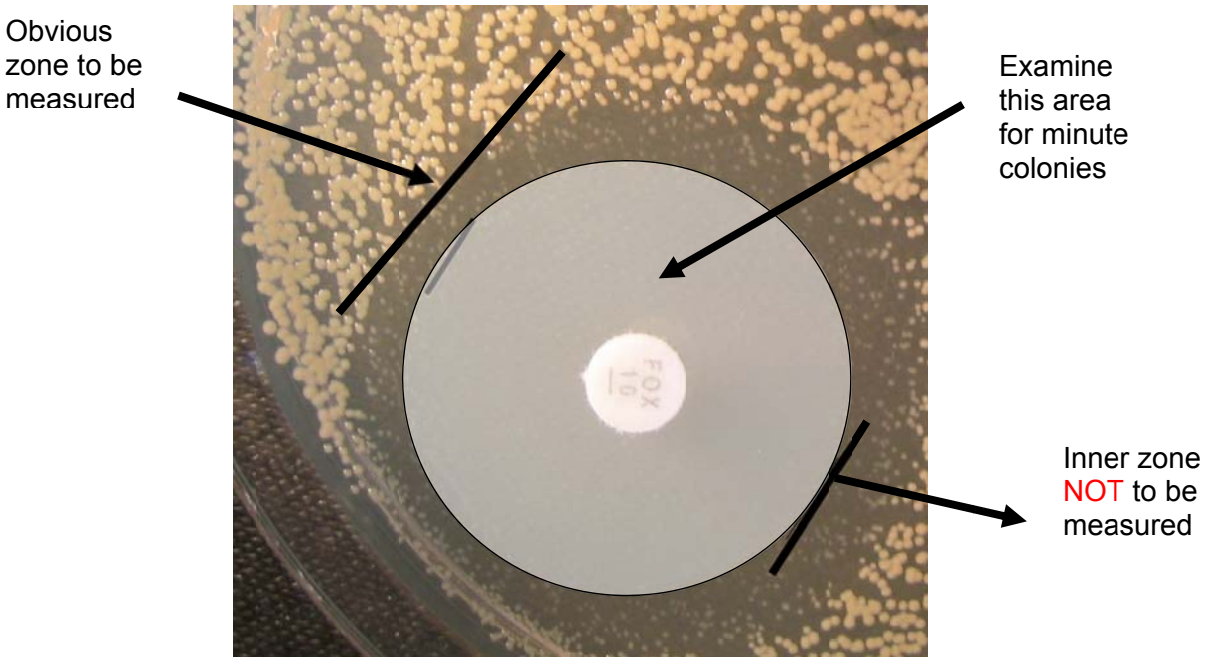
**NB**. It is important that the temperature does not exceed 36°C, as tests incubated at higher temperatures are less reliable.

8.2.6 Zone measurement

Measure zone diameters as previously described (section 7), reading the obvious zone edge (see Figure 3).

Examine zones carefully in good light to detect colonies, which may be minute, in zones. If there is suspicion that the colonies growing within zones are contaminants they should be identified and the isolate re-tested for resistance to cefoxitin if necessary.

Figure 3: Reading cefoxitin zones of inhibition with staphylococci




8.2.7 Interpretation:

For *S. aureus*

Susceptible = ≥22 mm diameter, resistant = ≤21 mm diameter

For *S. saprophyticus*

Susceptible = ≥20 mm diameter, resistant = ≤19 mm diameter

For coagulase staphylococci other than *S. saprophyticus*

Susceptible = ≥27 mm diameter, intermediate = 22-26 mm, resistant = ≤21 mm diameter

NB. Hyper -production of β-lactamase does not confer clinical resistance to penicillinase-resistant penicillins and such isolates should be reported susceptible to cefoxitin. Hyper-producers of β-lactamase give zones within the ranges of the susceptible population.

**Acknowledgment**

The BSAC acknowledges the assistance of the Swedish Reference Group for Antibiotics (SRGA) in supplying some breakpoint data for inclusion in this document.

**References**

1. Moosdeen, F., Williams, J.D. & Secker, A. (1988). Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. J. Antimicrob Chemother **21**, 439-43.

## Control of Antimicrobial Susceptibility Testing

### 1. Control strains

Control strains include susceptible strains to monitor test performance (not for the interpretation of susceptibility), and resistant strains to confirm that the method will detect particular mechanisms of resistance, for example, *Haemophilus influenzae* ATCC 49247 is a β-lactamase negative, ampicillin resistant strain (see table 2 of Disc Diffusion Method). Tables 2-6 provide zone diameters for recommended control organisms under a range of test conditions.

Control strains can be purchased from the National Collection of Type Cultures (NCTC; HPA Centre for Infections, 61 Colindale Avenue, London NW9 5HT). Alternatively, some may be obtained commercially (see section on suppliers)

### 2. Maintenance of control strains

Store control strains by a method that minimises the risk of mutations, for example, at $-70^0$C, on beads in glycerol broth. Ideally, two vials of each control strain should be stored, one as an ”in-use” supply, the other for archiving. Every week a bead from the ”in-use” vial should be subcultured on to appropriate non-selective media and checked for purity. From this pure culture, prepare one subculture for each of the following 7 days. Alternatively, for fastidious organisms that will not survive on plates for 7 days, subculture the strain daily for no more than 6 days.

### 3. Calculation of control ranges for disc diffusion tests

The acceptable ranges for the control strains have been calculated by combining zone diameter data from `field studies' and from multiple centres supplying their daily control data, from which cumulative distributions of zones of inhibition have been prepared. From these distributions, the 2.5 and 97.5 percentiles were read to provide a range that would contain 95% of observations. If distributions are normal, these ranges correspond to the mean $\pm$ 1.96 SD. The percentile ranges obtained by this method are, however, still valid even if the data do not show a normal distribution.

### 4. Frequency of routine testing with control strains

When the method is first introduced, daily testing is required until there are acceptable readings from 20 consecutive days (this also applies when new agents are introduced or when any test component changes). This provides sufficient data to support once weekly testing.

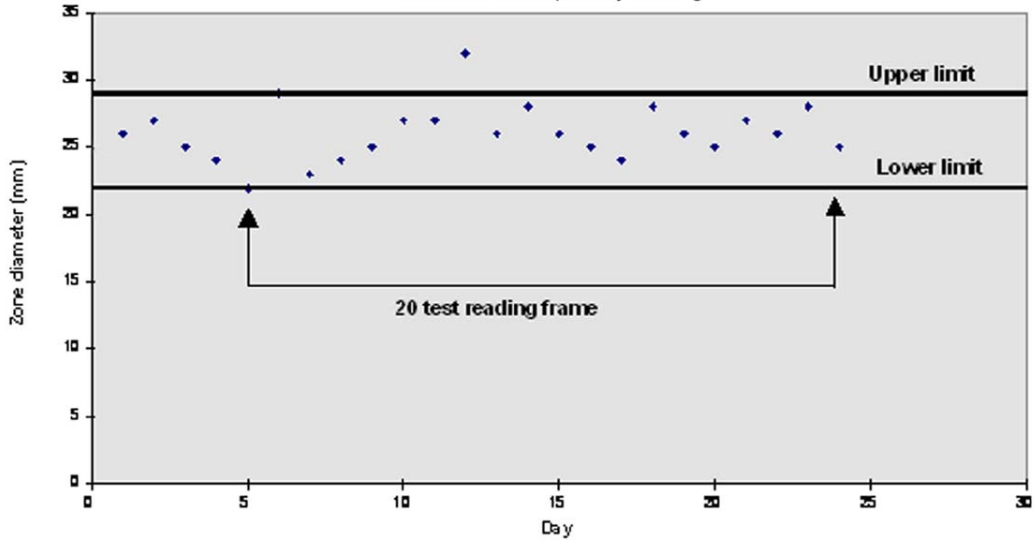
### 5. Use of control data to monitor the performance of disc diffusion tests

Use a reading frame of 20 consecutive results (remove the oldest result when adding a new one to make a total of 20) as illustrated in Figure 1. Testing is acceptable if no more than1 in every 20 results is outside the limits of acceptability. If 2 or more results fall out of the acceptable range this requires immediate investigation.

Look for trends within the limits of acceptability e.g. tendency for zones to be at the limits of acceptability; tendency for zones to be consistently above or below the mean;

gradual drift in zone diameters. Quality Assurance will often pick up trends before the controls go out of range.

Figure 1. Use of control strain to monitor performance of disc diffusion susceptibility testing.




## 6. Recognition of atypical results for clinical isolates

Atypical results with clinical isolates may indicate problems in testing that may or may not be reflected in zone diameters with control strains.

An organism with inherent resistance appears susceptible e.g. *Proteus* spp. susceptible to colistin or nitrofurantoin.

Resistance is seen in an organism when resistance has previously not been observed, e.g. penicillin resistance in Group A streptococci.

Resistance is seen in an organism when resistance is rare or has not been seen locally, e.g. vancomycin resistance in *Staphylococcus aureus*.

Incompatible susceptibilities are reported, e.g. a methicillin resistant staphylococcus reported susceptible to a β-lactam antibiotic.

In order to apply such rules related to atypical results it is useful to install an `expert' system for laboratory reporting to avoid erroneous interpretation.

## 7. Investigation of possible sources of error

If the control values are found to be outside acceptable limits on more than one occasion during a reading frame of twenty tests, investigation into the possible source of error is required. Possible problem areas are indicated in table 1.

Table 1: Potential sources of error in disc diffusion antimicrobial susceptibility testing.

| Possible source of error | Detail to check |
|---|---|
| Test conditions | Excessive pre-incubation before discs applied<br>Excessive pre-diffusion before plates incubated<br>Incorrect incubation temperature<br>Incorrect incubation atmosphere<br>Incorrect incubation time<br>Inadequate illumination of plates when reading<br>Incorrect reading of zone edges |
| Medium | Required susceptibility testing agar not used<br>Not prepared as required by the manufacturer's instructions<br>Batch to batch variation<br>Antagonists present (e.g. with sulphonamides and trimethoprim)<br>Incorrect pH<br>Incorrect divalent cation concentration<br>Incorrect depth of agar plates<br>Agar plates not level<br>Expiry date exceeded |
| Antimicrobial discs | Wrong agent or content used<br>Labile agent possibly deteriorated<br>Light sensitive agent left in light<br>Incorrect storage leading to deterioration<br>Disc containers opened before reaching room temperature<br>Incorrect labelling of disc dispensers<br>Expiry date exceeded |
| Control strains | Contamination<br>Mutation<br>Incorrect inoculum density<br>Uneven inoculation<br>Old culture used |

## 8. Reporting susceptibility results when controls indicate problems

Microbiologists must use a pragmatic approach, as results from repeat testing are not available on the same day. If results with control strains are out of range the implications for test results need to be assessed.

*Control results out of range*
If control zones are below range but test results are susceptible, or control zones are above range but test results are resistant, investigate possible sources of error but report the test results. Otherwise it may be necessary to suppress reports on affected agents, investigate and retest.

*Atypical results*
If results are atypical with clinical isolates, the purity of the isolate and identification should be confirmed and the susceptibility repeated. Suppress the results for individual agents and retest.

Table 2: Acceptable zone diameter (mm) ranges for control strains on Iso-Sensitest agar, plates incubated at 35-37 $^0$C in air for 18-20 h.

| Antimicrobial agent | Disc content (μg unless stated) | *Escherichia coli* | | | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | | *Enterococcus faecalis* |
|---|---|---|---|---|---|---|---|---|---|
| | | NCTC 10418 | ATCC 25922 | NCTC 11560[1] | NCTC 10662 | ATCC 27853 | NCTC 6571 | ATCC 25923 | ATCC 29212 |
| Amikacin | 30 | 24-27 | 23-27 | - | 21-30 | 26-32 | 25-30 | 25-29 | - |
| Ampicillin | 10 | 21-26 | 16-22 | - | - | - | - | - | 26-35 |
| Ampicillin | 25 | 24-30 | 21-28 | - | - | - | 42-50 | 40-46 | - |
| Amoxicillin | 10 | 20-24 | 13-18 | - | - | - | - | - | - |
| Aztreonam | 30 | 39-44 | 36-40 | - | 27-30 | 26-30 | - | - | - |
| Azithromycin | 15 | - | - | - | - | - | 27-33 | 25-30 | 15-21 |
| Carbenicillin | 100 | - | - | - | 20-25 | 18-23 | - | - | - |
| Cefamandole | 30 | 32-36 | 35-39 | - | - | - | - | - | - |
| Cefepime | 30 | 38-43 | 37-42 | - | - | - | - | - | - |
| Cefepime-clavulanic acid | 30/10 | 38-43 | 37-42 | - | - | - | - | - | - |
| Cefixime | 5 | 32-36 | 27-30 | - | - | - | - | - | - |
| Cefoxitin | 30 | 28-33 | 26-30 | - | - | - | - | - | - |
| Cefotaxime | 30 | 36-45 | 34-44 | - | 20-29 | 20-24 | - | - | - |
| Cefotaxime-clavulanic acid | 30/10 | 39-44 | 37-42 | - | - | - | - | - | - |
| Cefotetan | 30 | 36-41 | 34-38 | - | - | - | - | - | - |
| Cefpodoxime | 10 | 29-36 | 25-31 | - | - | - | - | - | - |
| Cefpodoxime-clavulanic acid | 10/1 | 29-36 | 25-31 | - | - | - | - | - | - |
| Cefpirome | 30 | 34-43 | 36-43 | - | - | - | - | - | - |
| Ceftazidime | 30 | 32-40 | 31-39 | - | 29-37 | 27-35 | - | - | - |
| Ceftazidime-clavulanic acid | 30/10 | 31-39 | 30-36 | - | - | - | - | - | - |
| Ceftizoxime | 30 | 44-49 | 40-44 | - | - | - | - | - | - |
| Ceftriaxone | 30 | 41-46 | 37-42 | - | - | - | - | - | - |
| Cefuroxime | 30 | 25-32 | 24-29 | - | - | - | - | - | - |
| Cefalexin | 30 | 21-28 | 16-21 | - | - | - | - | - | - |

| Antimicrobial agent | Disc content (μg unless stated) | *Escherichia coli* | | | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | | *Enterococcus faecalis* |
|---|---|---|---|---|---|---|---|---|---|
| | | NCTC 10418 | ATCC 25922 | NCTC 11560[1] | NCTC 10662 | ATCC 27853 | NCTC 6571 | ATCC 25923 | ATCC 29212 |
| Cefradine | 30 | 19-25 | 16-22 | - | - | - | - | - | - |
| Cephalothin | 30 | 22-26 | 17-21 | - | - | - | - | - | - |
| Chloramphenicol | 10 | 21-27 | 20-29 | - | - | - | 20-26 | 19-27 | - |
| Ciprofloxacin | 1 | 31-40 | 31-37 | - | 21-28 | 24-30 | 25-32 | 17-22 | 14-19 |
| Ciprofloxacin | 5 | - | - | - | 29-37 | 31-37 | - | - | 21-27 |
| Clarithromycin | 2 | - | - | - | - | - | 25-30 | 24-28 | - |
| Clindamycin | 2 | - | - | - | - | - | 30-35 | 26-33 | No zone |
| Co-amoxiclav | 3 | - | - | - | - | - | 32-38 | 27-32 | - |
| Co-amoxiclav | 30 | 18-31 | 20-26 | 12-18 | - | - | 42-50 | 37-44 | - |
| Colistin | 25 | 15-19 | 16-20 | - | 17-20 | 16-20 | - | - | - |
| Cotrimoxazole | 25 | 33-38 | 28-34 | - | - | - | - | 31-35 | - |
| Cotrimoxazole incubation @ $30^0$C | 25 | 35-39 | 31-34 | - | - | - | - | - | - |
| Doripenem | 10 | - | - | - | 33-37 | 41-45 | - | - | - |
| Doxycycline | 30 | - | - | - | - | - | 35-40 | 33-37 | - |
| Ertapenem | 10 | 35-41 | 35-39 | - | - | - | - | - | - |
| Erythromycin | 5 | - | - | - | - | - | 22-31 | 22-29 | - |
| Fosfomycin trometamol/G6P | 200/50 | 29-33 | 36-41 | - | - | - | 25-32 | 25-30 | 27-31 |
| Fusidic acid | 10 | - | - | - | - | - | 32-40 | 30-37 | - |
| Gentamicin | 10 | 21-27 | 21-27 | - | 20-26 | 22-28 | 24-30 | 22-29 | - |
| Gentamicin | 200 | - | - | - | - | - | - | - | 22-27 |
| Imipenem | 10 | 32-37 | 33-37 | - | 20-27 | 23-28 | - | - | 28-32 |
| Levofloxacin | 1 | 30-33 | 28-34 | - | - | - | - | - | - |
| Levofloxacin | 5 | - | - | - | 22-29 | 23-29 | - | - | - |
| Linezolid | 10 | - | - | - | - | - | 26-33 | 26-30 | 24-29 |
| Mecillinam | 10 | 34-39 | 30-35 | - | - | - | - | - | - |
| Meropenem | 10 | 38-42 | 27-39 | - | 26-33 | 32-39 | - | - | 22-28 |
| Mezlocillin | 75 | 31-36 | 27-32 | - | - | - | - | - | - |
| Minocycline | 30 | - | - | - | - | - | 34-39 | 33-36 | - |

| Antimicrobial agent | Disc content (μg unless stated) | *Escherichia coli* | | | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | | *Enterococcus faecalis* |
|---|---|---|---|---|---|---|---|---|---|
| | | NCTC 10418 | ATCC 25922 | NCTC 11560[1] | NCTC 10662 | ATCC 27853 | NCTC 6571 | ATCC 25923 | ATCC 29212 |
| Moxifloxacin | 1 | 31-35 | 29-33 | - | - | - | 33-40 | 33-38 | - |
| Moxifloxacin | 5 | - | - | - | 19-24 | 23-27 | - | - | - |
| Mupirocin | 5 | - | - | - | - | - | 26-35 | 24-34 | - |
| Mupirocin | 20 | - | - | - | - | - | 30-38 | 27-35 | - |
| Nalidixic acid | 30 | 28-36 | 26-32 | - | - | - | - | - | - |
| Neomycin | 10 | - | - | - | - | - | 18-22 | 21-27 | - |
| Netilmicin | 10 | 22-27 | 22-26 | - | 17-20 | 20-24 | - | 22-28 | - |
| Nitrofurantoin | 200 | 25-30 | 23-27 | - | - | - | 21-25 | 20-26 | - |
| Norfloxacin | 2 | 34-37 | 32-36 | - | - | - | - | - | - |
| Ofloxacin | 5 | 31-37 | 31-38 | - | 18-26 | 18-25 | - | - | |
| Penicillin | 1 unit | - | - | - | - | - | 32-40 | 28-36 | - |
| Piperacillin | 75 | 30-35 | 27-32 | - | 27-35 | 27-34 | - | - | - |
| Pip/tazobactam | 85 | 30-35 | 26-31 | - | 28-35 | 28-35 | - | - | 26-32 |
| Quinupristin-Dalfopristin | 15 | - | - | - | - | - | 27-31 | - | 12-19 |
| Rifampicin | 2 | - | - | - | - | - | 27-39 | 29-36 | - |
| Streptomycin | 10 | 18-24 | 17-22 | - | - | - | - | - | - |
| Streptomycin | 300 | - | - | - | - | - | - | - | 20-24 |
| Teicoplanin | 30 | - | - | - | - | - | 17-23 | 16-20 | 19-25 |
| Tetracycline | 10 | 23-29 | 22-28 | - | - | - | 31-40 | 26-35 | 9-13 |
| Ticarcillin | 75 | 32-35 | 27-30 | - | 24-28 | 23-27 | - | - | - |
| Ticarcillin-clavulanic acid | 85 | 33-37 | 27-31 | - | 25-29 | 24-27 | - | - | - |
| Tigecycline | 15 | 29-32 | 28-32 | - | - | - | 29-34 | 27-30 | 26-31 |
| Tobramycin | 10 | 24-27 | 23-27 | - | 23-30 | 26-32 | 26-31 | 29-35 | - |
| Trimethoprim | 2.5 | 30-37 | 25-31 | - | - | - | 25-30 | 20-28 | 28-35 |
| Trimethoprim | 5 | - | - | - | - | - | 24-34 | - | - |
| Vancomycin | 5 | - | - | - | - | - | 14-20 | 13-17 | 13-19 |

1 = β-Lactamase producing strain

Table 3: Acceptable zone diameter (mm) ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, plates incubated at 35-37$^0$C in air for 18-20 h.

| Antimicrobial agent | Disc content | *Staphylococcus aureus* | | Group A streptococci | |
|---|---|---|---|---|---|
| | (μg unless stated) | NCTC 6571 | ATCC 25923 | NCTC 8198 | ATCC 19615 |
| Amoxicillin | 2 | 25-29 | - | - | - |
| Cefuroxime | 5 | 20-27 | - | - | - |
| Chloramphenicol | 10 | 17-23 | - | - | - |
| Clindamycin | 2 | - | - | 25-28 | 29-35 |
| Co-amoxiclav | 2/1 | 29-36 | - | - | - |
| Erythromycin | 5 | 26-33 | 23-29 | - | - |
| Nalidixic acid | 30 | 6-9 | - | - | - |
| Penicillin | 1 unit | 30-41 | 27-35 | - | - |
| Tetracycline | 10 | 30-38 | 28-36 | - | - |

Table 4: Acceptable zone diameter ranges for control strains for detection of methicillin/oxacillin/cefoxitin resistance in staphylococci (methicillin/oxacillin incubated at 30$^0$C; cefoxitin incubated at 35$^0$C).

| Antimicrobial agent | Medium | Disc content (μg) | *Staphylococcus aureus* NCTC 6571 | ATCC 25923 | NCTC 12493[a] |
|---|---|---|---|---|---|
| Methicillin | Columbia/Mueller Hinton agar + 2% NaCl | 5 | 18-30 | 18-28 | No zone |
| Oxacillin | Columbia/Mueller Hinton agar + 2% NaCl | 1 | 19-30 | 19-29 | No zone |
| Cefoxitin | ISA | 10 | 26-31 | 24-29 | 10-20 |

[a] Methicillin/oxacillin/cefoxitin- resistant strain.

Table 5: Acceptable zone diameter (mm) ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood and NAD, plates incubated at 35-37$^0$C in 10% $CO_2$ /10% $H_2$/80% $N_2$ for 18-20 h.

| Antimicrobial agent | Disc content (μg unless stated) | *Bacteroides fragilis* NCTC 9343 | *Bacteroides thetaiotaomicron* ATCC 29741 | *Clostridium perfringens* NCTC 8359 |
|---|---|---|---|---|
| Clindamycin | 2 | 13-27 | 11-25 | 23-28 |
| Co-amoxiclav | 30 | 43-49 | - | 40-45 |
| Meropenem | 10 | 42-50 | 36-43 | 39-45 |
| Metronidazole | 5 | 34-43 | 26-40 | 11-23 |
| Penicillin | 1 unit | 6 | 6 | 26-30 |
| Piperacillin/tazobactam | 75/10 | 41-48 | - | 37-43 |

Table 6: Acceptable zone diameter (mm) ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood with or without the addition of NAD, plates incubated at 35-37$^0$C in 4-6% $CO_2$ for 18-20 h.

| Antimicrobial agent | Disc content ( μg unless stated) | *Pasteurella multocida* | *Neisseria gonorrhoeae* (with NAD) | *Staphylococcus aureus* | | *Haemophilus influenzae* (with NAD) | | *Streptococcus pneumoniae* |
|---|---|---|---|---|---|---|---|---|
| | | NCTC 8489 | NCTC 12700 | NCTC 6571 | ATCC 25923 | NCTC 11931 | ATCC 49247[a] | ATCC 49619 |
| Amoxicillin | 2 | - | - | 29-34 | - | 20-26 | No zone | - |
| Ampicillin | 2 | - | - | - | - | 22-30 | 6-13 | - |
| Ampicillin | 10 | 32-37 | - | - | - | - | - | - |
| Azithromycin | 15 | - | 30-40 | - | - | 28-32 | 23-27 | 25-30 |
| Cefaclor | 30 | - | - | - | - | 29-38 | No zone | 26-33 |
| Cefixime | 5 | - | 33-44 | - | - | - | - | - |
| Cefotaxime | 5 | 35-41 | 32-44 | 26-32 | - | 33-45 | 27-38 | 27-35 |
| Ceftazidime | 30 | - | - | - | - | 39-46 | 36-41 | - |
| Ceftizoxime | 30 | - | - | - | - | - | - | 36-44 |
| Ceftriaxone | 5 | - | 33-47 | - | - | 47-54 | 38-44 | - |
| Ceftriaxone | 30 | - | - | - | - | - | - | 38-47 |
| Cefuroxime | 5 | - | 23-32 | 22-29 | 24-29 | 22-28 | 6-16 | - |
| Chloramphenicol | 10 | - | - | 21-26 | - | 30-40 | 30-38 | 21-29 |
| Ciprofloxacin | 1 | 31-37 | 40-50 | 22-29 | 18-23 | 32-40 | 33-44 | 14-21 |
| Clarithromycin | 2 | - | - | - | - | 6-10 | No zone | 26-31 |
| Clindamycin | 2 | - | - | 21-25 | - | - | - | - |
| Co-amoxiclav | 3 | - | - | 29-36 | - | 20-27 | 10-20 | - |
| Co-trimoxazole | 25 | - | - | - | - | 40-47 | 38-42 | 21-25 |
| Ertapenem | 10 | - | - | - | - | 30-38 | 25-34 | 35-40 |
| Erythromycin | 5 | - | 20-29 | 25-29 | - | 12-23 | 9-16 | 23-36 |
| Imipenem | 10 | - | - | - | - | 32-39 | 31-36 | - |
| Levofloxacin | 1 | - | - | - | - | 38-43 | 35-41 | 17-21 |
| Linezolid | 10 | - | - | 22-26 | - | - | - | - |
| Meropenem | 10 | - | - | - | - | 38-45 | 33-39 | - |
| Moxifloxacin | 1 | - | - | - | - | 36-42 | 33-39 | 24-30 |
| Nalidixic acid | 30 | - | 32-40 | 9-17 | 9-17 | 33-38 | 33-39 | - |
| Ofloxacin | 5 | - | - | - | - | 39-49 | 38-44 | 21-26 |
| Oxacillin | 1 | - | - | - | - | - | - | 8-16 |
| Penicillin | 1 unit | 24-28 | 12-20 | 37-44 | 29-36 | - | - | - |

| Antimicrobial agent | Disc content ( μg unless stated) | *Pasteurella multocida* | *Neisseria gonorrhoeae* (with NAD) | *Staphylococcus aureus* | | *Haemophilus influenzae* (with NAD) | | *Streptococcus pneumoniae* |
|---|---|---|---|---|---|---|---|---|
| | | NCTC 8489 | NCTC 12700 | NCTC 6571 | ATCC 25923 | NCTC 11931 | ATCC 49247[a] | ATCC 49619 |
| Quinupristin-Dalfopristin | 15 | - | - | - | - | - | - | 21-29 |
| Rifampicin | 2 | - | 26-34 | 32-37 | - | - | - | - |
| Rifampicin | 5 | - | - | - | - | - | - | 28-35 |
| Spectinomycin | 25 | - | 17-23 | - | - | - | - | - |
| Teicoplanin | 30 | - | - | 14-19 | - | - | - | - |
| Telithromycin | 15 | - | - | - | - | 26-31 | 22-26 | 33-40 |
| Tetracycline | 10 | 29-34 | 27-35 | 33-40 | 27-34 | 27-35 | 9-14 | 26-36 |
| Tigecycline | 15 | - | - | 27-30 | 24-28 | - | - | 26-30 |
| Trimethoprim | 2.5 | - | - | - | - | 30-40 | 28-36 | - |
| Vancomycin | 5 | - | - | 12-16 | - | - | - | - |

[a] β-Lactamase-negative, ampicillin-resistant strain

## 9. Control of MIC determination

Tables 7-10 provide target MIC (mg/L) values for recommended control strains by BSAC methodology.[1,2] MICs should be within one two-fold dilution of the target values i.e. target MIC 1 mg/L acceptable range 0.5 – 2 mg/L.

Table 7: Target MICs (mg/L) for *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Bacteroides fragilis* and *Neisseria gonorrhoeae* control strains by BSAC methods

| Antimicrobial agent | *Haemophilus influenzae* | | *Enterococcus faecalis* | *Streptococcus pneumoniae* | *Bacteroides fragilis* | *Neisseria gonorrhoeae* |
|---|---|---|---|---|---|---|
| | NCTC 11931 | ATCC 49247 | ATCC 29212 | ATCC 49619 | NCTC 9343 | ATCC 49226 |
| Amikacin | - | - | 128 | - | - | - |
| Amoxicillin | 0.5 | 4 | 0.5 | 0.06 | 32 | 0.5 |
| Ampicillin | - | - | 1 | 0.06 | 32 | - |
| Azithromycin | 2 | 2 | - | 0.12 | - | - |
| Azlocillin | - | - | - | - | 4 | - |
| Aztreonam | - | - | >128 | - | 2 | - |
| Cefaclor | - | 128 | >32 | 2 | >128 | - |
| Cefamandole | - | - | - | - | 8 | - |
| Cefixime | 0.03 | 0.25 | - | 1 | 64 | - |
| Cefotaxime | - | 0.25 | 32 | 0.06 | 4 | - |
| Cefoxitin | - | - | - | - | 4 | - |
| Cefpirome | 0.06 | 0.5 | 16 | | 16 | - |
| Cefpodoxime | 0.12 | 0.5 | >32 | 0.12 | 32 | - |
| Ceftazidime | 0.12 | - | >32 | - | 8 | - |
| Ceftriaxone | - | - | >32 | 0.06 | 4 | - |
| Cefuroxime | 2 | 16 | >32 | 0.25 | 32 | - |
| Cephadroxil | - | - | >32 | - | 32 | - |
| Cephalexin | - | - | >32 | - | 64 | - |
| Cephalothin | - | - | 16 | - | - | - |
| Chloramphenicol | - | - | 4 | 4 | 4 | - |
| Ciprofloxacin | 0.008 | 0.008 | 1 | 1 | 2 | 0.004 |
| Clarithromycin | 8 | 4 | - | 0.03 | 0.25 | 0.5 |
| Clindamycin | - | - | 8 | 0.12 | 0.5 | - |
| Co-amoxiclav | 0.5 | 8 | 0.5 | 0.06 | 0.5 | 0.5 |
| Cotrimoxazole | - | 1 | 2 | 4 | - | - |
| Dalfopristin/ quinupristin | - | - | 1 | 0.5 | 16 | - |
| Enoxacin | - | - | - | - | 1 | - |
| Ertapenem | 0.12 | 0.5 | - | 0.12 | 0.25 | - |
| Erythromycin | 8 | 8 | 4 | 0.12 | 1 | 0.5 |
| Faropenem | - | - | - | 0.06 | 1 | - |
| Fleroxacin | - | - | - | - | 4 | - |
| Flucloxacillin | - | - | - | - | 16 | - |
| Fucidic acid | - | - | 2 | - | - | - |
| Gatifloxacin | 0.008 | - | 0.25 | 0.25 | 0.5 | 0.004 |
| Gemifloxacin | 0.12 | - | 0.03 | 0.03 | 0.25 | 0.002 |
| Gentamicin | - | - | 8 | - | 128 | - |
| Grepafloxacin | - | 0.004 | - | 0.25 | - | - |
| Imipenem | - | - | 0.5 | - | 0.06 | - |
| Levofloxacin | 0.008 | 0.015 | 1 | 0.5 | 0.5 | 0.008 |
| Linezolid | - | - | 1 | 2 | 4 | - |
| Loracarbef | - | 128 | >32 | 2 | >128 | - |
| Mecillinam | - | - | >128 | - | >128 | - |
| Meropenem | - | - | 2 | - | 0.06 | - |

| Antimicrobial agent | *Haemophilus influenzae* | | *Enterococcus faecalis* | *Streptococcus pneumoniae* | *Bacteroides fragilis* | *Neisseria gonorrhoeae* |
|---|---|---|---|---|---|---|
| | NCTC 11931 | ATCC 49247 | ATCC 29212 | ATCC 49619 | NCTC 9343 | ATCC 49226 |
| Metronidazole | - | - | - | - | 0.5 | - |
| Moxalactam | - | - | - | - | 0.25 | - |
| Moxifloxacin | 0.03 | 0.03 | 0.25 | 0.5 | - | 0.004 |
| Naladixic acid | - | 1 | - | >128 | 64 | - |
| Nitrofurantoin | - | - | 8 | - | - | - |
| Norfloxacin | - | - | 2 | - | 16 | - |
| Ofloxacin | - | - | 2 | - | 1 | - |
| Oxacillin | - | - | - | 1 | - | - |
| Pefloxacin | - | - | - | - | 1 | - |
| Penicillin | - | 4 | 2 | 0.5 | 16 | - |
| Piperacillin | - | - | 2 | - | 2 | - |
| Rifampicin | - | - | 2 | 0.03 | - | - |
| Roxithromycin | 16 | 16 | - | 0.12 | 2 | - |
| Rufloxacin | - | - | - | - | 16 | - |
| Sparfloxacin | - | 0.002 | - | 0.25 | 1 | - |
| Teicoplanin | - | - | 0.25 | - | - | - |
| Telithromycin | 1 | 2 | 0.008 | 0.008 | - | 0.03 |
| Tetracycline | - | 16 | 16 | 0.12 | 0.5 | - |
| Ticarcillin | - | - | - | - | 4 | - |
| Tigecycline | - | - | 0.12 | 0.06 | - | - |
| Tobramycin | - | - | 16 | - | - | - |
| Trimethoprim | - | - | 0.25 | 4 | 16 | - |
| Trovafloxacin | 0.008 | 0.002 | 0.06 | 0.12 | 0.12 | - |
| Vancomycin | - | - | 2 | 0.25 | 16 | - |

Table 8: Target MICs (mg/L) for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* control strains by BSAC methods

| Antimicrobial agent | *Escherichia coli* | | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | | |
|---|---|---|---|---|---|---|---|
| | NCTC 10418 | ATCC 25922 | NCTC 10662 | ATCC 27853 | NCTC 6571 | ATCC 25923 | ATCC 29213 |
| Amikacin | 0.5 | 1 | 2 | 2 | 1 | - | 2 |
| Amoxicillin | 2 | 4 | >128 | >128 | 0.12 | 0.25 | 0.5 |
| Ampicillin | 2 | 4 | >128 | >128 | 0.06 | - | - |
| Azithromycin | - | - | - | - | 0.12 | 0.12 | 0.12 |
| Azlocillin | 4 | - | 4 | - | 0.25 | - | - |
| Aztreonam | 0.03 | 0.25 | 4 | 2 | >128 | - | >128 |
| Carbenicillin | 2 | - | 32 | - | 0.5 | - | - |
| Cefaclor | 1 | 2 | >128 | >128 | 1 | - | 1 |
| Cefamandole | 0.25 | - | >128 | >128 | 0.25 | - | - |
| Cefixime | 0.06 | 0.25 | 16 | - | 8 | 8 | 16 |
| Cefotaxime | 0.03 | 0.06 | 8 | 8 | 0.5 | - | 1 |
| Cefotetan | 0.06 | - | >128 | >128 | 4 | - | - |
| Cefoxitin | 4 | - | >128 | >128 | 2 | **2** | - |
| Cefpirome | 0.03 | 0.03 | 4 | 1 | 0.25 | - | 0.5 |
| Cefpodoxime | 0.25 | 0.25 | 128 | >128 | 1 | 4 | 2 |
| Ceftazidime | 0.06 | 0.25 | 1 | 1 | 4 | - | 8 |
| Ceftizoxime | 0.008 | - | - | - | 2 | - | - |
| Ceftriaxone | 0.03 | 0.06 | 8 | 8 | 1 | - | 2 |
| Cefuroxime | 2 | 4 | >128 | >128 | 0.5 | 1 | 1 |
| Cephadroxil | 8 | 8 | >128 | >128 | 1 | - | 2 |
| Cephalexin | 4 | 8 | >128 | >128 | 1 | - | 4 |
| Cephaloridine | - | - | >128 | >128 | 0.06 | - | - |
| Cephalothin | 4 | 8 | >128 | >128 | 0.5 | - | 0.25 |
| Cephradine | - | - | >128 | >128 | 2 | - | - |
| Chloramphenicol | 2 | 4 | 128 | - | 2 | - | 2 |
| Ciprofloxacin | 0.015 | 0.015 | 0.25 | 0.25 | 0.12 | 0.5 | 0.5 |
| Clarithromycin | - | - | - | - | 0.12 | 0.12 | 0.12 |
| Clindamycin | - | - | - | - | 0.06 | 0.12 | 0.06 |
| Co-amoxiclav | 2 | 4 | >128 | 128 | 0.12 | 0.12 | 0.25 |
| Colistin | 0.5 | - | 2 | 4 | 128 | - | - |
| Cotrimoxazole | 0.25 | 0.25 | - | - | - | - | 2 |
| Dalfopristin/ Quinupristin | - | - | - | - | 0.12 | 0.25 | 0.25 |
| Daptomycin Mueller Hinton | - | - | - | - | 1 | 2 | - |
| Dirythromycin | - | - | - | - | 1 | - | 1 |
| Doripenem | 0.008 | 0.008 | 0.5 | 0.25 | - | - | - |
| Doxycycline | - | - | - | - | 0.06 | 0.12 | - |
| Enoxacin | 0.25 | - | 1 | - | 0.5 | - | - |
| Ertapenem | 0.008 | 0.015 | - | - | - | - | - |
| Erythromycin | - | - | - | - | 0.12 | 0.5 | 0.25 |
| Farapenem | 0.25 | - | >128 | >128 | 0.12 | - | - |
| Fleroxacin | 0.06 | 0.12 | 1 | - | 0.5 | - | - |
| Flucloxacillin | - | - | >128 | >128 | 0.06 | - | - |
| Flumequine | 2 | - | >128 | >128 | - | - | - |
| Fosfomycin | 4 | - | >128 | >128 | 8 | - | - |
| Fusidic acid | >128 | - | - | - | 0.06 | 0.12 | 0.06 |
| Gatifloxacin | 0.015 | 0.015 | 1 | 1 | 0.03 | 0.12 | 0.12 |
| Gemifloxacin | 0.008 | 0.008 | 0.25 | 0.25 | 0.015 | 0.03 | 0.03 |
| Gentamicin | 0.25 | 0.5 | 1 | 1 | 0.12 | 0.25 | 0.25 |
| Grepafloxacin | 0.03 | 0.03 | 0.5 | - | 0.03 | - | - |
| Imipenem | 0.06 | 0.12 | 2 | 1 | 0.015 | - | 0.015 |

| Antimicrobial agent | *Escherichia coli* | | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | | |
|---|---|---|---|---|---|---|---|
| | NCTC 10418 | ATCC 25922 | NCTC 10662 | ATCC 27853 | NCTC 6571 | ATCC 25923 | ATCC 29213 |
| Kanamycin | 1 | - | 1 | - | 2 | - | - |
| Levofloxacin | 0.03 | 0.03 | 0.5 | 0.5 | 0.12 | 0.25 | 0.25 |
| Linezolid | - | - | - | - | 0.5 | 1 | - |
| Lomefloxacin | - | - | - | - | 0.5 | - | - |
| Loracarbef | 0.5 | 1 | >128 | >128 | 0.5 | - | 1 |
| Mecillinam | 0.12 | 0.12 | 8 | - | 8 | - | 64 |
| Meropenem | 0.015 | 0.008 | 2 | 0.25 | 0.03 | - | 0.06 |
| Methicillin | - | - | >128 | >128 | 1 | 2 | 2 |
| Mezlocillin | 2 | - | 8 | - | 0.5 | - | - |
| Minocycline | - | - | - | - | 0.06 | 0.06 | - |
| Moxalactam | 0.03 | - | 8 | - | 8 | - | - |
| Moxifloxacin | 0.03 | 0.03 | 2 | 2 | 0.06 | 0.06 | 0.06 |
| Mupirocin | - | - | - | - | 0.25 | 0.25 | 0.12 |
| Nalidixic acid | 2 | 4 | >128 | >128 | >128 | 128 | 128 |
| Neomycin | - | - | 32 | - | 0.12 | - | - |
| Netilmicin | - | - | 1 | 0.5 | - | - | - |
| Nitrofurantoin | 4 | 8 | - | - | 8 | - | 16 |
| Norfloxacin | 0.06 | 0.06 | 1 | 1 | 0.25 | - | 1 |
| Ofloxacin | 0.06 | 0.03 | 1 | 1 | 0.25 | - | 0.5 |
| Oxacillin | - | - | >128 | >128 | 0.25 | 0.25 | 0.5 |
| Pefloxacin | 0.06 | - | 0.5 | - | 0.25 | - | - |
| Penicillin | - | - | >128 | >128 | 0.03 | 0.03 | 0.12 |
| Piperacillin | 0.5 | 2 | 4 | 2 | 0.25 | - | 1 |
| Piperacillin/ tazobactam | 0.5 | 2 | 4 | 4 | - | - | - |
| Rifampicin | 16 | - | - | - | 0.004 | 0.015 | 0.004 |
| Roxithromycin | - | - | - | - | 0.25 | 0.5 | 0.5 |
| Rufloxacin | 0.5 | - | 8 | - | 1 | - | - |
| Sparfloxacin | 0.015 | 0.015 | 0.5 | 0.5 | 0.03 | - | - |
| Sulphonamide | 16 | - | >128 | >128 | 64 | - | - |
| Sulphamethoxazole | 0.06 | 0.12 | - | - | - | - | - |
| Teicoplanin | - | - | - | - | 0.25 | 1 | 1 |
| Telithromycin | - | - | - | - | 0.03 | 0.06 | 0.06 |
| Temocillin | 2 | - | >128 | - | 128 | - | - |
| Tetracycline | 1 | 2 | - | 32 | 0.06 | 0.06 | 0.5 |
| Ticarcillin | 1 | - | 16 | - | 0.5 | - | - |
| Ticarcillin/ 4mg/L clavulanate | - | - | 32 | 16 | - | - | - |
| Tigecycline | 0.12 | 0.12 | - | - | 0.12 | - | - |
| Tobramycin | 0.25 | 0.5 | 0.5 | 0.5 | 0.12 | - | 0.5 |
| Trimethoprim | 0.12 | 0.25 | 32 | - | 0.25 | - | 0.5 |
| Trovafloxacin | 0.015 | 0.015 | 0.5 | 0.5 | 0.015 | 0.03 | 0.03 |
| Vancomycin | - | - | - | - | 0.5 | 0.5 | 1 |

Table 9: Target MICs (mg/L) for *Pasteurella multocida* control strain by BSAC methods

| Antimicrobial agents | *Pasteurella multocida* NCTC 8489 |
|---|---|
| Ampicillin | 0.12 |
| Cefotaxime | 0.004 |
| Ciprofloxacin | 0.008 |
| Penicillin | 0.12 |
| Tetracycline | 0.25 |

Table 10: Target MICs (mg/L) for anaerobic control strains by BSAC methods on Iso-Sensitest agar supplemented with 5% defibrinated horse blood and 20 mg/L NAD

| Antimicrobial agent | *Bacteroides fragilis* NCTC 9343 | *Bacteroides thetaiotaomicron* ATCC 29741 | *Clostridium perfringens* NCTC 8359 |
|---|---|---|---|
| Clindamycin | 0.5 | 2 | 0.06 |
| Co-amoxiclav (2:1 ratio) | 0.5 | 0.5 | ≤ 0.06 |
| Meropenem | 0.06 | 0.12 | ≤ 0.015 |
| Metronidazole | 0.5 | 4 | 8 |
| Penicillin | 16 | 16 | 0.06 |
| Piperacillin/tazobactam (fixed 4 mg/L tazobactam) | ≤ 0.12 | 8 | 0.5 |

Table 11: Target MICs (mg/L) for Group A streptococci control strains by BSAC methods

| | Group A streptococci | |
|---|---|---|
| Antimicrobial agent | NCTC 8198 | ATCC 19615 |
| Clindamycin | 0.03 | 0.06 |

**References**


1. Andrews, J.M. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, Suppl S1 to Volume 48 July 2001.

2. Andrews, J. M., Jevons, G., Brenwald, N. and Fraise, A. for the BSAC Working Party on Sensitivity Testing. Susceptibility testing *Pasteurella multocida* by BSAC standardized methodology. *Journal of Antimicrobial Chemotherapy.*

## Useful web sites

| | | |
|---|---|---|
| BSAC | British Society for Antimicrobial Chemotherapy | http://www.bsac.org.uk |
| CDC | Centre for Disease Control (Atlanta, USA) | http://www.cdc.gov |
| WHO | World Health Organisation (Geneva, Switzerland) | http://www.who.int |
| CLSI | Clinical and Laboratory Standards Institute | http://www.clsi.org |
| NEQAS | National External Quality Assessment Scheme | http://www.ukneqas.org.uk |
| NCTC | National Collection of Type Cultures | http://www.ukncc.co.uk |
| JAC | The Journal of Antimicrobial Chemotherapy | http://www.jac.oupjournals.org |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing | http://www.eucast.org |