BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing

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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
Pseudomonas spp.	ISA
Staphylococci *other than for the detection of methicillin/oxacillin resistance*	ISA
Staphylococci *for the detection of methicillin/oxacillin resistance*	Mueller-Hinton or Columbia agar (Oxoid CM331 or equivalent) with 2% NaCl [1]
Enterococci	ISA
S. pneumoniae	ISA + 5 % defibrinated horse blood [2]
Haemolytic streptococci	ISA + 5 % defibrinated horse blood [2]
M. catarrhalis	ISA + 5 % defibrinated horse blood [2]
N. meningitidis	ISA + 5 % defibrinated horse blood [2]
N. gonorrhoeae	ISA + 5 % defibrinated horse blood [2]
Haemophilus spp.	ISA + 5 % defibrinated horse blood + 20 mg/L NAD

NB [1]see Section 8.

[2]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 mean depth of 4.0 mm ± 0.5 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
E. coli	NCTC 12241 (ATCC 25922)	NCTC 10418
E. coli	NCTC 11560 [1]	
S. aureus	NCTC 12981 (ATCC 25923)	NCTC 6571
S. aureus	NCTC 12493 [2]	
Ps. aeruginosa	NCTC 12934 (ATCC 27853)	NCTC 10662
Ent. faecalis	NCTC 12697 (ATCC 29212)	
H. influenzae	NCTC 12699 (ATCC 49247)	NCTC 11931
S. pneumoniae	NCTC 12977 (ATCC 49619)	
N. gonorrhoeae	NCTC 12700 (ATCC 49226)	

[1] β-lactamase producing strain

[2] Methicillin/oxacillin resistant strain

NB

- The control strains listed include sensitive strains that 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 β-lactam antibiotics.

- To minimise the risk of mutations store control strains 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 that 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 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 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 $BaCL_2$ (1.17% w/v $BaCl_2.2H_20$) to 99.5 ml of 0.18M H_2SO_4 (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 that 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	*N. gonorrhoeae*
Enterococci	*Serratia* spp.	
Enterobacteriaceae	*S. pneumoniae*	
Pseudomonas spp.	*N. meningitidis*	
Acinetobacter spp.	*M. catarrhalis*	
Haemophilus 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)[1] 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.]

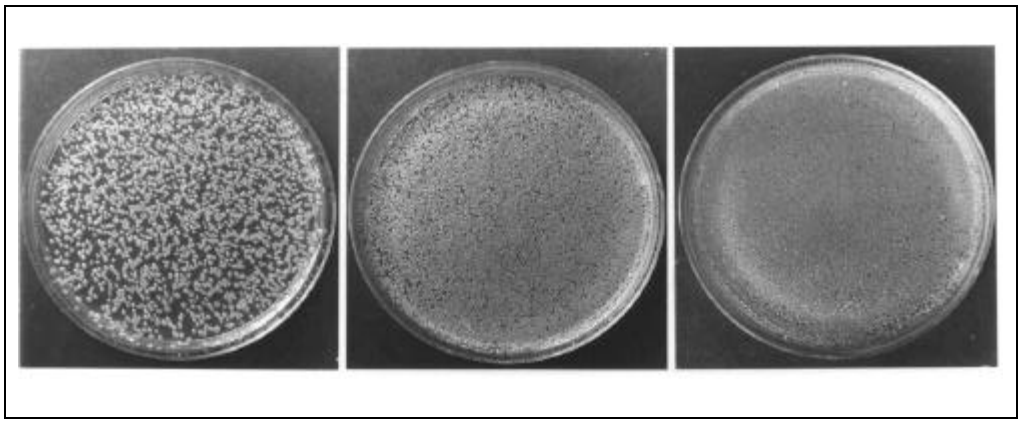
3.2.3. From the table select the volume to transfer (with the appropriate fixed volume micropipette) to 5 mL sterile distilled water.

[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 (µL) to transfer to 5 mL sterile distilled water
Enterobacteriaceae	0.01 - 0.05	250
Enterococci	>0.05 - 0.1	125
Pseudomonas	>0.1 - 0.3	40
Staphylococci	>0.3 - 0.6	20
	>0.6 - 1.0	10
Haemophilus	0.01 - 0.05	500
Streptococci	>0.05 - 0.1	250
Miscellaneous fastidious	>0.1 - 0.3	125
organisms	>0.3 - 0.6	80
	>0.6 - 1.0	40

3.3 Acceptable ranges for inoculum densities are shown below:

Lightest acceptable Ideal Heaviest acceptable



Example of the acceptable inoculum density 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*

Organisms	***Incubation conditions***
Enterobacteriaceae	35-37°C in air for 18-20h
Pseudomonas spp.	"
Staphylococci[1]	"
M. catarrhalis	"
Haemolytic streptococci	"
Enterococci	35-37°C in air for 24h[2]
N. meningitidis	35-37°C in 4-6% CO_2 for 18-20h
S. pneumoniae	"
Haemophilus spp.	"
N. gonorrhoeae	"

[1] Incubation conditions for the detection of methicillin/oxacillin resistance given in Section 8.

[2] It is essential that plates be 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.

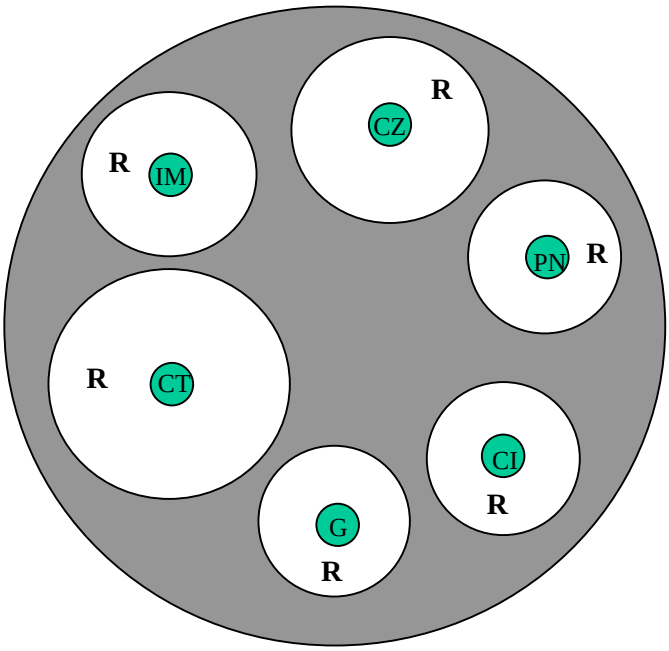
7. Measuring zones

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*). 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 = ≥15 mm diameter, resistant = ≤14mm diameter

8.1.7 NB. Some hyper-producers of β-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 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.

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) 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*[1].

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R [3]	I	S £	Disc content (µg)	R £	I	S [3]
Ampicillin[2]	16	-	8	10	17	-	18
Cefuroxime (axetil)	2	-	1	30	24	-	25
Cefuroxime (parenteral)	16	-	8	30	19	-	20
Ceftazidime	4	-	2	30	27	-	28
Ceftazidime [3] *E. coli* & *Klebsiella* spp.	4	-	2	30	21	-	22
Ciprofloxacin[4]	2	-	1	1	17	-	18
Gentamicin	2	-	1	10	19	-	20
Amikacin	8	-	4	30	19	-	20
Aztreonam[5]	2	-	1	30	23	-	24
Cefaclor	2	-	1	30	34	-	35
Cefamandole[6,7]	16	-	8	30	19	-	20
Cefepime	2	-	1	30	31	-	32
Cefixime	2	-	1	5	19	-	20
Cefoperazone[6]	8	-	4	30	24	-	25
Cefotaxime	2	-	1	30	29	-	30
Cefotetan[6]	8	-	4	30	23	-	24
Cefoxitin[7]	16	-	8	30	19	-	20
Cefpirome	2	-	1	20	24	-	25
Cefpodoxime	2	-	1	5	33	-	34
Ceftibuten	2	-	1	10	27	-	28
Ceftizoxime	2	-	1	30	29	-	30
Ceftriaxone	2	-	1	30	27	-	28
Cephalothin[7]	16	-	8	30	26	-	27
Cephradine[7]	16	-	8	30	11	-	12

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

1. 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 repeated if the inoculum density is outside the acceptable range.

2. Amoxycillin - report as for ampicillin

3. 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 β-lactamases and until a mechanism of resistance has been identified this tentative zone diameter breakpoint is recommended.

4. 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.

5. The MIC breakpoint for aztreonam has been lowered to ensure that ESBL producers with aztreonam MIC values of 4 mg/L are not interpreted susceptible to this antibiotic.

6. Zone breakpoints only valid for *E. coli*, *Klebsiella* spp. and *Pr. mirabilis*.

7. The MIC breakpoints for this β-lactam antibiotic has been amended based on MIC distribution data for the population lacking a mechanism of resistance.

8. 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*

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

Table 3. MIC and zone breakpoints for staphylococci.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R [3]	I	S £	Disc content (µg unless stated)	R £	I	S [3]
Gentamicin	2	-	1	10	19	-	20
Penicillin[1]	0.25	-	0.12	1 unit	24	-	25
Methicillin [2,3]	8	-	4	5	14	-	15
Oxacillin [2,3,4]	4	-	2	1	14	-	15
Vancomycin[5]	8	-	4	5	11	-	12
Teicoplanin[5,6]	8	-	4	30	14	-	15
Rifampicin	0.12	-	0.06	2	29	-	30
Erythromycin	1	-	0.5	5	19	-	20
Quinupristin/ Dalfopristin[7]	4	-	2	15	19	-	20
Amikacin coagulase-negative staphylococci	32	8-16	4	30	21	22-24	25
Amikacin *S. aureus*	32	8-16	4	30	18	-	19
Azithromycin	2	-	1	15	19	-	20
Chloramphenicol	16	-	8	10	14	-	15
Ciprofloxacin	2	-	1	1	17	-	18
Clarithromycin	1	-	0.5	2	19	-	20
Clindamycin	1	-	0.5	2	25	-	26
Co-amoxyclav[2]	2	-	1	3	17	-	18
Co-trimoxazole[8]	64	-	32	25	16	-	17
Fusidic acid	2	-	1	10	29	-	30
Gatifloxacin	2	-	1	2	19	-	20
Gemifloxacin	0.5	-	0.25	1	19	-	20
Linezolid [9]	8	-	4	10	19	-	20
Moxifloxacin	2	-	1	1	19	-	20
Mupirocin [10]	8	-	4	5	21	-	22
Neomycin				10	16	-	17
Netilmicin for *S. aureus*	2	-	1	10	23	-	24

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R [3]	I	S £	Disc content (µg unless stated)	R £	I	S [3]
Netilmicin for coagulase-negative staphylococci	2	-	1	10	29	-	30
Ofloxacin	4	-	2	1	23	-	24
Tetracycline	2	-	1	10	19	-	20
Tobramycin for *S. aureus*	2	-	1	10	20	-	21
Tobramycin for coagulase-negative staphylococci	2	-	1	10	29	-	30
Trimethoprim[11]	1	-	0.5	5	19	-	20

1. Penicillin; check for heaped zone edge (= resistant)
2. Staphylococci exhibiting resistance to methicillin/oxacillin should be regarded as resistant to other penicillins, cephalosporins, carbapenems and combinations of β-lactam and β-lactamase inhibitors
3. Recommendations for Mueller-Hinton or Columbia agar with 2% NaCl.
4. MIC breakpoint for coagulase-negative staphylococci is currently under review.
5. 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.
6. Teicoplanin - disc testing not recommended for coagulase-negative staphylococci. An MIC method of testing should be used to determine susceptibility.
7. 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 = ≥15 mm.
8. Based on sulphamethoxazole MIC.
9. Information on clinical response in patients with serious staphylococcal infections is not yet available. In such patients an MIC determination might be appropriate.
10. 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, ≥512 mg/L). The latter is clinically significant the former is not.
11. Amended zone diameter breakpoints are biological breakpoints for the treatment of MRSA infections. However, this does not imply there is proven correlation with clinical efficacy.

Table 4. MIC and zone breakpoints for *S. pneumoniae*.

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

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R [3]	I	S £	Disc content (µg)	R £	I	S [3]
Vancomycin	8	-	4	5	12	-	13

1. Organisms with reduced susceptibility to penicillin: confirm resistance with a test for penicillin MIC. Organisms with an MIC ≤ 1 mg/L are considered susceptible to β-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.

2. Penicillin resistance in *S. pneumoniae* is detected with an oxacillin 1 µg disc.

3. The MIC BPs for penicillin has been changed to reflect international standards.

4. Isolates with MICs of ciprofloxacin ≤2 mg/L are considered as having intermediate susceptibility.

5. Advice on testing the susceptibility of *S. pneumoniae* to co-trimoxazole is not provided because of the following CSM recommendations.

`Co-trimoxazole should be limited to the role of drug of choice in *Pneumocyctis carinii* pneumonia; it is also indicated for *toxoplasmosis* and *nocardiasis*. It should now only be considered for use in *acute exacerbations of chronic bronchitis* and *infections of the urinary tract* when there is good bacteriological evidence of sensitivity to co-trimoxazole and good reason to prefer this combination to a single antibiotic; similarly it should only be used in *acute otitis media in children* when there is good reason to prefer it. Review of the safety of co-trimoxazole using spontaneous adverse drug reaction data has indicated that the profile of reported adverse reactions with trimethoprim is similar to that with co-trimoxazole; *blood and generalised skin disorders* are the most serious reactions with both drugs and predominantly have been reported to occur in **elderly patients**. A recent large post-marketing study has demonstrated that such reactions are very rare with co-trimoxazole; the study did not distinguish between co-trimoxazole and trimethoprim with respect to *serious hepatic, renal*, *blood or skin disorders*.'

Table 5. MIC and zone breakpoints for enterococci.

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

1. High-level gentamicin resistant enterococci usually give no zone or only a trace of inhibition around gentamicin 200 µg discs. Occasionally, however, a plasmid carrying the resistance may be unstable and the resistance is seen as a zone of inhibition with a few small colonies within the zone. Retesting of resistant colonies results in growth to the disc or increased numbers of colonies within the zone. Zones should be carefully examined to avoid missing such resistant organisms. If in doubt, isolates may be sent to the reference laboratory for confirmation.
2. It is essential that plates be 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 = ≥15 mm.

Table 6. MIC and zone breakpoints for haemolytic streptococci.

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

1. Active metabolite not taken into consideration.
2. Based on sulphamethoxazole MIC.

Table 7. MIC and zone breakpoints for *M. catarrhalis* .

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

1. Test for β-lactamase. It must be remembered that β-lactamase positive isolates of *M. catarrhalis* are often slow to become positive and tests for β-lactamase production must be examined after the longest recommended time for the test before being interpreted as negative.
2. Active metabolite not taken into consideration.

Table 8. MIC and zone breakpoints for *N. gonorrhoeae* .

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

1. Quinolone resistance is most reliably detected with nalidixic acid. Strains with reduced susceptibility to fluoroquinolones have no zone of inhibition with nalidixic acid.
2. Test for β-lactamase.
3. Confirm resistance by MIC if β-lactamase negative.

Table 9. MIC and zone breakpoints for *N. meningitidis* .

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

Table 10. MIC and zone breakpoints for *H. influenzae* .

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R [3]	I	S £	Disc content (µg unless stated)	R £	I	S [3]
Ampicillin[1]	2	-	1	2	17	-	18
Amoxycillin[1]	2	-	1	2	16	-	17
Cefuroxime	2	-	1	5	16	-	17
Cefotaxime	2	-	1	5	24	-	25
Tetracycline	2	-	1	10	21	-	22
Chloramphenicol	4	-	2	10	24	-	25
Azithromycin[2]	8	0.5-4	0.25	15	19	20-34	35
Cefaclor	2	-	1	30	36	-	37
Ceftazidime	4	-	2	30	29	-	30
Ceftriaxone	2	-	1	30	34	-	35
Ciprofloxacin[3]	2	-	1	1	27	-	28
Clarithromycin[4]	32	1-16	0.5	5	9	10-24	25
Co-amoxyclav	2	-	1	2/1	16	-	17
Co-trimoxazole	64	-	32	25	21	-	22
Erythromycin	16	1-8	0.5	5	14	15-27	28
Gatifloxacin[3]	2	-	1	2	19	-	20
Gemifloxacin[3]	0.5	-	0.25	1	19	-	20
Imipenem	8	-	4	10	19	-	20
Levofloxacin[3]	4	-	2	1	19	-	20
Moxifloxacin[3]	2	-	1	1	17	-	18
Meropenem	8	-	4	10	27	-	28
Nalidixic acid [3]	-	-	-	30	-	-	-
Trimethoprim	1	-	0.5	2.5	20	-	21

1. Test for β-lactamase.
2. No resistant strains.
3. 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.
4. Active metabolite not taken into consideration.

Table 11. MIC and zone breakpoints for urinary tract infections (Gram-negative rods) [1-4].

Antibiotic	MIC BP (mg/L)			Interpretation of zone diameters (mm)									
					Coliforms			*E. coli*			*Pr. mirabilis*		
	R [3]	I	S £	Disc content (µg)	R£	I	S [3]	R £	I	S [3]	R £	I	S [3]
Ampicillin	64	-	32	25				15	-	16	24	-	25
Cephalexin[5]	64	-	32	30				15	-	16	11	-	12
Ciprofloxacin	8	-	4	1	19	-	20	19	-	20	19	-	20
Co-amoxyclav	64	-	32	20/10				17	-	18	17	-	18
Fosfomycin[6,7]	256	-	128	200/50				19	-	20	33	-	34
Mecillinam	16	-	8	10				13	-	14	13	-	14
Nalidixic acid	32	-	16	30	17	-	18	17	-	18	17	-	18
Nitrofurantoin	64	-	32	200				19	-	20			
Norfloxacin	8	-	4	2	15	-	16	15	-	16	15	-	16
Trimethoprim	4	-	2	2.5	16	-	17	16	-	17	16	-	17

1. 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.
2. For agents not listed criteria given for systemic isolates may be used for urinary tract isolates (see Tables 1 and 2).
3. Direct susceptibility tests on urine samples may be performed as long as the inoculum obtained is equivalent to semi-confluent growth.
4. In the absence of 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.
5. Cefadroxil report as for cephalexin.
6. = fosfomycin/glucose 6 phosphate
7. Fosfomycin - the sensitivity of *Proteus* that swarm up to the disc, can be difficult to interpret.

Table 12. MIC and zone breakpoints for urinary tract infections (Gram-positive cocci) [1-3] .

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

1. 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.
2. Direct susceptibility tests on urine samples may be performed as long as the inoculum obtained is equivalent to semi-confluent growth.
3. Isolates of *Staphylococcus epidermidis* and *Staphylococcus aureus* should be treated as from systemic infections because they are usually associated with more serious infections.
4. Cefadroxil report as for cephalexin.
5. Fosfomycin/glucose 6 phosphate.
6. Trimethoprim not active *in vivo* against enterococci.

13. Susceptibility testing of anaerobic and fastidious bacteria

Anaerobic bacteria

The dynamics of the disc diffusion technique, in which the size of the zone is related to a critical concentration of antimicrobial agent, a critical population of organisms and a critical time, makes it an unsuitable method for testing slow-growing organisms. The BSAC Working Party recommendation for slow growing anaerobic organisms is to test for susceptibility by MIC determinations. The media recommended for the Etest by the manufacturer is Wilkins-Chalgren with 5% sheep blood or Brucella agar with 5% sheep blood and 1 mg/L vitamin K. Sheep blood is not often used in the UK and horse blood can be substituted, but results for control organisms may be slightly different (follow Etest technical guide instructions).

Anaerobes that grow well within 24 hours can be tested by disc diffusion as a screen for resistance. The medium of choice for susceptibility testing of anaerobic bacteria is Wilkins-Chalgren agar supplemented with 5% horse blood. This medium adequately supports the growth of most fast growing anaerobic bacteria and has no adverse affect on the activity of antimicrobial agents.

1. Select colonies from a fresh plated culture. Suspend colonies in sterile distilled water and adjust the density to that of a 0.5 McFarland standard.
2. Dilute the suspension in sterile distilled water, 1 in 100 for Gram-negative species and 1 in 10 for Gram-positive species.
3. Use a sterile swab to inoculate evenly the surface of the medium.
4. Incubate plates for 18-24 hours at 37^0C under strict anaerobic conditions.
5. Interpret zone diameters according to the tentative breakpoints given in the Table.

Resistance must be confirmed by MIC determinations. There are insufficient data available to recommend zone diameter breakpoints for other agents at this time. There are particular

problems when interpreting results for combinations containing β-lactamase inhibitors as these have *in vitro* activity alone against many anaerobic species.

Tests for complete anaerobiosis, such as anaerobic indicator BR55 (Oxoid), growth of an obligate anaerobe such as *Bacteroides thetaiotaomicron* ATCC 29741 and lack of growth of *Pseudomonas aeruginosa* on Simmonds Citrate agar, are essential when performing any susceptibility tests on anaerobes. Metronidazole and clindamycin are particularly affected by the presence of any oxygen, which will result in raised MICs or reduced zone sizes.

Tentative zone diameter breakpoints for rapidly growing anaerobic organisms.

Antibiotic	MIC breakpoint (mg/L)		Disc content (μg)	Interpretation of zone diameter (mm)	
	R	S		R	S
penicillin	≥ 2	≤ 1	2	≤ 17	≥ 18
clindamycin	≥ 4	≤ 2	2	≤ 17	≥ 18
metronidazole	≥ 16	≤ 8	5	≤ 17	≥ 18

Helicobacter pylori

Disc diffusion methods are not suitable for testing *H. pylori* as this species is slow growing and results may not be accurate. The recommended method of susceptibility testing is Etest (follow technical guide instructions).

i. Suspend colonies from a 2-3 day culture on a blood agar plate in sterile distilled water and adjust the density to equal a MacFarland 3 standard.
ii. Use a swab dipped in the suspension to inoculate evenly the entire surface of the plate. The medium of choice is Mueller-Hinton agar or Wilkins-Chalgren agar with 5-10% horse blood.
iii. Allow the plate to dry and apply Etest strip.
iv. Incubate at 35^0C in microaerophilic conditions for 3-5 days.

v. Read the MIC at the point of complete inhibition of all growth, including hazes and isolated colonies. Tentative interpretative criteria for MICs are given in the Table below.

Tentative MIC breakpoints for *H. pylori*

	MIC breakpoint (mg/L)	
Antibiotic	R	S
Amoxycillin	≥ 2	≤ 1
Clarithromycin	≥ 2	≤ 1
Tetracycline	≥ 4	≤ 2
Metronidazole	≥ 8	≤ 4

Campylobacter species

The most commonly isolated *Campylobacter* spp. are those associated with gastrointestinal infection, *Campylobacter coli* and *Campylobacter jejuni*. Most *Campylobacter* spp. require a microaerophilic atmosphere for growth. Susceptibility tests for *Campylobacter* spp. are not standardized and therefore there is some variability in the susceptibility data reported in the literature. However, disc diffusion methods are suitable for detecting resistance to the commonly used antimicrobials. Nalidixic acid discs should be used to detect quinolone resistance.

i. Suspend colonies from a fresh plated culture in sterile distilled water. Adjust the density of the suspension to that of a 0.5 McFarland standard.

ii. Use a swab dipped in the undiluted suspension to inoculate evenly the entire surface of Iso-Sensitest agar supplemented with 5% horse blood.

iii. Incubate for 18-24h at 42^0C in microaerophilic conditions. *Campylobacter fetus*, which is primarily associated with extraintestinal infections, does not grow well at 42^0C and should be incubated at 35-37^0C.

iv. Measure zone diameters. Tentative zone diameter breakpoints are given below.

Tentative zone diameter breakpoints for *Campylobacter* spp.

Antibiotic	MIC breakpoint (mg/L) R	MIC breakpoint (mg/L) S	Disc content (µg)	Interpretation of zone diameter (mm) R	Interpretation of zone diameter (mm) S
Erythromycin	≥ 2	≤ 1	5	≤ 19	≥ 20
Ciprofloxacin	≥ 4	≤ 2	1	≤ 17	≥ 18
Nalidixic acid			30	≤ 15	≥ 16

Brucella species

Brucella spp. are Hazard Group 3 pathogens and all work must be done in containment level 3 accommodation. The antimicrobial agents most commonly used for treatment are doxycycline, rifampicin, ciprofloxacin, tetracycline and streptomycin and, from the limited information available, there is little or no resistance to these drugs. *Brucella* spp. are uncommon isolates and interpretative standards are not available. Since *Brucella* spp. are highly infectious, the Working Party recommends that no susceptibility testing is done in clinical laboratories. The FAO/WHO Collaborating Centre for Reference and Research on Brucellosis (Veterinary Laboratory Agency, New Haw, Addlestone, Surrey, KT15 3NB, UK, Tel: 01932 357216, contact Mr. A. MacMillan) are willing to receive any isolates for confirmation of identification and susceptibility and are particularly interested in isolates from patients with treatment failure.

Legionella species

Legionella spp. are slow growing and have particular growth requirements. Disc diffusion methods for susceptibility are unsuitable. Susceptibility should be determined by agar dilution MICs on buffered yeast extract agar with 5% water-lysed horse blood[1]. The antimicrobial agents commonly used for treatment are macrolides, rifampicin and fluoroquinolones. Validated MIC breakpoints are not established for *Legionella* spp. If results for test isolates are within range of the normal wild-type distribution, listed below, clinical susceptibility may be assumed.

MIC ranges for normal wild-type *Legionella* spp.

Antibiotic	Range of MICs (mg/L)
Erythromycin	0.06-0.5
Clarithromycin	0.004-0.06
Rifampicin	0.004-0.06
Ciprofloxacin	0.016-0.06

Stenotrophomonas maltophilia

Susceptibility tests on *S. maltophilia* are affected by temperature and should be incubated at 30^0C to achieve the correct results. Aminoglycoside and polymixin susceptibility tests are particularly vulnerable to temperature variation and isolates will often appear erroneously susceptible if incubated at 37^0C [2-4]. Tests on β-lactam antibiotics are particularly affected by the medium. Most β-lactams have poor activity against this species and organisms may appear falsely susceptible in diffusion tests. The medium, dilution and interpretative standards are as recommended for *P. aeruginosa* in Table 2.

References

1. Felmingham D., Robbins M. J., Sangharajkam., Leakey A. & Ridgway G. L. (1991). In-vitro activity of some 14-, 15-, and 16- membered macrolides against *Staphylococcus* spp., *Legionella* spp., *Mycoplasma* spp. and *Ureoplasma ureolyticum*. *Drugs under experimental and Clinical Research* **17**, 91-9.

2. Wheat, P. F., Winstanley, T. G. & Spencer, R. C.(1985). Effect of temperature on antimicrobial susceptibilities of *Pseudomonas maltophilia*. *Journal of Clinical Pathology* **38**, 1055-8.

3. Rahmati-Bahram, A., Magee, J. T. & Jackson, S. K. (1995). Growth temperature-dependent variation of cell envelope lipids and antibiotic susceptibility in *Stenotrophomonas* (*Xanthomonas*) *maltophilia*. *Journal of Antimicrobial Chemotherapy* **36**, 317-26.

4. Rahmati-Bahram, A., Magee, J. T. & Jackson, S. K. (1997). Effect of temperature on aminoglycoside binding sites in *Stenotrophomonas maltophilia*. *Journal of Antimicrobial Chemotherapy* **39**, 19-24.

14. Susceptibility testing of topical antibiotics

MIC breakpoints, specifically for topical antibiotics, are not given because the Working Party is unaware of pharmacological, pharmacodynamic or clinical response data on which to base recommendations. The Working Party would be very grateful for relevant data to be submitted to it for consideration.

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 β-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; TCS Biosciences Ltd. Buckingham,UK).

1. Maintenance of control strains.

Store control strains to minimise the risk of mutations, for example, at -70^{0}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 ± 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

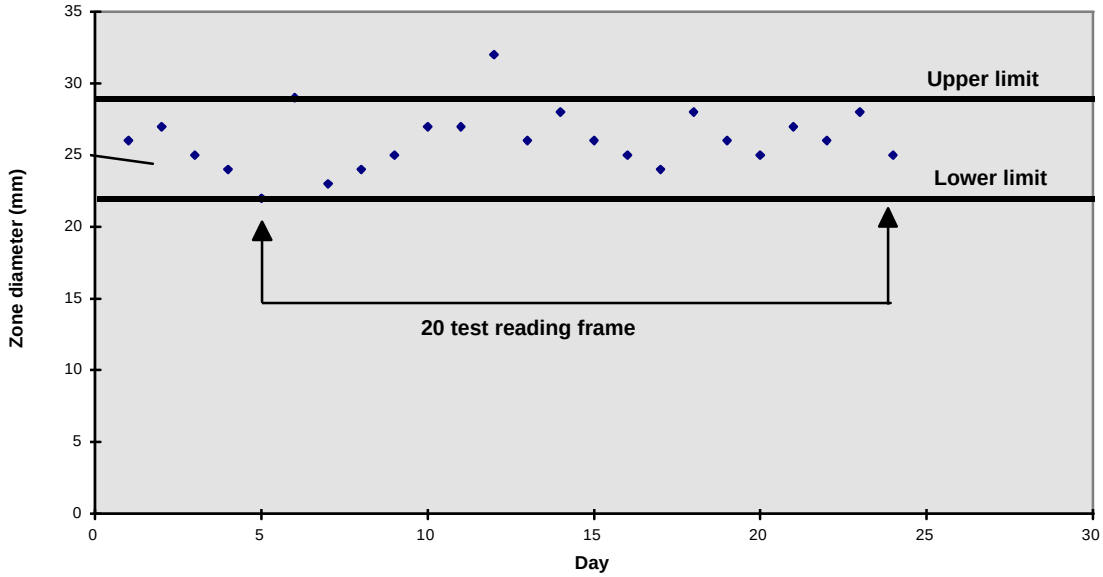
When the method is first introduced, daily testing is required until there are acceptable readings from 20 consecutive days (this also applies when new agents are introduced or when any test component changes). This provides sufficient data to support once weekly testing. If any result from weekly

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 than1 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.

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



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	Labelling
		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 β-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 ^{0}C in air for 18-20 h.

Antibiotic	Disc content (µg unless stated)	*E. coli* NCTC 10418	*E. coli* ATCC 25922	*E. coli* NCTC 11560*	*Ps. aeruginosa* NCTC 10662	*Ps. aeruginosa* ATCC 27853	*S. aureus* NCTC 6571	*S. aureus* ATCC 25923	*E. faecalis* 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^{0}C in air for 18-20 h.

		S. aureus	
Antibiotic	Disc content (μg unless stated)	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.

			S. aureus	
			NCTC	ATCC
Antibiotic	Media	Disc content (μg)	6571	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^{0}C in 4-6% CO_2 for 18-20 h.

		S. aureus		*H. influenzae* (with NAD)		*S. pneumoniae*
Antibiotic	Disc content (μg unless stated)	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-amoxyclav	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[1]

Table 1. Expected MICs for control strains with BSAC methodology[1]

Antibiotic	*H. influenzae* NCTC 11931	*H. influenzae* ATCC 49247	*Ent. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*B. fragilis* NCTC 9343	*N. gonorrhoeae* ATCC 49226
Amikacin			128			
Gentamicin			8		128	
Tobramycin			16			
Azithromycin	2	2		0.12		
Amoxycillin	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-amoxyclav	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	
Piperacillin			2		2	

Antibiotic	***H. influenzae*** **NCTC 11931**	***H. influenzae*** **ATCC 49247**	***Ent. faecalis*** **ATCC 29212**	***S. pneumoniae*** **ATCC 49619**	***B. fragilis*** **NCTC 9343**	***N. gonorrhoeae*** **ATCC 49226**
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[1]

Antibiotic	*Esch. coli* NCTC 10418	*Esch .coli* ATCC 25922	*Ps. aeruginosa* NCTC 10662	*Ps. aeruginosa* ATCC 27853	*S. aureus* NCTC 6571	*S. aureus* ATCC 25923	*S. aureus* 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
Amoxycillin	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-amoxyclav	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	***Esch. coli* NCTC 10418**	***Esch .coli* ATCC 25922**	***Ps. aeruginosa* NCTC 10662**	***Ps. aeruginosa* ATCC 27853**	***S. aureus* NCTC 6571**	***S. aureus* ATCC 25923**	***S. aureus* 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		
Grepafloxacin	0.03	0.03	0.5		0.03		

Antibiotic	*Esch. coli* NCTC 10418	*Esch .coli* ATCC 25922	*Ps. aeruginosa* NCTC 10662	*Ps. aeruginosa* ATCC 27853	*S. aureus* NCTC 6571	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213
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

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

Useful web sites

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

JAC: The Journal of Antimicrobial Chemotherapy.

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Working Party on Susceptibility Testing of the

British Society for Antimicrobial Chemotherapy.

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