

# BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing

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## Contents

Page

### Standardized method for disc susceptibility testing:

1.	Preparation of plates	4
2.	Selection of control organisms	5
3.	Preparation of inoculum	5
4.	Inoculation of agar plates	8
5.	Antimicrobial discs	8
6.	Incubation	9
7.	Measuring zones	9
8.	Methicillin/oxacillin testing of staphylococci	10

### MIC and zone breakpoints Tables for:

1.	Enterobacteriaceae and Acinetobacter	13
2.	Pseudomonas	16
3.	Staphylococci	17
4.	<i>S. pneumoniae</i>	19
5.	Enterococci.	21
6.	Haemolytic Streptococci.	22
7.	<i>M. catarrhalis</i> .	23
8.	<i>N. gonorrhoeae</i> .	24
9.	<i>N. meningitidis</i> .	25
10.	<i>H. influenzae</i> .	26
11.	Urinary Tract Infections (Gram-negative rods).	27
12.	Urinary Tract Infections (Gram-positive cocci).	29

**Control of susceptibility testing**

1. Maintenance of control strains.	31
2. Calculation of control ranges for disc diffusion.	31
3. Frequency of testing.	31
4. Methods of using control data to monitor the performance of susceptibility testing.	32
5. Investigation of possible sources of error.	32
6. Reporting.	33
7. Acceptable zone diameter ranges for control strains.	34
8. Control of MIC determinations.	38

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## 1. Preparation of plates

- 1.1 Prepare Iso-Sensitest agar (ISA, CM471, Oxoid, Basingstoke, UK), or media shown to have the same performance as ISA, according to the manufacturer's instructions. Media for fastidious organisms are supplemented with 5% defibrinated horse blood or 5% defibrinated horse blood and 20 mg/L β-nicotinamide adenine dinucleotide [NAD] (e.g. Sigma Diagnostics 260-120 or N-3014, Merck BDH 42032 6H) as follows :

Organisms	Medium to be used
Enterobacteriaceae	ISA
<i>Pseudomonas</i> spp.	ISA
Staphylococci <i>other than for the detection of methicillin/oxacillin resistance</i>	ISA
Staphylococci <i>for the detection of methicillin/oxacillin resistance</i>	Mueller-Hinton or Columbia agar (Oxoid CM331 or equivalent) with 2% NaCl <sup>1</sup>
Enterococci	ISA
<i>S. pneumoniae</i>	ISA + 5 % defibrinated horse blood <sup>2</sup>
Haemolytic streptococci	ISA + 5 % defibrinated horse blood <sup>2</sup>
<i>M. catarrhalis</i>	ISA + 5 % defibrinated horse blood <sup>2</sup>
<i>N. meningitidis</i>	ISA + 5 % defibrinated horse blood <sup>2</sup>
<i>N. gonorrhoeae</i>	ISA + 5 % defibrinated horse blood <sup>2</sup>
<i>Haemophilus</i> spp.	ISA + 5 % defibrinated horse blood + 20 mg/L NAD

**NB** <sup>1</sup> see Section 8.

<sup>2</sup>ISA supplemented with 5 % defibrinated horse blood + 20 mg/L NAD may be used for testing.

- 1.2 Pour sufficient molten agar into 90 mm sterile petri dishes to give a depth of 4 mm (25 ml).
- 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 whether a fan-assisted drying cabinet or 'still air' incubator is used and whether (and under what conditions) plates are dried before storage.

### IMPORTANT : DO NOT OVER-DRY PLATES

- 1.4 Ideally, plates should be stored in vented plastic boxes at 8-10°C prior to use. Alternatively, 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, tests should confirm that excess moisture is not produced in a sealed environment or that plates are not over-dried in an unsealed environment.

## 2. Selection of control organisms

- 2.1 The following control strains should be included, as appropriate, with every batch of sensitivity tests.

Organism	Either	Or
<i>E. coli</i>	NCTC 12241 (ATCC 25922)	NCTC 10418
<i>E. coli</i>	NCTC 11560 <sup>1</sup>	
<i>S. aureus</i>	NCTC 12981 (ATCC 25923)	NCTC 6571
<i>S. aureus</i>	NCTC 12493 <sup>2</sup>	
<i>Ps. aeruginosa</i>	NCTC 12934 (ATCC 27853)	NCTC 10662
<i>Ent. faecalis</i>	NCTC 12697 (ATCC 29212)	
<i>H. influenzae</i>	NCTC 12699 (ATCC 49247)	NCTC 11931
<i>S. pneumoniae</i>	NCTC 12977 (ATCC 49619)	
<i>N. gonorrhoeae</i>	NCTC 12700 (ATCC 49226)	

<sup>1</sup>  $\beta$ -lactamase producing strain

<sup>2</sup> Methicillin/oxacillin resistant strain

### NB

- The control strains listed include sensitive strains which have been chosen to monitor test performance, and resistant strains which can also be used to confirm that the method will detect a mechanism of resistance, for example, *H. influenzae* ATCC 49247 is resistant to  $\beta$ -lactam antibiotics.
- Store control strains to minimise the risk of mutations, for example at -20°C (not adequate for fastidious organisms) or preferably at -70°C, in glycerol broth, on beads. Ideally, two vials of each control 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 which will not survive on plates for 7 days subculture the strain daily for no more than 6 days.

## 3. Preparation of inoculum

This standardized method of testing has been developed with an inoculum giving semi-confluent growth of colonies after overnight incubation. Use of an inoculum which 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 decreased inoculum will have the opposite effect. The following methods reliably give semi-confluent growth.

**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 0.5 McFarland Standard

#### 3.1.1 Preparation of 0.5 McFarland Standard

Add 0.5 ml of 0.048M BaCl<sub>2</sub> (1.17% w/v BaCl<sub>2</sub>.2H<sub>2</sub>O) to 99.5 ml of 0.18M H<sub>2</sub>SO<sub>4</sub> (1% w/v) with constant stirring. Distribute the standard into screw cap tubes of the same size and with the same 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. Alternatively, prepared standards can be purchased (e.g. from bioMérieux, Basingstoke, UK).

#### 3.1.2 Inoculum preparation by the growth method (for non-fastidious organisms, e.g. *Enterobacteriaceae*, *Pseudomonas* spp. & staphylococci)

Touch at least four morphologically similar colonies with a sterile loop. Transfer growth into Iso-Sensitest broth or equivalent which has been shown not to interfere with the test. Incubate broth with shaking at 35-37°C until the visible turbidity is equal to or greater than the 0.5 McFarland standard.

#### 3.1.3 Inoculum preparation by the direct colony suspension method (the method of choice for fastidious organisms, e.g. *Haemophilus* spp., *N. gonorrhoeae* and *S. pneumoniae*)

Colonies are taken directly from the plate into Iso-Sensitest broth (or equivalent) or sterile distilled water. The suspension should match or exceed the 0.5 McFarland standard.

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

#### 3.1.4 Adjustment of the organism suspension to the density of the 0.5 McFarland standard.

Adjust the density of the organism suspension prepared as in 3.1.2 or 3.1.3 to equal that of the 0.5 McFarland standard by adding sterile distilled water. To aid comparison, compare the test and standard against a white background with a contrasting black line.

**NB. Suspension should be used within 10 minutes.**

#### 3.1.5 Dilution of suspension equivalent to a 0.5 McFarland standard in distilled water before inoculation.

1:100	1:10	No dilution
Haemolytic streptococci	Staphylococci	<i>N. gonorrhoeae</i>
Enterococci	<i>Serratia</i> spp.	
Enterobacteriaceae	<i>S. pneumoniae</i>	
<i>Pseudomonas</i> spp.	<i>N. meningitidis</i>	
<i>Acinetobacter</i> spp.	<i>M. catarrhalis</i>	
<i>Haemophilus</i> spp.		

**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)<sup>1</sup> and from this the following simplified procedure has been developed.

- 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. Do not leave the organisms standing in water. It is essential to get an even suspension.
- 3.2.2** Zero the spectrophotometer with a sterile water or broth blank (as appropriate) at a wavelength of 500 nm. Measure the absorbance of the bacterial suspension.

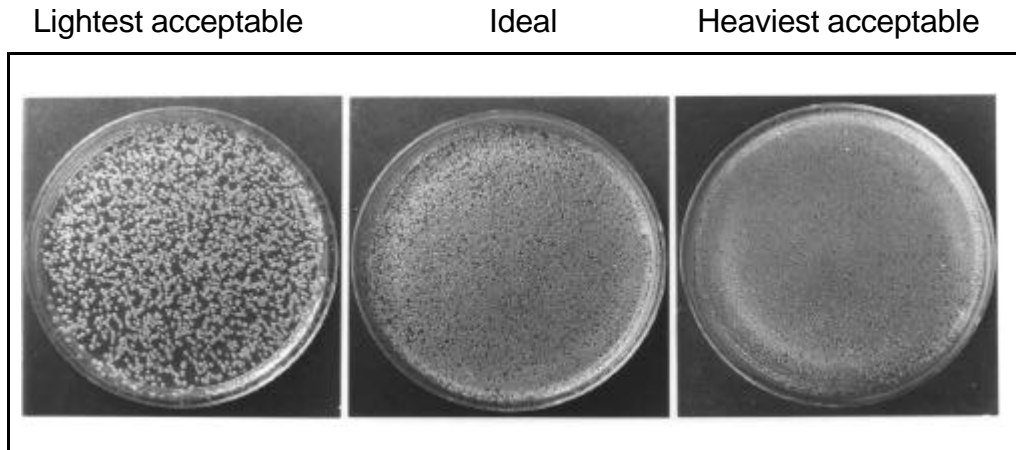
[Spectrophotometer must have a cellholder for 100 x 12 mm test tubes. A much simpler photometer would also probably be acceptable. The 100 x 12 mm test tubes could also be replaced with another tube/cuvette system if required, but the dilutions would need to be recalibrated.]

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

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

Organisms	Absorbance reading at 500 nm	Volume ( $\mu$ L) to transfer to 5 mL sterile distilled water
Enterobacteriaceae	0.01 - 0.05	250
Enterococci	>0.05 - 0.1	125
<i>Pseudomonas</i>	>0.1 - 0.3	40
Staphylococci	>0.3 - 0.6	20
	>0.6 - 1.0	10
<i>Haemophilus</i>	0.01 - 0.05	500
Streptococci	>0.05 - 0.1	250
Miscellaneous fastidious organisms	>0.1 - 0.3	125
	>0.3 - 0.6	80
	>0.6 - 1.0	40

### 3.3 Acceptable ranges for inoculum densities are shown below:



Example of semi-confluent growth and the acceptable range for a Gram-negative rod.

## 4. Inoculation of agar plates

Use the adjusted suspension within 15 min to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess 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 any length of time before the discs are applied, the organism may begin to grow, and this will result in reduced zones of inhibition. Discs should therefore be applied to the surface of the agar within 15 min of inoculation.**

## 5. Antimicrobial discs

### 5.1. *Disc contents*

*These are given in Tables 1-11.*

### 5.2. *Application of discs*

Discs should be firmly applied to the surface of an agar plate which has previously been dried. The contact with the agar should be even. A 90 mm plate will accommodate six discs without unacceptable overlapping of zones.

### 5.3. *Storage and handling of discs*

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

**5.3.1** 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 quinolones).

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



- 5.3.3** Store working supplies of discs at < 8°C.
- 5.3.4** To prevent condensation, allow discs to warm to room temperature before opening containers.
- 5.3.5** Store disc dispensers in sealed containers with an indicating desiccant.
- 5.3.6** Discard any discs on the expiry data shown on the side of the container.

## 6. Incubation

- 6.1** If plates are left at room temperature, after discs have been applied, larger zones of inhibition may be obtained compared with zones produced when plates are incubated immediately. Plates therefore should be incubated within 15 min of disc application.
- 6.2** *Conditions of incubation*

<b>Organisms</b>	<b>Incubation conditions</b>
Enterobacteriaceae	35-37°C in air for 18-20h
<i>Pseudomonas</i> spp.	"
Staphylococci <sup>1</sup>	"
<i>M. catarrhalis</i>	"
Haemolytic streptococci	"
Enterococci	35-37°C in air for 24h <sup>2</sup>
<i>N. meningitidis</i>	35-37°C in 4-6% CO <sub>2</sub> for 18-20h
<i>S. pneumoniae</i>	"
<i>Haemophilus</i> spp.	"
<i>N. gonorrhoeae</i>	"

<sup>1</sup> Incubation conditions for the detection of methicillin/oxacillin resistance given in Section 8.

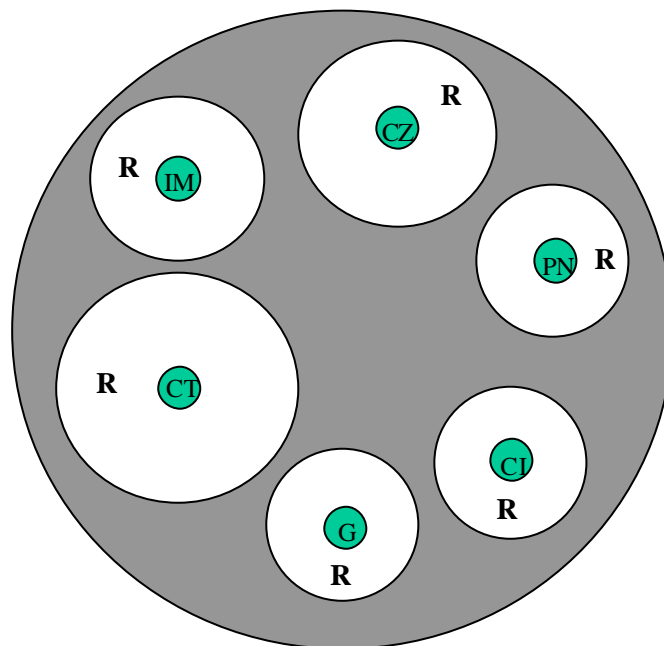
<sup>2</sup> It is essential that plates are incubated for at least 24h before reporting a strain as sensitive to vancomycin or teicoplanin.

**NB.** Avoid stacking plates too high in the incubator as this may affect results due to uneven heating of plates. The efficiency of incubators varies and therefore the control of incubation, including height of plate stacking, should be part of the laboratory's Quality Assurance programme.

## 7. Measuring zones

- 7.1** Measure (mm) diameters of zones of inhibition (edge should be taken as the point of inhibition as judged by the naked eye) of the control strain and test with a ruler, calipers or an automated zone reader (a template may also be used for interpreting susceptibility. A programme for constructing templates is given on the BSAC web site [bsac.org.uk](http://bsac.org.uk)). Tiny colonies at the edge, films of growth due to swarming of *Proteus* spp. and slight growth within sulphonamide or trimethoprim zones should be ignored. Colonies growing within the zone of inhibition should be subcultured and identified and the test repeated if necessary.
- 7.2** Confirm that the zone of inhibition for the control strain falls within the acceptable ranges in before interpreting the test (see section on control of the standardized method).
- 7.3** When the template is used for interpreting susceptibility (example below) the test plate is placed over the template and the zones of inhibition examined in relation to the template zones. If the zone of inhibition of the test strain is within the area marked with an **R** it is regarded as resistant. If the zone of inhibition is equal to or larger than the marked area it is considered susceptible.

### Template for interpreting susceptibility



## 8. Methicillin/oxacillin testing of staphylococci

### 8.1. Introduction

Methicillin susceptibility testing can be difficult. 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 number of resistant strains that can be detected. 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 *S. aureus*. Detection of methicillin resistance in coagulase-negative staphylococci may require incubation for 48h.

## Method of detecting methicillin/oxacillin resistance in *S. aureus* and coagulase-negative staphylococci

### 8.1.1. Medium

Prepare Columbia (Oxoid) or Mueller-Hinton agar (Oxoid) 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 in a 90 mm sterile petri dish (25 ml). Dry and store plates as detailed in Sections 1.3 and 1.4.

### 8.1.2 Inoculum

Prepare inoculum as detailed in Section 3.

### 8.1.3 Control

Susceptible controls (ATCC 25923 or NCTC 6571) test disc content. NCTC 12493 is a resistant strain used to check that the test will detect resistant strains (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 methicillin 5µg or an oxacillin 1µg disc on to the surface of inoculated agar. Discs should be stored and handled as detailed in Section 5.3.

### 8.1.5 Incubation

Incubate for 24 h at 30°C.

### 8.1.6 Reading and Interpretation

Measure zone diameters (mm) as detailed in section 7. Examine zones carefully in good light to detect colonies, which may be minute, in zones. Colonies growing within zones should be identified and re-tested for resistance to methicillin/oxacillin.

For both methicillin and oxacillin interpretation is as follows:  
susceptible =  $\geq 15$  mm diameter, resistant =  $\leq 14$ mm diameter

**8.1.7 NB.** Some hyper-producers of  $\beta$ -lactamase give zones within the range of 6-14 mm and, if possible, should be checked by PCR for *mecA* or by latex agglutination tests for PBP2a. Increase in methicillin/oxacillin zone size in the presence of clavulanic acid is not a reliable test for hyper-producers of  $\beta$ -lactamase as zones of inhibition with some MRSA also increase in the presence of clavulanic acid. Rarely, hyper-producers of  $\beta$ -lactamase give no zone in this test and would therefore not be distinguished from MRSA.

## 9. MIC and zone diameter breakpoints

All breakpoints are subject to review in the light of additional data and the Working Party would welcome any data relating to breakpoints and control zone ranges. *Ad hoc* modifications to breakpoints are not acceptable. The Working Party has set up a mechanism to modify and publish changes to breakpoints in a timely fashion, *via* the BSAC www site ( [www.bsac.org.uk](http://www.bsac.org.uk)) and any changes will be dated.

## 10. Acknowledgment

The BSAC would like to acknowledge the assistance of The Swedish Reference Group for Antibiotics (SRGA) in supplying some data for inclusion in these Tables.

## 11. 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. *Journal of Antimicrobial Chemotherapy* 21, 439-43.

Table 1. MIC and zone breakpoints for Enterobacteriaceae and *Acinetobacter*<sup>1,2</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg)	R <sup>£</sup>	I	S <sup>3</sup>
Ampicillin <sup>3</sup>	16	-	8	10	17	-	18
Cefuroxime (axetil)	2	-	1	30	24	-	25
Cefuroxime (parenteral)	<b>16</b>	-	<b>8</b>	30	<b>19</b>	-	<b>20</b>
Ceftazidime	4	-	2	30	27	-	28
Ceftazidime <sup>4</sup> <i>E. coli</i> & <i>Klebsiella</i> spp.	4	-	2	30	21	-	22
Ciprofloxacin <sup>5</sup>	2	-	1	1	17	-	18
Gentamicin	2	-	1	10	19	-	20
Amikacin	8	-	4	30	<b>19</b>	-	<b>20</b>
Aztreonam	16	-	8	30	<b>23</b>	-	<b>24</b>
Cefaclor	<b>2</b>	-	<b>1</b>	30	<b>34</b>	-	<b>35</b>
Cefamandole <sup>6</sup>	<b>16</b>	-	<b>8</b>	30	<b>19</b>	-	<b>20</b>
Cefepime	<b>2</b>	-	<b>1</b>	30	<b>31</b>	-	<b>32</b>
Cefixime	2	-	1	5	<b>19</b>	-	<b>20</b>
Cefoperazone <sup>6</sup>	<b>8</b>	-	<b>4</b>	30	<b>24</b>	-	<b>25</b>
Cefotaxime	2	-	1	30	<b>29</b>	-	<b>30</b>
Cefotetan <sup>6</sup>	8	-	4	30	<b>23</b>	-	<b>24</b>
Cefoxitin	8	-	4	30	<b>19</b>	-	<b>20</b>
Cefpirome	2	-	1	20	<b>24</b>	-	<b>25</b>
Cefpodoxime	2	-	1	5	<b>33</b>	-	<b>34</b>
Ceftibuten	2	-	1	10	<b>27</b>	-	<b>28</b>
Ceftizoxime	2	-	1	30	<b>29</b>	-	<b>30</b>
Ceftriaxone	2	-	1	30	<b>27</b>	-	<b>28</b>
Cephalothin	<b>2</b>	-	<b>1</b>	30	<b>26</b>	-	<b>27</b>
Cephradine	4	-	2	30	<b>11</b>	-	<b>12</b>

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg)	Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>		R <sup>£</sup>	I	S <sup>3</sup>
Chloramphenicol	16	-	8	30	<b>20</b>	-	<b>21</b>
Co-amoxycylav	16	-	8	20/10	<b>17</b>	-	<b>18</b>
Colistin <sup>7</sup>	8	-	4	25	<b>14</b>	-	<b>15</b>
Co-trimoxazole	<b>64</b>	-	<b>32</b>	25	<b>15</b>	-	<b>16</b>
Doxycycline	<b>2</b>	-	<b>1</b>	30	<b>28</b>	-	<b>29</b>
Gatifloxacin	<b>2</b>	-	<b>1</b>	2	<b>19</b>	-	<b>20</b>
Gemifloxacin	<b>0.5</b>	-	<b>0.25</b>	1	<b>19</b>	-	<b>20</b>
Imipenem	8	-	4	10	<b>22</b>	-	<b>23</b>
Levofloxacin	<b>4</b>	-	<b>2</b>	1	<b>19</b>	-	<b>20</b>
Meropenem	8	-	4	10	<b>22</b>	-	<b>23</b>
Mezlocillin	32	-	16	75	<b>21</b>	-	<b>22</b>
Moxifloxacin	<b>2</b>	-	<b>1</b>	1	<b>19</b>	-	<b>20</b>
Netilmicin	2	-	1	10	<b>24</b>	-	<b>25</b>
Ofloxacin	4	-	2	1	<b>23</b>	-	<b>24</b>
Piperacillin	32	-	16	85	<b>23</b>	-	<b>24</b>
/Tazobactam							
Piperacillin	32	-	16	75	<b>23</b>	-	<b>24</b>
Streptomycin <sup>6</sup>	<b>16</b>	-	<b>8</b>	10	<b>12</b>	-	<b>13</b>
Sulphamethoxazole	64	-	32	100	<b>13</b>	-	<b>14</b>
Tetracycline	2	-	1	30	<b>33</b>	-	<b>34</b>
Timentin	32	-	16	85	<b>20</b>	-	<b>21</b>
Tobramycin	2	-	1	10	<b>17</b>	-	<b>18</b>
Trimethoprim	4	1-2	0.5	2.5	<b>14</b>	<b>15-19</b>	<b>20</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.

2. Problems with testing *Acinetobacter* and *Serratia* spp. have been related to obtaining the correct inoculum. Once a clinically significant strain of *Acinetobacter* sp. or *Serratia* sp. has been identified, it might be prudent to determine the susceptibility by an MIC method, or the disc testing must be reported if the inoculum density is outside the acceptable range.
3. Amoxicillin - report as for ampicillin
4. Strains of *E. coli* and *Klebsiella* spp. have been identified with MICs of ceftazidime of 1 mg/L. These MICs are higher than the 'wild sensitive' population (c. 0.12 mg/L). These strains do not possess extended spectrum  $\beta$ -lactamases and until a mechanism of resistance has been identified this tentative zone diameter breakpoint is recommended.
5. Strains of *E. coli* and *Klebsiella* spp. with ciprofloxacin MICs of 0.25 and 0.5 mg/L may be reported as resistant. These MICs are higher than the 'wild sensitive' population for these species and may indicate a mechanism of resistance with clinical significance.
6. Zone breakpoints only valid for *E. coli*, *Klebsiella* spp. and *Pr. mirabilis*.
7. Colistin - some strains of Enterobacteriaceae (particularly *Serratia*, *Providencia*, *Citrobacter* and *Enterobacter* spp.) have been shown to produce clear zones of inhibition with small colonies around the disc. These strains should be regarded as resistant as the MICs typically exceed 128 mg/L.



Table 2. MIC and zone breakpoints for *Pseudomonas* <sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg)	R <sup>£</sup>	I	S <sup>3</sup>
Gentamicin	8	2-4	1	10	14	15-21	22
Amikacin	32	8-16	4	30	17	18-21	22
Ceftazidime	16	-	8	30	23	-	24
Imipenem	8	-	4	10	21	-	22
Meropenem	8	-	4	10	<b>21</b>	-	<b>22</b>
Piperacillin	32	-	16	75	23	-	24
Piperacillin /tazobactam	32	-	16	75/10	23	-	24
Ciprofloxacin	8	2-4	1	1	9	-	10
Ciprofloxacin	8	2-4	1	5	<b>15</b>	<b>16-19</b>	<b>20</b>
Aztreonam	16	-	8	30	<b>22</b>	-	<b>23</b>
Carbenicillin	256	-	128	100	<b>12</b>	-	<b>13</b>
Cefotaxime	2	-	1	30	<b>26</b>	-	<b>27</b>
Cefpirome	2	-	1	20	<b>19</b>	<b>20-24</b>	<b>25</b>
Ceftriaxone	2	-	1	30	<b>29</b>	-	<b>30</b>
Colistin	8	-	4	25	<b>13</b>	-	<b>14</b>
Gatifloxacin	<b>2</b>	-	1	2	<b>19</b>	-	<b>20</b>
Gemifloxacin	<b>0.5</b>	-	<b>0.25</b>	5	<b>19</b>	-	<b>20</b>
Levofloxacin	<b>4</b>	-	<b>2</b>	5	<b>17</b>	-	<b>18</b>
Moxifloxacin	<b>8</b>	<b>2-4</b>	<b>1</b>	5	<b>17</b>	<b>18-24</b>	<b>25</b>
Netilmicin	8	2-4	1	30	<b>15</b>	<b>16-18</b>	<b>19</b>
Ofloxacin	16	4-8	2	5	<b>12</b>	<b>13-19</b>	<b>20</b>
Timentin	128	32-64	16	85	<b>19</b>	-	<b>20</b>
Tobramycin	8	2-4	1	10	<b>16</b>	<b>17-19</b>	<b>20</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.

Table 3. MIC and zone breakpoints for staphylococci <sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg unless stated)	R <sup>£</sup>	I	S <sup>3</sup>
Gentamicin	2	-	1	10	19	-	20
Penicillin <sup>2</sup>	0.25	-	0.12	1 unit	24	-	25
Methicillin <sup>3,4</sup>	8	-	4	5	<b>14</b>	-	<b>15</b>
Oxacillin <sup>3,4,5</sup>	<b>4</b>	-	<b>2</b>	1	<b>14</b>	-	<b>15</b>
Vancomycin <sup>6</sup>	8	-	4	5	11	-	12
Teicoplanin <sup>6,7</sup>	8	-	4	30	14	-	15
Rifampicin	0.12	-	0.06	2	29	-	30
Erythromycin	1	-	0.5	5	19	-	20
Quinupristin/ Dalfopristin <sup>8</sup>	<b>4</b>	-	<b>2</b>	15	<b>19</b>	-	<b>20</b>
Amikacin coagulase- negative staphylococci	32	8-16	4	30	<b>21</b>	<b>22-24</b>	<b>25</b>
Amikacin <i>S. aureus</i>	32	8-16	4	30	<b>18</b>	-	<b>19</b>
Azithromycin	2	-	1	15	<b>19</b>	-	<b>20</b>
Chloramphenicol	16	-	8	10	<b>19</b>	-	<b>20</b>
Ciprofloxacin	2	-	1	1	<b>17</b>	-	<b>18</b>
Clarithromycin	1	-	0.5	2	<b>19</b>	-	<b>20</b>
Clindamycin	1	-	0.5	2	<b>25</b>	-	<b>26</b>
Co-amoxyclav <sup>3</sup>	2	-	1	3	<b>17</b>	-	<b>18</b>
Co-trimoxazole <sup>9</sup>	<b>64</b>	-	<b>32</b>	25	<b>16</b>	-	<b>17</b>
Fusidic acid	2	-	1	10	<b>29</b>	-	<b>30</b>
Gatifloxacin	<b>2</b>	-	<b>1</b>	2	<b>19</b>	-	<b>20</b>
Gemifloxacin	<b>0.5</b>	-	<b>0.25</b>	1	<b>19</b>	-	<b>20</b>
Linezolid <sup>10</sup>	<b>8</b>	-	<b>4</b>	10	<b>19</b>	-	<b>20</b>
Moxifloxacin	<b>2</b>	-	<b>1</b>	1	<b>19</b>	-	<b>20</b>
Mupirocin <sup>11</sup>	<b>8</b>	-	<b>4</b>	5	<b>21</b>	-	<b>22</b>
Neomycin				10	<b>16</b>	-	<b>17</b>

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg unless stated)	R <sup>£</sup>	I	S <sup>3</sup>
Netilmicin for <i>S. aureus</i>	2	-	1	10	<b>23</b>	-	<b>24</b>
Netilmicin for coagulase-negative staphylococci	2	-	1	10	<b>29</b>	-	<b>30</b>
Ofloxacin	4	-	2	1	<b>23</b>	-	<b>24</b>
Tetracycline	2	-	1	10	<b>19</b>	-	<b>20</b>
Tobramycin for <i>S. aureus</i>	2	-	1	10	<b>20</b>	-	<b>21</b>
Tobramycin for coagulase-negative staphylococci	2	-	1	10	<b>29</b>	-	<b>30</b>
Trimethoprim	1	-	0.5	5	<b>27</b>	-	<b>28</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Penicillin ; check for heaped zone edge (= resistant)
3. Staphylococci exhibiting resistance to methicillin/oxacillin should be regarded as resistant to other penicillins, cephalosporins, carbapenems and combinations of  $\beta$ -lactam and  $\beta$ -lactamase inhibitors
4. Recommendations for Mueller-Hinton or Columbia agar with 2% NaCl.
5. MIC breakpoint for coagulase-negative staphylococci is currently under review.
6. Glycopeptide intermediate *S. aureus* (GISA) cannot be detected by this method or any other method of disc testing. Population analysis is the most reliable method for detecting heterogeneous resistance to vancomycin. If, on clinical grounds, resistance to vancomycin is suspected, it is recommended that the organism be sent to a specialist laboratory, such as Southmead Hospital in Bristol or the Antibiotic Resistance Monitoring and Reference Laboratory at Colindale for further investigation.
7. Teicoplanin - disc testing not recommended for coagulase-negative staphylococci. An MIC method of testing should be used to determine susceptibility.
8. The presence of blood has a marked effect on the activity of quinupristin/ dalfopristin. On the rare occasions when blood needs to be added to enhance the growth of staphylococci, susceptible =  $\geq 15$  mm.
9. Based on sulphamethoxazole MIC.
10. Information on clinical response in patients with serious staphylococcal infections is not yet available. In such patients an MIC determination might be appropriate.

11. Mupirocin - an Etest or other MIC method should be performed on any strain designated resistant by the disc method. This will identify whether the strain has low-level resistance (MIC 8 - 256 mg/L) or high-level resistance (MIC,  $\geq 512$  mg/L). The latter is clinically significant, the former is not.

Table 4. MIC and zone breakpoints for *S. pneumoniae*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg)	R <sup>£</sup>	I	S <sup>3</sup>
Penicillin <sup>2,3,4</sup>	2	0.12-1	0.06	Oxacillin 1	19	-	20
Erythromycin	1	-	0.5	5	19	-	20
Tetracycline	2	-	1	10	19	-	20
Chloramphenicol	16	-	8	10	17	-	18
Ciprofloxacin <sup>5</sup>	<b>4</b>	<b>£2</b>	-	1	9	≥10	-
Azithromycin	2	-	1	15	<b>19</b>	-	<b>20</b>
Cefaclor <sup>3</sup>	<b>2</b>	-	<b>1</b>	30	<b>24</b>	-	<b>25</b>
Cefixime <sup>3</sup>	2	-	1	5	<b>19</b>	-	<b>20</b>
Cefotaxime <sup>3</sup>	2	-	1	5	<b>29</b>	-	<b>30</b>
Cefpodoxime <sup>3</sup>	2	-	1	1	<b>21</b>	-	<b>22</b>
Ceftibuten <sup>3</sup>	2	-	1	10	<b>27</b>	-	<b>28</b>
Ceftizoxime <sup>3</sup>	2	-	1	30	<b>29</b>	-	<b>30</b>
Ceftriaxone <sup>3</sup>	<b>2</b>	-	<b>1</b>	30	<b>27</b>	-	<b>28</b>
Cefuroxime <sup>3</sup>	2	-	1	5	<b>24</b>	-	<b>25</b>
Cephadroxil <sup>3</sup>	<b>2</b>	-	<b>1</b>	30	<b>24</b>	-	<b>25</b>
Cephalexin <sup>3</sup>	4	-	2	30	<b>24</b>	-	<b>25</b>
Clarithromycin	1	-	0.5	2	<b>19</b>	-	<b>20</b>
Gatifloxacin	<b>2</b>	-	<b>1</b>	2	<b>19</b>	-	<b>20</b>
Gemifloxacin	<b>0.5</b>	-	<b>0.25</b>	1	<b>19</b>	-	<b>20</b>
Imipenem <sup>3</sup>	8	-	4	10	<b>24</b>	-	<b>25</b>
Levofloxacin	<b>4</b>	-	<b>2</b>	1	<b>19</b>	-	<b>20</b>
Linezolid	<b>8</b>	-	<b>4</b>	10	<b>19</b>	-	<b>20</b>
Meropenem <sup>3</sup>	8	-	4	10	<b>27</b>	-	<b>28</b>
Moxifloxacin	<b>2</b>	-	<b>1</b>	1	<b>17</b>	-	<b>18</b>
Ofloxacin	<b>4</b>	-	<b>2</b>	1	<b>19</b>	-	<b>20</b>
Quinupristin /dalfopristin	<b>4</b>	-	<b>2</b>	15	<b>19</b>	-	<b>20</b>
Rifampicin	2	-	1	5	<b>21</b>	-	<b>22</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Organisms with reduced susceptibility to penicillin: confirm resistance with a test for penicillin MIC. Organisms with an MIC  $\leq 1$  mg/L are considered susceptible to  $\beta$ -lactam antibiotics except in infections of the central nervous system. In addition, an MIC of cefotaxime for strains isolated from meningitis or other invasive infections is advised.
3. Penicillin resistance in *S. pneumoniae* is detected with an oxacillin 1  $\mu$ g disc.
4. The MIC BPs for penicillin have been changed to reflect international standards.
5. Isolates with MICs of ciprofloxacin  $\leq 2$  mg/L are considered as having intermediate susceptibility.

Table 5. MIC and zone breakpoints for enterococci <sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg)	Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>		R <sup>£</sup>	I	S <sup>3</sup>
Gentamicin	1024	-	512	200	9	-	10
Ampicillin	16	-	8	10	19	-	20
Vancomycin <sup>2</sup>	8	-	4	5	12	-	13
Teicoplanin <sup>2</sup>	8	-	4	30	19	-	20
Quinupristin/ dalfopristin <sup>3</sup>	<b>4</b>	-	<b>2</b>	15	<b>19</b>	-	<b>20</b>
Azithromycin	2	-	1	15	<b>29</b>	-	<b>30</b>
Imipenem	8	-	4	10	<b>19</b>	-	<b>20</b>
Meropenem	8	-	4	10	<b>19</b>	-	<b>20</b>
Linezolid	<b>8</b>	-	<b>4</b>	10	<b>19</b>	-	<b>20</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. It is essential that plates are incubated for at least 24 h before reporting a strain as sensitive to vancomycin or teicoplanin.
3. Although not fully explained, the presence of blood has a marked effect on the activity of quinupristin/dalfopristin. On the rare occasions when blood needs to be added to enhance the growth of enterococci, susceptible =  $\geq 15$  mm.

Table 6. MIC and zone breakpoints for haemolytic streptococci <sup>1</sup> .

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg unless stated)	R <sup>£</sup>	I	S <sup>3</sup>
Penicillin	0.25	-	0.12	1 unit	19	-	20
Tetracycline	2	-	1	10	19	-	20
Erythromycin	1	-	0.5	5	19	-	20
Clarithromycin <sup>2</sup>	1	-	0.5	<b>2</b>	<b>19</b>	-	<b>20</b>
Azithromycin	2	-	1	15	<b>19</b>	-	<b>20</b>
Cefadroxil	<b>2</b>	-	<b>1</b>	30	<b>24</b>	-	<b>25</b>
Cefixime	2	-	1	5	<b>19</b>	-	<b>20</b>
Cefotaxime	2	-	1	5	<b>27</b>	-	<b>28</b>
Cephalexin	4	-	2	30	<b>24</b>	-	<b>25</b>
Cephalothin	<b>2</b>	-	<b>1</b>	30	<b>28</b>	-	<b>29</b>
Co-trimoxazole <sup>3</sup>	<b>64</b>	-	<b>32</b>	25	<b>16</b>	-	<b>17</b>
Linezolid	<b>8</b>	-	<b>4</b>	10	<b>19</b>	-	<b>20</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Active metabolite not taken into consideration.
3. Based on sulphamethoxazole MIC.



Table 7. MIC and zone breakpoints for *M. catarrhalis*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg)	R <sup>£</sup>	I	S <sup>3</sup>
Ampicillin <sup>2</sup>	2	-	1	2	<b>29</b>	-	<b>30</b>
Cefaclor	<b>2</b>	-	<b>1</b>	30	<b>22</b>	-	<b>23</b>
Cefuroxime	2	-	1	5	<b>19</b>	-	<b>20</b>
Chloramphenicol	4	-	2	10	<b>22</b>	-	<b>23</b>
Ciprofloxacin	2	-	1	1	<b>17</b>	-	<b>18</b>
Clarithromycin <sup>3</sup>	1	-	0.5	2	<b>19</b>	-	<b>20</b>
Co-amoxyclav	2	-	1	2/1	<b>18</b>	-	<b>19</b>
Erythromycin	1	-	0.5	5	<b>27</b>	-	<b>28</b>
Gatifloxacin	<b>2</b>	-	<b>1</b>	2	<b>19</b>	-	<b>20</b>
Gemifloxacin	<b>0.5</b>	-	<b>0.25</b>	1	<b>19</b>	-	<b>20</b>
Levofloxacin	<b>4</b>	-	<b>2</b>	1	<b>19</b>	-	<b>20</b>
Moxifloxacin	<b>2</b>	-	<b>1</b>	1	<b>17</b>	-	<b>18</b>
Tetracycline	2	-	1	10	<b>21</b>	-	<b>22</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Test for  $\beta$ -lactamase. It must be remembered that  $\beta$ -lactamase positive isolates of *M. catarrhalis* are often slow to become positive and tests for  $\beta$ -lactamase production must be examined after the longest recommended time for the test before being interpreted as negative.
3. Active metabolite not taken into consideration.

Table 8. MIC and zone breakpoints for *N. gonorrhoeae*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg unless stated)	R <sup>£</sup>	I	S <sup>3</sup>
Spectinomycin	<b>128</b>	-	<b>64</b>	25	<b>13</b>	-	<b>14</b>
Nalidixic acid <sup>2</sup>	-	-	-	30	-	-	-
Penicillin <sup>3,4</sup>	2	0.12-1	0.06	1 unit	<b>17</b>	<b>18-25</b>	<b>26</b>
Cefuroxime	2	-	1	5	<b>19</b>	-	<b>20</b>
Tetracycline	2	-	1	10	<b>19</b>	-	<b>20</b>
Rifampicin	2	-	1	2	<b>20</b>	-	<b>21</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Quinolone resistance is most reliably detected with nalidixic acid. Strains with reduced susceptibility to fluoroquinolones have no zone of inhibition with nalidixic acid.
3. Test for  $\beta$ -lactamase.
4. Confirm resistance by MIC if  $\beta$ -lactamase negative.

Table 9. MIC and zone breakpoints for *N. meningitidis*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>	Disc content (µg unless stated)	R <sup>£</sup>	I	S <sup>3</sup>
Penicillin	0.12	-	0.06	1 unit	<b>24</b>	-	<b>25</b>
Cefotaxime	2	-	1	5	<b>29</b>	-	<b>30</b>
Chloramphenicol	4	-	2	10	<b>19</b>	-	<b>20</b>
Rifampicin	2	-	1	2	<b>29</b>	-	<b>30</b>
Erythromycin	1	-	0.5	5	<b>26</b>	-	<b>27</b>
Tetracycline	2	-	1	10	<b>21</b>	-	<b>22</b>
Ciprofloxacin	2	-	1	1	<b>31</b>	-	<b>32</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.

Table 10. MIC and zone breakpoints for *H. influenzae*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg unless stated)	Interpretation of zone diameters (mm)		
	R <sup>3</sup>	I	S <sup>£</sup>		R <sup>£</sup>	I	S <sup>3</sup>
Ampicillin <sup>2</sup>	2	-	1	2	19	-	20
Cefuroxime	2	-	1	5	19	-	20
Cefotaxime	2	-	1	5	24	-	25
Tetracycline	2	-	1	10	21	-	22
Chloramphenicol	4	-	2	10	24	-	25
Azithromycin <sup>3</sup>	8	0.5-4	0.25	15	<b>19</b>	<b>20-34</b>	<b>35</b>
Cefaclor	<b>2</b>	-	<b>1</b>	30	<b>36</b>	-	<b>37</b>
Ceftazidime	4	-	2	30	<b>29</b>	-	<b>30</b>
Ceftriaxone	<b>2</b>	-	<b>1</b>	30	<b>34</b>	-	<b>35</b>
Ciprofloxacin <sup>4</sup>	2	-	1	1	<b>27</b>	-	<b>28</b>
Clarithromycin <sup>5</sup>	32	1-16	0.5	5	<b>9</b>	<b>10-24</b>	<b>25</b>
Co-amoxyclav	2	-	1	2/1	<b>19</b>	-	<b>20</b>
Co-trimoxazole	64	-	32	25	<b>21</b>	-	<b>22</b>
Erythromycin	16	1-8	0.5	5	<b>14</b>	<b>15-27</b>	<b>28</b>
Gatifloxacin <sup>4</sup>	<b>2</b>	-	<b>1</b>	2	<b>19</b>	-	<b>20</b>
Gemifloxacin <sup>4</sup>	<b>0.5</b>	-	<b>0.25</b>	1	<b>19</b>	-	<b>20</b>
Imipenem	8	-	4	10	<b>19</b>	-	<b>20</b>
Levofloxacin <sup>4</sup>	<b>4</b>	-	<b>2</b>	1	<b>19</b>	-	<b>20</b>
Meropenem	8	-	4	10	<b>27</b>	-	<b>28</b>
Nalidixic acid <sup>4</sup>	-	-	-	30	-	-	-
Trimethoprim	1	-	0.5	2.5	<b>20</b>	-	<b>21</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. Test for  $\beta$ -lactamase.
3. No resistant strains.

4. Quinolone resistance is most reliably detected with nalidixic acid. Strains with reduced susceptibility to fluoroquinolones give no zone of inhibition with a 30 µg nalidixic acid disc.
5. Active metabolite not taken into consideration.

Table 11. MIC and zone breakpoints for urinary tract infections (Gram-negative rods) <sup>1-5</sup>.

Antibiotic	MIC BP (mg/L)			Disc content (µg)	Interpretation of zone diameters (mm)								
					Coliforms			<i>E. coli</i>			<i>Pr. mirabilis</i>		
	R <sup>3</sup>	I	S <sub>£</sub>		R <sub>£</sub>	I	S <sup>3</sup>	R <sub>£</sub>	I	S <sup>3</sup>	R <sub>£</sub>	I	S <sup>3</sup>
Ampicillin	<b>64</b>	-	<b>32</b>	25				<b>15</b>	-	<b>16</b>	<b>24</b>	-	<b>25</b>
Cephalexin <sup>8</sup>	<b>64</b>	-	<b>32</b>	30				<b>15</b>	-	<b>16</b>	<b>11</b>	-	<b>12</b>
Ciprofloxacin	<b>8</b>	-	<b>4</b>	1	<b>19</b>	-	<b>20</b>	<b>19</b>	-	<b>20</b>	<b>19</b>	-	<b>20</b>
Co-amoxyclov	<b>64</b>	-	<b>32</b>	20/10				<b>17</b>	-	<b>18</b>	<b>17</b>	-	<b>18</b>
Fosfomycin <sup>6,7</sup>	<b>256</b>	-	<b>128</b>	200/50				<b>19</b>	-	<b>20</b>	<b>33</b>	-	<b>34</b>
Mecillinam	<b>16</b>	<b>2-8</b>	<b>1</b>	10				<b>13</b>	<b>14-23</b>	<b>24</b>	<b>13</b>	<b>14-18</b>	<b>19</b>
Nalidixic acid	<b>32</b>	-	<b>16</b>	30	<b>17</b>	-	<b>18</b>	<b>17</b>	-	<b>18</b>	<b>17</b>	-	<b>18</b>
Nitrofurantoin	<b>64</b>	-	<b>32</b>	200				<b>19</b>	-	<b>20</b>			
Norfloxacin	<b>8</b>	-	<b>4</b>	2	<b>15</b>	-	<b>16</b>	<b>15</b>	-	<b>16</b>	<b>15</b>	-	<b>16</b>
Trimethoprim	<b>4</b>	-	<b>2</b>	2.5	<b>16</b>	-	<b>17</b>	<b>16</b>	-	<b>17</b>	<b>16</b>	-	<b>17</b>

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. If an organism is isolated from multiple sites, for example from blood and urine, interpretation of susceptibility should be made with regard to the systemic site, that is, if the blood isolate is resistant and the urine isolate sensitive, both should be reported similarly (resistant) irrespective of the results obtained using interpretative criteria for the urine isolates.
3. For agents not listed criteria given for systemic isolates may be used for urinary tract isolates (see Tables 1 and 2).

4. Direct susceptibility tests on urine samples may be performed as long as the inoculum obtained is equivalent to semi-confluent growth.
5. In the absence of a definitive organism identification, use the recommendations most appropriate for the presumptive identification, accepting that on some occasions the interpretation may be incorrect. A more cautious approach is to use the systemic recommendations.
6. = fosfomicin/glucose 6 phosphate
7. Fosfomicin - the sensitivity of *Proteus*. which swarm up to the disc, can be difficult to interpret.
8. Cefadroxil report as for cephalexin.

Table 12. MIC and zone breakpoints for urinary tract infections (Gram-positive cocci) <sup>1-4</sup> .

Antibiotic	Interpretation of zone diameters (mm)												
	MIC BP (mg/L)				Enterococci			<i>S. saprophyticus</i>			Group B streptococci		
	R <sup>3</sup>	I	S <sub>£</sub>	Disc content (µg)	R <sub>£</sub>	I	S <sup>3</sup>	R <sub>£</sub>	I	S <sup>3</sup>	R <sub>£</sub>	I	S <sup>3</sup>
Ampicillin	<b>64</b>	-	<b>32</b>	<b>25</b>	<b>19</b>	-	<b>20</b>	<b>25</b>	-	<b>26</b>	<b>25</b>	-	<b>26</b>
Cephalexin <sup>6</sup>	<b>64</b>	-	<b>32</b>	<b>30</b>							<b>23</b>	-	<b>24</b>
Ciprofloxacin	<b>8</b>	-	<b>4</b>	<b>1</b>				<b>17</b>	-	<b>18</b>			
Co-amoxyclov	<b>64</b>	-	<b>32</b>	<b>20/10</b>	<b>20</b>	-	<b>21</b>	<b>27</b>	-	<b>28</b>	<b>27</b>	-	<b>28</b>
Fosfomycin <sup>5</sup>	<b>256</b>	-	<b>128</b>	<b>200/50</b>	<b>19</b>	-	<b>20</b>	<b>19</b>	-	<b>20</b>			
Mecillinam	<b>128</b>	-	<b>64</b>	<b>50</b>				<b>9</b>	-	<b>10</b>			
Nalidixic acid	<b>32</b>	-	<b>16</b>	<b>30</b>	<b>17</b>	-	<b>18</b>						
Nitrofurantoin	<b>64</b>	-	<b>32</b>	<b>200</b>	<b>14</b>	-	<b>15</b>	<b>19</b>	-	<b>20</b>	<b>19</b>	-	<b>20</b>
Norfloxacin	<b>8</b>	-	<b>4</b>	<b>2</b>	<b>15</b>	-	<b>16</b>						
Trimethoprim <sup>7</sup>	<b>4</b>	-	<b>2</b>	<b>2.5</b>				<b>14</b>	-	<b>15</b>			

1. The information in bold is tentative. Tentative breakpoints are based on information that is currently available to the Working Party, and for some organism/antimicrobial combinations few data are available or breakpoints are based on information from one single laboratory. Breakpoints will remain tentative for one year from when first published.
2. If an organism is isolated from multiple sites, for example from blood and urine, interpretation of susceptibility should be made with regard to the systemic site, that is, if the blood isolate is resistant and the urine isolate sensitive, both should be reported similarly (resistant) irrespective of the results obtained using interpretative criteria for the urine isolates.
3. Direct susceptibility tests on urine samples may be performed as long as the inoculum obtained is equivalent to semi-confluent growth.



4. Isolates of *Staphylococcus epidermidis* and *Staphylococcus aureus* should be treated as from systemic infections because they are usually associated with more serious infections.
5. Fosfomycin/glucose 6 phosphate.
6. Cefadroxil report as for cephalexin.
7. Trimethoprim not active *in vivo* against enterococci.

## Control of Susceptibility Testing

Control strains have been chosen to include susceptible strains to monitor test performance (not for the interpretation of susceptibility), and resistant strains to confirm that the method will detect a mechanism of resistance, for example, *H. influenzae* ATCC 49247 a  $\beta$ -lactamase negative ampicillin resistant strain (refer to Table 5). Tables 2 - 5 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; CPHL, 61, Colindale Avenue, London NW9 5HT). Alternatively, some may be obtained commercially (e.g. Oxoid, Basingstoke, UK : Mast Laboratories, Merseyside, UK: Becton Dickinson, Oxford, UK).

### **1. Maintenance of control strains.**

Store control strains to minimise the risk of mutations, for example, at  $-70^{\circ}\text{C}$ , on beads in glycerol broth. Ideally, two vials of each control 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 which will not survive on plates for 7 days, subculture the strain daily for no more than 6 days.

### **2. Calculation of control ranges for disc diffusion.**

The acceptable ranges for the control strains have been calculated by combining zone diameter data from 'field studies' and from centres supplying their daily control data, from which cumulative distributions of zones of inhibition have been deduced. From these distributions, the 2.5 and 97.5 percentiles were read off empirically, to obtain a 'middle-range' that would contain 95% of the observations. If any distribution were normal, the above ranges correspond to the mean  $\pm$  1.96 SD respectively. The percentile ranges obtained by this method are, however, still valid even if the data are 'non-normal'.

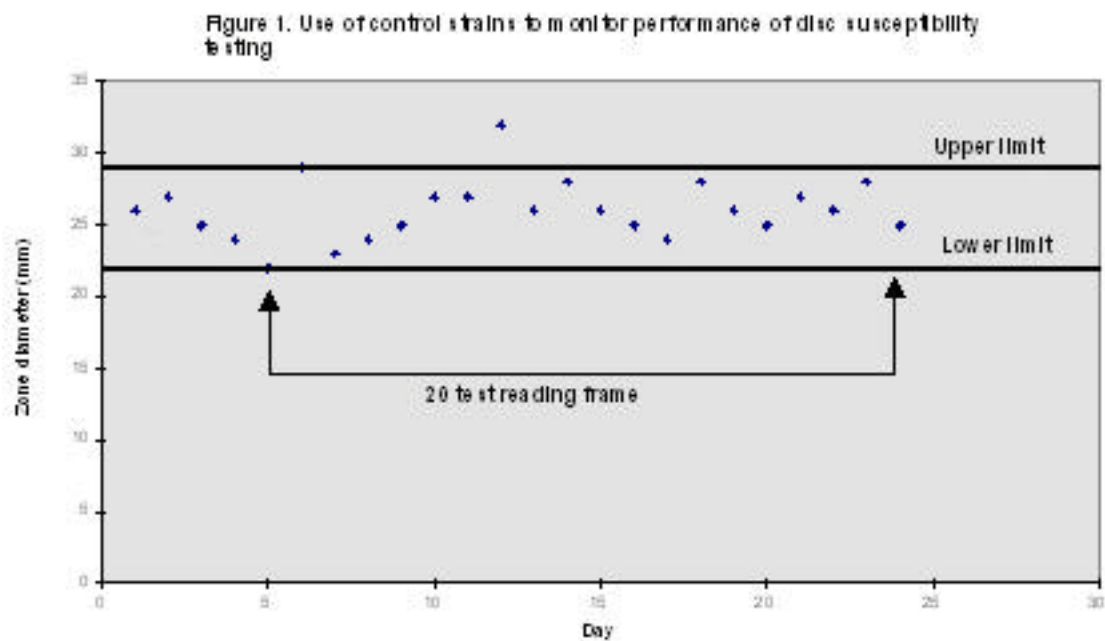
### **3. Frequency of testing**

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. If any result from weekly

testing (or results from testing new batches of media or discs) is out of range corrective action must be taken.

#### **4. Methods of using control data to monitor the performance of susceptibility testing.**

- 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.
- Acceptable if not more than 1 in every 20 results are outside the limits of acceptability.
- If 2 or more results fall out of the range this is a violation and requires immediate investigation.
- Look for trends within the limits of acceptability eg. tendency for zones to be at lower limits of acceptability or for zones to be consistently above or below the mean.



#### **5. 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. Areas for consideration are indicated in Table 1.

Table 1. Recommended areas for consideration as potential sources of error in disc susceptibility testing.

TEST CONDITIONS	CONTROLS	MEDIUM	DISCS
Pre-incubation	Contamination	Non-susceptibility testing agar	Wrong agent or content
Pre-diffusion	Mutation	Not prepared as per the manufacturer's instructions	Labile agent
Incubation temperature	Inoculum density	Batch to batch variation	Light sensitive agent
Incubation atmosphere	Uneven inoculation	Antagonists	Incorrect storage
Incubation time	Age of culture	pH	Disc containers opened before reaching room temperature
Illumination		Divalent cations	Labeling
		Depth	Expiry date
		Expiry date	

## 6. Reporting

Microbiologists require a pragmatic approach as results from repeat testing are not available on the same day. Quality Assurance will often pick up trends before the controls go out of range. The following procedure is suggested for reporting:

### 1. *Erroneous control results*

- Control zones are small, tests are susceptible or control zones are large, tests are resistant  
*investigate but report*

### 2. *Recognition of atypical results in clinical isolates*

- Inherent resistance - susceptibility where resistance is expected eg. *Proteus* spp. susceptible to colistin or nitrofurantoin.
- Resistance previously not observed - eg. penicillin resistant Group A Streptococci
- Resistance rare locally
- Knowledge application - useful to install an 'expert' system for laboratory reporting to avoid erroneous interpretation, for example, a methicillin resistant staphylococcus reported susceptible to a  $\beta$ -lactam antibiotic.

- All atypical results - the purity of the isolate and identification should be confirmed and the susceptibility repeated.
- Suppress results for individual antibiotics and retest.

### ***7. Acceptable zone ranges for control strains***

Tables 1 to 4 give the acceptable zone diameter ranges for the control strains obtained with the stated media and conditions of incubation.

**Table1. Iso-Sensitest agar, plates incubated at 35-37 °C in air for 18-20 h.**

Antibiotic	Disc content (µg unless stated)	<i>E. coli</i>			<i>Ps. aeruginosa</i>		<i>S. aureus</i>		<i>E. faecalis</i>
		NCTC 10418	ATCC 25922	NCTC 11560*	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29212
Amikacin	30	24-27	23-27	-	21-30	26-32	-	-	-
Ampicillin	10	21-26	16-22	-	-	-	-	-	26-35
"	25	24-30	-	-	-	-	-	-	-
Aztreonam	30	39-44	36-40	-	27-30	26-30	-	-	-
Cefixime	5	32-36	27-30	-	-	-	-	-	-
Cefoxitin	30	28-33	26-30	-	-	-	-	-	-
Cefotaxime	30	36-45	34-44	-	20-29	20-24	-	-	-
Ceftazidime	30	32-40	31-39	-	29-37	27-35	-	-	-
Cefuroxime	30	25-32	24-29	-	-	-	-	-	-
Cephalexin	30	21-28	16-21	-	-	-	-	-	-
Chloramphenicol	10	-	20-29	-	-	-	20-26	19-27	-
Ciprofloxacin	1	31-40	31-37	-	21-28	24-30	25-32	17-22	-
Clindamycin	2	-	-	-	-	-	30-35	26-33	29-33
Co-amoxyclav	3	-	-	-	-	-	-	27-32	-
"	30	18-31	20-26	18-23	-	-	-	-	-
Colistin	25	-	16-20	-	17-20	16-20	-	-	-
Erythromycin	5	-	-	-	-	-	22-31	22-29	-
Fusidic acid	10	-	-	-	-	-	32-40	30-37	-
Gentamicin	10	21-27	21-27	-	20-26	22-28	24-30	20-26	-
"	200	-	-	-	-	-	-	-	22-27
Imipenem	10	32-37	33-37	-	20-27	23-28	-	-	-
Levofloxacin	1	30-33	-	-	-	-	-	-	-
Meropenem	10	38-42	27-39	-	32-39	30-38	-	-	-
Mupirocin	5	-	-	-	-	-	26-35	24-34	-
Nalidixic acid	30	28-36	-	-	-	-	-	-	-
Neomycin	10	-	-	-	-	-	-	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	5	34-37	32-36	-	-	-	-	-	-
Ofloxacin	5	-	-	-	18-26	18-25	-	-	-
Penicillin	1 unit	-	-	-	-	-	32-40	29-36	-
Piperacillin	75	-	-	-	27-35	27-34	-	-	-
Pip/tazobactam	85	-	-	-	28-35	29-37	-	-	-
Quinupristin/dalfopristin	15	-	-	-	-	-	27-31	-	12-19
Rifampicin	2	-	-	-	-	-	27-39	29-36	-
Teicoplanin	30	-	-	-	-	-	17-23	16-20	19-25
Tetracycline	10	-	-	-	-	-	31-40	26-35	-
Ticarcillin	75	32-35	27-30	-	24-28	23-27	-	-	-
Timentin	85	33-37	27-31	-	25-29	24-27	-	-	-
Tobramycin	10	24-27	-	-	23-30	26-32	-	29-35	-
Trimethoprim	2.5	28-34	20-26	-	-	-	25-30	20-28	-
"	5	-	-	-	-	-	24-34	-	-
Vancomycin	5	-	-	-	-	-	14-20	13-17	13-19

\* = β-lactamase producing strain

**Table 2. Iso-Sensitest agar supplemented with 5% defibrinated horse blood with, or without, the addition of NAD , plates incubated at 35-37<sup>0</sup>C in air for 18-20 h.**

Antibiotic	Disc content (µg unless stated)	<i>S. aureus</i>	
		NCTC 6571	ATCC 25923
Erythromycin	5	22-29	23-29
Penicillin	1 unit	30-41	27-35
Tetracycline	10	30-38	28-36

**Table 3. Detection of methicillin/oxacillin resistance in staphylococci.**

Antibiotic	Media	Disc content (µg)	<i>S. aureus</i>	
			NCTC 6571	ATCC 25923
Methicillin	Columbia/Mueller Hinton agar + 2% NaCl	5	18-30	18-28
Oxacillin	Columbia/Mueller Hinton agar + 2% NaCl	1	19-30	19-29

**Table 4. Iso-Sensitest agar supplemented with 5% defibrinated horse blood with, or without, the addition of NAD, plates incubated at 35-37<sup>0</sup>C in 4-6% CO<sub>2</sub> for 18-20 h.**

	Disc content (µg unless stated)	<i>S. aureus</i>		<i>H. influenzae</i> (with NAD)		<i>S. pneumoniae</i>
		NCTC 6571	ATCC 25923	NCTC 11931	ATCC 49247	ATCC 49619
Ampicillin	2	-	-	22-30	6-13	-
Azithromycin	15	-	-	24-36	20-30	-
Cefotaxime	5	26-32	-	33-45	27-38	-
Cefuroxime	5	-	-	22-28	6-16	-
Chloramphenicol	10	21-26	-	30-40	30-38	21-29
Ciprofloxacin	1	-	-	32-40	33-44	14-21
Co-amoxycylav	3	-	-	20-27	10-20	-
Erythromycin	5	25-29	-	12-23	9-16	23-36
Nalidixic acid	30	-	-	33-38	-	-
Oxacillin	1	-	-	-	-	8-16
Penicillin	1 unit	39-43	-	--	-	-
Quinupristin/ dalfopristin	15	-		-		21-29
Rifampicin	2	32-37	-	-	-	-
Rifampicin	5	-	-	-	-	32-37
Tetracycline	10	-	-	27-35	9-14	26-36
Trimethoprim	2.5	-	-	30-40	28-36	-



## 8. Control of MIC determinations.

The following tables provide MIC values for recommended control strains using BSAC methodology as described by Andrews<sup>1</sup>

**Table 1. Expected MICs for control strains with BSAC methodology<sup>1</sup>**

Antibiotic	<i>H. influenzae</i>	<i>H. influenzae</i>	<i>Ent. faecalis</i>	<i>S. pneumoniae</i>	<i>B. fragilis</i>	<i>N. gonorrhoeae</i>
	NCTC 11931	ATCC 49247	ATCC 29212	ATCC 49619	NCTC 9343	ATCC 49226
Amikacin			128			
Gentamicin			8		128	
Tobramycin			16			
Azithromycin	2	2		0.12		
Amoxicillin	0.5	4	0.5	0.06	32	0.5
Ampicillin			1	0.06	32	
Azlozillin					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			
Co-amoxycylav	0.5	8	0.5	0.06	0.5	0.5
Faropenem				0.06	1	
Flucloxacillin					16	
Imipenem			0.5		0.06	
Loracarbef		128	>32	2	>128	
Mecillinam			>128		>128	
Meropenem			2		0.06	
Moxalactam					0.25	
Oxacillin				1		
Penicillin		4	2	0.5	16	

<b>Antibiotic</b>	<i>H. influenzae</i>	<i>H. influenzae</i>	<i>Ent. faecalis</i>	<i>S. pneumoniae</i>	<i>B. fragilis</i>	<i>N. gonorrhoeae</i>
	NCTC 11931	ATCC 49247	ATCC 29212	ATCC 49619	NCTC 9343	ATCC 49226
Piperacillin			2		2	
Ticarcillin					4	
Cotrimoxazole		1	2	4		
Trimethoprim			0.25	4	16	
Teicoplanin			0.25			
Vancomycin			2	0.25	16	
Telithromycin	1	2	0.008	0.008		0.03
Clarithromycin	8	4		0.03	0.25	0.5
Clindamycin			8	0.12	0.5	
Erythromycin	8	8	4	0.12	1	0.5
Linezolid				2	4	
Roxithromycin	16	16		0.12	2	
Chloramphenicol			4	4	4	
Fucidic acid			2			
Metronidazole					0.5	
Nitrofurantoin			8			
Rifampicin			2	0.03		
Synercid			1	0.5	16	
Ciprofloxacin	0.008	0.008	1	1	2	0.004
Enoxacin					1	
Fleroxacin					4	
Gatifloxacin					0.5	
Grepafloxacin		0.004		0.25		
Levofloxacin		0.015		0.5	0.5	
Moxifloxacin	0.03	0.03	0.25	0.5		0.004
Naladixic acid		1		>128	64	
Norfloxacin			2		16	
Ofloxacin			2		1	
Pefloxacin					1	
Rufloxacin					16	
Sparfloxacin		0.002		0.25	1	
Trovafloxacin	0.008	0.002	0.06	0.12	0.12	
Tetracycline		16	16	0.12	0.5	

**Table 2. Expected MICs for control strains with BSAC methodology<sup>1</sup>**

Antibiotic	<i>Esch. coli</i>	<i>Esch. coli</i>	<i>Ps. aeruginosa</i>	<i>Ps. aeruginosa</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
	NCTC 10418	ATCC 25922	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29213
Amikacin	0.5	1	2	2	1		2
Gentamicin	0.25	0.5	1	1	0.12	0.25	0.25
Kanamycin	1		1		2		
Neomycin			32		0.12		
Tobramycin	0.25	0.5	0.5	0.5	0.12		0.5
Azithromycin					0.12	0.12	0.12
Amoxicillin	2	4	>128	>128	0.12	0.25	
Ampicillin	2	4	>128	>128	0.06		
Azlozillin	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		
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		
Co-amoxyclov	2	4	>128	128	0.12	0.12	0.25
Farapenem	0.25		>128	>128	0.12		
Flucloxacillin			>128	>128	0.06		
Imipenem	0.06	0.12	2	1	0.015		0.015
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

Antibiotic	<i>Esch. coli</i>	<i>Esch. coli</i>	<i>Ps. aeruginosa</i>	<i>Ps. aeruginosa</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
	NCTC 10418	ATCC 25922	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29213
Methicillin			>128	>128	1	2	2
Mezlocillin	2		8		0.5		
Moxalactam	0.03		8		8		
Oxacillin			>128	>128	0.25	0.25	0.5
Penicillin			>128	>128	0.03	0.03	0.12
Piperacillin	0.5	2	4	2	0.25		1
Temocillin	2		>128		128		
Ticarcillin	1		16		0.5		
Ticarcillin/ 4mg/L clavulanate			32	16			
Cotrimoxazole	0.25	0.25					2
Sulphonamide	16		>128	>128	64		
Trimethoprim	0.12	0.25	32		0.25		0.5
Teicoplanin					0.25	0.5	0.5
Vancomycin					0.5	0.5	1
ABT 773					0.015	0.03	0.03
HMR 3647					0.03	0.06	0.06
Clarithromycin					0.12	0.12	0.12
Clindamycin					0.06	0.12	0.06
Dirythromycin					1		1
Erythromycin					0.12	0.5	0.25
Linezolid					0.5	1	
Roxithromycin					0.25	0.5	0.5
Chloramphenicol	2	4	128		2		2
Colistin	0.5		2		128		
Fosfomycin	4		>128	>128	8		
Fucidic acid	>128				0.06	0.12	0.06
Mupirocin					0.25	0.25	0.12
Nitrofurantoin	4	8			8		16
Rifampicin	16				0.004	0.015	0.004
Synercid					0.12	0.25	0.25
Ciprofloxacin	0.015	0.015	0.25	0.25	0.12	0.5	0.5
Enoxacin	0.25		1		0.5		
Fleroxacin	0.06	0.12	1		0.5		
Flumequine	2		>128	>128			
Gatifloxacin	0.015		1		0.03		

Antibiotic	<i>Esch. coli</i>	<i>Esch. coli</i>	<i>Ps. aeruginosa</i>	<i>Ps. aeruginosa</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
	NCTC 10418	ATCC 25922	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29213
Grepafloxacin	0.03	0.03	0.5		0.03		
Levofloxacin	0.03	0.03	0.5	0.5	0.12	0.25	0.25
Lomefloxacin					0.5		
Moxifloxacin	0.03	0.03	2	2	0.06	0.06	
Naladixic acid	2	4	>128	>128	>128	128	128
Norfloxacin	0.06	0.06	1	1	0.25		1
Ofloxacin	0.06	0.03	1	1	0.25		0.5
Pefloxacin	0.06		0.5		0.25		
Rufloxacin	0.5		8		1		
Sparfloxacin	0.015	0.015	0.5	0.5	0.03		
Trovafloxacin	0.015	0.015	0.5	0.5	0.015	0.03	0.03
Tetracycline	1	2		32	0.06		0.5

<sup>1</sup> Andrews, J.M. Determination of Minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, Suppl S1 to Volume 48 July 2001.

**Useful web sites**

<b>SRGA :</b>	The Swedish Reference Group for Antibiotics	<a href="http://www.srga.org">www.srga.org</a>
<b>CDC :</b>	Centre for Disease Control (Atlanta, USA)	<a href="http://www.cdc.gov">www.cdc.gov</a>
<b>WHO :</b>	World Health Organisation (Geneva, Switzerland)	<a href="http://www.who.int">www.who.int</a>
<b>NCCLS :</b>	National Committee for Clinical Laboratory Standards	<a href="http://www.nccls.org">www.nccls.org</a>
<b>NEQAS :</b>	National External Quality Assessment Scheme	<a href="http://www.ukneqas.org.uk">www.ukneqas.org.uk</a>
<b>NCTC :</b>	National Collection of Type Cultures	<a href="http://www.phls.org.uk">www.phls.org.uk</a>