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BSAC standardized disc susceptibility testing method (version 5)

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Preface

The interpretative criteria have been expanded to include recommendations for testing *Stenotrophomonas maltophilia*, *Campylobacter* spp.[1] and Coryneform organisms and there is limited information for the fast-growing anaerobes *Bacteroides fragilis*, *Bacteroides thetaiotaomicron* and *Clostridium perfringens*. In the section 'Additional Information' advice on testing *Helicobacter pylori*, *Brucella* and *Legionella* spp. can be found.[1]

It is the intention of the British Society for Antimicrobial Chemotherapy (BSAC) and the *Journal of Antimicrobial Chemotherapy* to publish this document annually. However, as with all methods, it will require constant review and updating. We therefore advise that all interested parties frequently consult the BSAC website (http://www.bsac.org.uk) where the latest updates will be made available.

New or altered text compared with version 4[2] is indicated in boldface.

Introduction

The *BSAC Guide to Sensitivity Testing* was first published in 1991 and one of its most important sections was that dealing with breakpoints for clinically relevant bacteria.[3] These breakpoints have been used extensively to interpret MIC results and for single concentration 'breakpoint' tests. However, a criticism of the guidelines was that they did not provide a standardized method of disc diffusion testing with zone limits that correlated with these MIC breakpoints. The limitations of the widely used Stokes' comparative method were also a cause for concern.

The task of developing such a method of disc testing is immense and the Working Party and the Council of the BSAC needed evidence that there was sufficient interest to warrant the investment required not only in the short term, but also for continuing support and development. This necessary confirmation was obtained from a questionnaire survey,[4] which indicated that 90.6% of UK laboratories would be prepared to switch to an upgraded disc test, and the development and 'field testing' of the standardized method were therefore undertaken.[5]

Fortuitously, the introduction of the standardized method has coincided with the availability of automated zone measuring devices, which aid measuring and interpretation considerably. With laboratories using the same method there is a real opportunity to combine zone diameter data, so that levels of resistance in the UK and Ireland can be surveyed and subtle changes in susceptibility detected.

The method, like all standardized disc tests, cannot be adapted by the user, with the exception that various methods of inoculum preparation can be used to achieve semi-confluent growth.

For microorganisms not included in this document, work is either ongoing (e.g. anaerobes) or reported elsewhere (e.g. mycobacteria).[6]

1 Preparation of plates

1.1 Prepare Iso-Sensitest agar (ISA; Oxoid, Basingstoke, UK), or media shown to have the same performance as ISA, according to the manufacturer's instructions. Supplement media for fastidious organisms with 5% defibrinated horse blood or 5% defibrinated horse blood and 20 mg/L β-nicotinamide adenine dinucleotide [NAD] as indicated in Table 1. **Use Columbia agar with 2% NaCl for methicillin/oxacillin susceptibility testing of staphylococci.**
1.2 Pour sufficient molten agar into sterile Petri dishes to give a depth of 4 ± 0.5 mm (25 mL in a 90 mm Petri dish).
1.3 Dry the surface of the agar to remove excess moisture before use. The length of time needed to dry the surface of the agar depends on the drying conditions, e.g. whether a fan-assisted drying cabinet or 'still air' incubator is used, whether plates are dried before storage and storage conditions. **It is important that plates are not over-dried.**
1.4 Store the plates in vented plastic boxes at 8–10°C prior to use. Alternatively the plates may be stored at 4–8°C in sealed plastic bags. Plate drying, method of storage and storage time should be determined by individual laboratories as part of their

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

Organisms	Medium to be used
Enterobacteriaceae	ISA
Pseudomonas spp.	ISA
Stenotrophomonas maltophilia	ISA
Staphylococci (other than methicillin/oxacillin)	ISA
Staphylococcus aureus (tests using cefoxitin to detect methicillin/oxacillin/cefoxitin resistance)	ISA
Staphylococci (tests using methicillin or oxacillin for the detection of methicillin/oxacillin/cefoxitin resistance[a])	Columbia agar (Oxoid CM331 or equivalent) with 2% NaCl
Enterococci	ISA
Streptococcus pneumoniae	ISA + 5% defibrinated horse blood[b]
α-Haemolytic streptococci	ISA + 5% defibrinated horse blood + 20 mg/L NAD
β-Haemolytic streptococci	ISA + 5% defibrinated horse blood[b]
Moxarella catarrhalis	ISA + 5% defibrinated horse blood[b]
Haemophilus spp.	ISA + 5% defibrinated horse blood + 20 mg/L NAD
Neisseria gonorrhoeae	ISA + 5% defibrinated horse blood[b]
Neisseria meningitidis	ISA + 5% defibrinated horse blood[b]
Pasteurella multocida	ISA + 5% defibrinated horse blood + 20 mg/L NAD
Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Clostridium perfringens	**ISA + 5% defibrinated horse blood + 20 mg/L NAD**
***Campylobacter* spp.**	**ISA + 5% defibrinated horse blood[b]**
Coryneform organisms	**ISA + 5% defibrinated horse blood + 20 mg/L NAD**

[a]See refs 5 and 7.
[b]ISA supplemented with 5% defibrinated horse blood + 20 mg/L NAD may be used for testing.

Table 2. Control strains for antimicrobial susceptibility testing

Organism	Strain		Characteristics
	either	or	
Escherichia coli	NCTC 12241 (ATCC 25922)	NCTC 10418	**susceptible**
Escherichia coli	NCTC 11560		**TEM-1 β-lactamase producer**
Staphylococcus aureus	NCTC 12981 (ATCC 25923)	NCTC 6571	**susceptible**
Staphylococcus aureus	NCTC 12493		***mecA*-positive, methicillin resistant**
Pseudomonas aeruginosa	NCTC 12934 (ATCC 27853)	NCTC 10662	**susceptible**
Enterococcus faecalis	NCTC 12697 (ATCC 29212)		**susceptible**
Haemophilus influenzae	NCTC 11931		**susceptible**
Haemophilus influenzae	NCTC 12699 (ATCC 49247)		**resistant to β-lactams (β-lactamase negative)**
Streptococcus pneumoniae	NCTC 12977 (ATCC 49619)		**intermediate resistance to penicillin**
Neisseria gonorrhoeae	NCTC 12700 (ATCC 49226)		**low-level resistant to penicillin**
Pasteurella multocida	NCTC 8489		**susceptible**
Bacteroides fragilis	**NCTC 9343 (ATCC 25285)**		**susceptible**
Bacteroides thetaiotaomicron	**ATCC 29741**		**susceptible**
Clostridium perfringens	**NCTC 8359 (ATCC 12915)**		**susceptible**

quality assurance programme. In particular, quality control tests should confirm that excess surface moisture is not produced and that plates are not over-dried.

2 Selection of control organisms

2.1 The performance of the tests should be monitored by the use of appropriate control strains. The control strains listed (Table 2) include susceptible strains that have been chosen to monitor test performance and resistant strains that can be used to confirm that the method will detect phenotypically resistant isolates.

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

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

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

Table 4. Preparation of inoculum

Organisms	Optical density 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	0.1–0.3	125
fastidious organisms	0.3–0.6	80
	0.6–1.0	40

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

3 Preparation of inoculum

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

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

3.1 Comparison with 0.5 McFarland standard

3.1.1 Preparation of the McFarland standard

Add 0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.2H_2O$) to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring. Thoroughly mix the McFarland standard to ensure that it is evenly suspended. Using matched cuvettes with a 1 cm light path and water as a blank standard, measure the optical density in a spectrophotometer at a wavelength of 625 nm. The acceptable range for the standard is 0.08–0.13. Distribute the standard into screw cap tubes of the same size and volume as those used to prepare the test inoculum. 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 6 months, after which time they should be discarded. Alternatively, prepared standards can be purchased (e.g. from bioMérieux, Basingstoke, UK) but commercial standards should be checked to ensure that absorbance is within the acceptable range as indicated above.

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

Touch at least four morphologically similar colonies with a sterile loop. Transfer the growth into Iso-Sensitest broth or an equivalent that has been shown to have no adverse effect on the test. Incubate the 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, i.e. Haemophilus spp., Neisseria gonorrhoeae, ***Neisseria meningitidis, Moraxella catarrhalis,*** *Streptococcus pneumoniae,* ***α- and β-haemolytic streptococci, Clostridium perfringens, Bacteroides fragilis, Bacteroides thetaiotaomicron, Campylobacter spp., Pasteurella multocida and Coryneform organisms****)*

Colonies are taken directly from the plate into Iso-Sensitest broth (or equivalent) or distilled water. The suspension should match or exceed the density of the 0.5 McFarland standard. Note that 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. Note that the suspension should be used within 15 min.

3.2 Dilution of suspension adjusted to the turbidity of a 0.5 McFarland standard

See Table 3 for details. These suspensions should be used with 15 min of preparation.

3.3 Photometric standardization of turbidity of suspensions

A photometric method of preparing inocula was described by Moosdeen *et al.*[8] and from this the following simplified procedure has been developed.

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

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

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

Table 6. MIC and zone breakpoints for Enterobacteriaceae (including *Salmonella* and *Shigella* spp.) and *Acinetobacter* spp.

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Amikacin[a]	16	16	8	30	15	16–18	19
Amoxicillin[b]	16	16	8	10	11	12–14	15
Ampicillin[b]	16	16	8	10	11	12–14	15
Aztreonam[c]	1	–	1	30	23	–	24
Cefaclor	1	–	1	30	34	–	35
Cefamandole[d,e]	8	–	8	30	19	–	20
Cefepime	1	–	1	30	31	–	32
Cefixime	1	–	1	5	19	–	20
Cefoperazone[d]	4	–	4	30	24	–	25
Cefotaxime	1	–	1	30	29	–	30
Cefotetan[d]	4	–	4	30	23	–	24
Cefoxitin[e]	8	–	8	30	19	–	20
Cefpirome	1	–	1	20	24	–	25
Cefpodoxime[f,g]	1	–	1	10	**19**	–	**20**
Ceftazidime	2	–	2	30	27	–	28
Ceftazidime[h,i]	2	–	2	30	21	–	22
Ceftibuten	1	–	1	10	27	–	28
Ceftizoxime	1	–	1	30	29	–	30
Ceftriaxone	1	–	1	30	27	–	28
Cefuroxime (axetil)	1	–	1	30	24	–	25
Cefuroxime (parenteral)	8	–	8	30	19	–	20
Cefalotin[e]	8	–	8	30	26	–	27
Cefradine[e]	8	–	8	30	11	–	12
Chloramphenicol	8	–	8	30	20	–	21
Ciprofloxacin [j,k]	1	1	0.5	1	16	17–19	20
Co-amoxiclav[b]	16	16	8	20/10	11	12–14	15

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Table 6. (*Continued*)

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Colistin[l]	4	–	4	25	14	–	15
Co-trimoxazole[m]	32	–	32	25	15	–	16
Doxycycline	1	–	1	30	28	–	29
Ertapenem	2	–	2	10	*27*	–	*28*
Gatifloxacin	1	–	1	2	19	–	20
Gemifloxacin	0.25	–	0.25	1	19	–	20
Gentamicin[a]	4	4	2	10	16	17–19	20
Imipenem[n]	4	–	4	10	22	–	23
Levofloxacin	2	2	1	1	13	14–16	17
Meropenem	4	–	4	10	22	–	23
Mezlocillin	16	–	16	75	21	–	22
Moxifloxacin	1	1	0.5	1	16	17–19	20
Ofloxacin	1	1	0.5	5	25	26–28	29
Piperacillin/tazobactam	16	–	16	75/10	21	–	22
Piperacillin	16	–	16	75	23	–	24
Streptomycin[d]	8	–	8	10	12	–	13
Sulfamethoxazole	32	–	32	100	13	–	14
Ticarcillin/clavulanic acid	16	–	16	85	20	–	21
Tobramycin[a]	4	4	2	10	17	18–20	21
Trimethoprim	2	1–2	0.5	2.5	14	15–19	20

The information in italics is tentative. Breakpoints will remain tentative for 1 year from when first published.
Some problems with testing *Acinetobacter* and *Serratia* spp. have been related to difficulties in achieving the correct inoculum. Once a clinically significant isolate of *Acinetobacter* sp. or *Serratia* sp. has been identified, it might be prudent to determine the susceptibility by an MIC method, or the disc diffusion test must be repeated if the inoculum density is outside the acceptable range.
The identification of Enterobacteriaceae to the species level is essential for the application of expert rules for the interpretation of susceptibility. Species that typically have inducible AmpC enzymes (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella morganii and Providencia* spp.) readily mutate to stably derepressed AmpC production during treatment (in 20% cases with *Enterobacter* spp.), conferring resistance to all first, second and third generation cephalosporins.
[a]**Individual aminoglycoside agents must be tested; susceptibility to other aminoglycosides cannot be inferred from the gentamicin result and *vice versa*.**
[b]These interpretative standards apply only to *Escherichia coli* and *Proteus mirabilis*.
[c]The MIC breakpoint for aztreonam has been set to ensure that ESBL producers with aztreonam MIC values of 4 mg/L are not interpreted as susceptible to this agent.
[d]Zone diameter breakpoints are valid only for *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis*.
[e]The MIC breakpoints have been adjusted to take account of the MIC distribution for the population lacking a mechanism of resistance.
[f]All Enterobacteriaceae isolates should be tested with cefpodoxime or both cefotaxime (or ceftriaxone) and ceftazidime. Enterobacteriaceae with resistance to cefpodoxime, ceftriaxone, cefotaxime or ceftazidime should be tested for the presence of ESBLs. Organisms inferred to have ESBLs should be reported as resistant to all penicillins (except temocillin) and cephalosporins, including the fourth-generation cephalosporins cefepime and cefpirome. For serious infections, carbapenems (imipenem, meropenem and ertapenem) are the treatment of choice.
[g]**Organisms with cefpodoxime zone diameters of <20 mm have a substantive mechanism of resistance. Organisms with zone diameters of 21–25 mm are uncommonly ESBL-producers and may require further investigation.**
[h]**These interpretative standards apply only to *Escherichia coli* and *Klebsiell*a spp.**
[i]Isolates of *Escherichia coli* and *Klebsiella* spp. have been identified with ceftazidime MICs of 1 mg/L, which is higher than those for the 'wild susceptible' population (c. 0.12 mg/L). These isolates do not possess extended-spectrum β-lactamases and until a mechanism of resistance has been identified the zone diameter breakpoint is tentative.
[j]Isolates of *Escherichia coli* and *Klebsiella* spp. with ciprofloxacin MICs of 0.25 and 0.5 mg/L might be reported as resistant by disc testing. These MICs are higher than those for the 'wild susceptible' populations for the species and may indicate a mechanism of resistance with clinical significance.
[k]For ciprofloxacin, there is clinical evidence to indicate a poor response in systemic infections caused by *Salmonella* spp. with reduced susceptibility to fluoroquinolones (ciprofloxacin MICs 0.125–1 mg/L). This reduced susceptibility is most reliably detected with nalidixic acid 30 µg discs as isolates with reduced susceptibility show no zone of inhibition.
[l]Some strains of Enterobacteriaceae (particularly *Serratia*, *Providencia*, *Citrobacter* and *Enterobacter* spp.) produce clear zones of inhibition with small colonies around the colistin disc. These isolates are resistant as the MICs typically exceed 128 mg/L.
[m]**MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim**. For advice on testing susceptibility to co-trimoxazole, see Appendix 1.
[n]***Proteus* spp. and *Morganella morganii* are considered poor targets for imipenem.**

3.3.1 Suspend colonies (touch 4–5 when possible) in 3 mL distilled water or broth in a 100 × 12 mm glass tube (note that tubes are not reused) to give turbidity that is just visible. Do not leave the organisms standing in water. It is essential to get an even suspension.

3.3.2 Zero the spectrophotometer with a sterile water or broth blank (as appropriate) at a wavelength of 500 nm. Measure the optical density of the bacterial suspension. (The spectrophotometer must have a cellholder for 100 × 12 mm test tubes. A much simpler photometer would also probably be acceptable.

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Table 7. MIC and zone diameter breakpoints for *Pseudomonas* spp. and *Stenotrophomonas maltophilia*

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Amikacin	16	16	8	30	15	16–18	19
Aztreonam	8	–	8	30	22	–	23
Carbenicillin	128	–	128	100	12	–	13
Cefotaxime	1	–	1	30	26	–	27
Cefpirome	1	–	1	20	19	20–24	25
Ceftazidime	8	–	8	30	23	–	24
Ceftriaxone	1	–	1	30	29	–	30
Ciprofloxacin	1	1	0.5	1	12	13–22	23
Ciprofloxacin	1	1	0.5	5	19	20–29	30
Colistin	4	–	4	25	13	–	14
Gatifloxacin	1	–	1	2	19	–	20
Co-trimoxazole[a]	**32**	–	**32**	**25**	**19**	–	**20**
Gemifloxacin	0.25	–	0.25	5	19	–	20
Gentamicin	4	–	4	10	17	–	18
Imipenem[b]	4	–	4	10	21	–	22
Levofloxacin	2	2	1	5	16	17–21	22
Meropenem[b]	4	–	4	10	21	–	22
Moxifloxacin	4	2–4	1	5	17	18–24	25
Netilmicin	4	2–4	1	30	15	16–18	19
Piperacillin	16	–	16	75	23	–	24
Piperacillin/tazobactam	16	–	16	75/10	21	–	22
Ticarcillin	64	32–64	16	75	19	–	20
Ticarcillin/clavulanic acid	64	32–64	16	85	19	–	20
Tobramycin	4	–	4	10	19	–	20

[a]For *Stenotrophomonas maltophilia*, susceptibility testing is not recommended except for co-trimoxazole (see www.bsac.org.uk BSAC Standardized Susceptibility Testing Method, Additional Methodology, *Stenotrophomonas maltophilia*). MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

[b]The detection of resistance mediated by carbapenemase is difficult, particularly if resistance is not fully expressed. **Refer carbapenem/ceftazidime-resistant isolates to a Reference Laboratory.**

The 100 × 12 mm test tubes could also be replaced with another tube/cuvette system if required, but the dilutions would need to be recalibrated.)

3.3.3 From Table 4 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.)

3.4 Direct susceptibility testing

The Working Party does not advocate direct susceptibility testing, as the control of inoculum is impossible. However, we are aware that this is a common practice in many laboratories and therefore we are suggesting methods that will achieve the correct inoculum size for a reasonable proportion of infected urines. The following methods have been developed and recommended by laboratories that use the BSAC method and we suggest adopting whichever method best suits individual laboratory working practice. If the inoculum is not correct and growth is not semi-confluent, or the culture is mixed, the test must be repeated.

3.4.1 Direct susceptibility testing of urines

(i) Method 1: thoroughly mix the urine, place a 10 μL loop of urine in the centre of the susceptibility plate and spread with a dry swab.
(ii) Method 2: thoroughly mix the urine, then dip a sterile cotton-wool swab in the urine and remove excess. Make a cross in the centre of the susceptibility plate then spread with a sterile dry swab. If only small numbers of organisms are seen under the microscope, the initial cotton-wool swab may be used to inoculate and spread the susceptibility plate.

3.4.2.1 *Direct susceptibility testing of positive blood cultures*
The method suggested gives the correct inoculum size for a reasonable proportion of positive blood cultures. The method varies according to the Gram reaction of the infecting organism.
3.4.2.2 *Gram-negative bacilli*
Using a venting needle, place one drop in 5 mL of sterile water and use this to inoculate Iso-Sensitest or equivalent agar.
3.4.2.2.1 *Gram-positive organisms*
It is not always possible to accurately assume the genera of Gram-positive organisms from the Gram's stain. However, careful observation of the morphology, coupled with some clinical information, should make an 'educated guess' correct most of the time.

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Table 8. MIC and zone diameter breakpoints for staphylococci

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg unless stated)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Amikacin (*Staphylococcus aureus*)	16	16	8	30	15	16–18	19
Amikacin (coagulase-negative staphylococci)	16	16	8	30	21	22–24	25
Azithromycin	1	–	1	15	19	–	20
Cefoxitin[a] *Staphylococcus aureus*	4	–	4	10	21	–	22
Chloramphenicol	8	–	8	10	14	–	15
Ciprofloxacin[b]	1	–	1	1	17	–	18
Clarithromycin	0.5	–	0.5	2	19	–	20
Clindamycin[c]	0.5	–	0.5	2	25	–	26
Co-amoxiclav[a]	1	–	1	3	17	–	18
Co-trimoxazole[d,e]	32		32	25	16	–	17
Erythromycin	0.5	–	0.5	5	19	–	20
Fusidic acid	1	–	1	10	29	–	30
Gatifloxacin	1	–	1	2	19	–	20
Gemifloxacin	0.25	–	0.25	1	19	–	20
Gentamicin	1	–	1	10	19	–	20
Linezolid[e]	4	–	4	10	19	–	20
Methicillin[a,f]	4	–	4	5	14	–	15
Moxifloxacin	1	–	1	1	19	–	20
Mupirocin [g,h]	4	–	4	5	21	–	22
Mupirocin[h]	256	8–256	4	20	6	7–26	27
Neomycin	–	–	–	10	16	–	17
Ofloxacin	1	–	1	5	27	–	28
Oxacillin[a,f,i,j]	2	–	2	1	14	–	15
Penicillin[j]	0.12	–	0.12	1 U	24	–	25
Quinupristin/dalfopristin[k]	2	–	2	15	19	–	20
Rifampicin	0.06	–	0.06	2	29	–	30
Teicoplanin[l,m]	8	8	4	30	14	–	15
Telithromycin	0.5	–	0.5	15	26	–	27
Tetracycline	1	–	1	10	19	–	20
Tobramycin for *Staphylococcus aureus*	1	–	1	10	20	–	21
Tobramycin for coagulase-negative staphylococci	1	–	1	10	29	–	30
Trimethoprim[n]	0.5	–	0.5	5	19	–	20
Vancomycin[m]	8	8	4	5	11	–	12

[a]Staphylococci exhibiting resistance to methicillin/oxacillin/cefoxitin should be regarded as resistant to other penicillins, cephalosporins, carbapenems and combinations of β-lactam and β-lactamase inhibitors. Some hyper-producers of β-lactamase give zones within the range of 7–14 mm and if possible, should be checked by a PCR method for *mecA* or a latex agglutination test 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.
[b]MIC breakpoints relate to high-dose therapy (750 mg oral twice daily).
[c]Organisms that appear resistant to erythromycin, but susceptible to clindamycin, should be checked for the presence of inducible resistance (see www.bsac.org.uk/Susceptibility Testing/BSAC Standardized Disc Susceptibility Method/Additional Methods).
[d]For advice on testing for susceptibility to co-trimoxazole see Appendix 1. **MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.**
[e]Information on clinical response in patients with serious staphylococcal infections is not yet available. In such patients an MIC determination might be appropriate.
[f]Recommendations for tests on Mueller–Hinton or Columbia agars with 2% NaCl.
[g]An Etest or other MIC method should be performed on any strain designated mupirocin resistant when tested with a 5 μg disc. The MIC will indicate whether the strain has low-level (MIC 8–256 mg/L) or high-level (MIC ≥ 512 mg/L) resistance.
[h]Isolates with low-level resistance to mupirocin (MICs 8–256 mg/L) may be eradicated more slowly than susceptible isolates.[9]
[i]MIC breakpoint for coagulase-negative staphylococci is currently under review.
[j]Penicillin; check for heaped zone edge (=resistant).
[k]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, resistant ≤ 14 mm. The corresponding MIC breakpoints are the same.
[l]Teicoplanin—disc testing not recommended for coagulase-negative staphylococci. An MIC method should be used to determine susceptibility.
[m]Glycopeptide-intermediate *Staphylococcus aureus* (GISA) cannot be detected by the BSAC disc method or any other disc diffusion method. The Etest 'macro-method' (see **www.bsac.org.uk/Susceptibility Testing/BSAC Standardized Disc Susceptibility Method/Additional Methods**) may be used to screen for GISA and GISA with heterogenous resistance to vancomycin (hetero-GISA) but positive results require confirmation. Population analysis is the most reliable method for confirming resistance and for distinguishing susceptible, hetero-GISA and GISA isolates. 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.
[n]Amended zone diameter breakpoints are microbiological breakpoints based on the MIC distribution for the wild-type population. However, there is no clear evidence correlating these breakpoints with clinical efficacy.

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Table 9. MIC and zone diameter breakpoints for *Streptococcus pneumoniae*

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Azithromycin	1	–	1	15	19	–	20
Cefaclor[a]	1	–	1	30	24	–	25
Cefixime[a]	1	–	1	5	19	–	20
Cefotaxime[a]	1	–	1	5	29	–	30
Cefpodoxime[a]	1	–	1	1	21	–	22
Ceftibuten[a]	1	–	1	10	27	–	28
Ceftizoxime[a]	1	–	1	30	29	–	30
Ceftriaxone[a]	1	–	1	30	27	–	28
Cefuroxime[a]	1	–	1	5	24	–	25
Cefadroxil[a]	1	–	1	30	24	–	25
Cefalexin[a]	2	–	2	30	24	–	25
Chloramphenicol	8	–	8	10	17	–	18
Ciprofloxacin	2	0.25–2	0.12	1	9	10–24	25
Clarithromycin	0.5	–	0.5	2	19	–	20
Co-trimoxazole[b]	32	–	32	25	16	–	17
Ertapenem[a]	1	0.06–1	0.03	10	27	28–39	40
Erythromycin	0.5	–	0.5	5	19	–	20
Gatifloxacin	1	–	1	2	19	–	20
Gemifloxacin	0.25	–	0.25	1	19	–	20
Imipenem[a]	4	–	4	10	24	–	25
Levofloxacin	2	–	2	1	9	–	10
Linezolid	4	4	2	10	19	–	20
Meropenem[a]	4	–	4	10	27	–	28
Moxifloxacin	0.5	–	0.5	1	17	–	18
Ofloxacin	4	0.25–4	0.12	5	15	16–27	28
Penicillin[c]	1	0.12–1	0.06	oxacillin 1	19	–	20
Quinupristin/dalfopristin	2	–	2	15	19	–	20
Rifampicin	1	–	1	5	21	–	22
Telithromycin	0.5	–	0.5	15	28	–	29
Tetracycline	1	–	1	10	19	–	20
Vancomycin	4	–	4	5	12	–	13

[a]Organisms with reduced susceptibility to penicillin: confirm resistance with a test for penicillin MIC. Organisms for which penicillin MIC ≤ 1 mg/L are considered susceptible to β-lactam agents except in infections of the CNS. In addition, cefotaxime MIC determination is advised for strains isolated from meningitis or other invasive infections.
[b]For advice on testing susceptibility to co-trimoxazole see Appendix 1. **MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.**
[c]Penicillin resistance in *Streptococcus pneumoniae* is detected with an oxacillin 1 μg disc.

3.4.2.2.2 *Staphylococci and Enterococci*
Using a venting needle, place three drops in 5 mL of sterile water and use this to inoculate Iso-Sensitest or equivalent agar.
3.4.2.2.3 *Pneumococci, 'viridans' streptococci and diphtheroids*
Using a venting needle, place one drop in the centre of an Iso-Sensitest or equivalent agar supplemented with 5% horse blood, and spread evenly over the entire surface of the plate. If the inoculum is not correct and growth is not semi confluent, or the culture is mixed, the test must be repeated.

4 Inoculation of agar plates

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

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

4.2 *Use of rotary platers for susceptibility testing*

Rotary platers can be used for inoculating susceptibility tests but care must be taken. The swab must be moved at an even pace to ensure that the inoculum is semi-confluent and that no gaps are present between the swab streaks.

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Table 10. MIC and zone diameter breakpoints for *Enterococci*

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (µg)	Interpretation of zone diameters (mm) R≤	I	S≥
Ampicillin	8	–	8	10	19	–	20
Azithromycin	1	–	1	15	29	–	30
Gentamicin[a]	128	–	128	200	*14*	–	*15*
Imipenem	4	–	4	10	19	–	20
Linezolid	4	–	4	10	19	–	20
Meropenem	4	–	4	10	19	–	20
Quinupristin/dalfopristin[b]	2	–	2	15	19	–	20
Teicoplanin[c]	8	8	4	30	19	–	20
Vancomycin[c]	8	8	4	5	12	–	13

The information in italics is tentative. Breakpoints will remain tentative for 1 year from when first published.
[a]High-level gentamicin-resistant Enterococci usually give no zone or only a trace of inhibition around gentamicin 200 µg discs. Occasionally, however, the plasmid carrying the resistance gene 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.
[b]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, resistant = ≤14 mm. MIC breakpoint recommendations will follow.
[c]It is essential that plates be incubated for at least 24 h before reporting a strain as susceptible to vancomycin or teicoplanin.

Table 11. MIC and zone diameter breakpoints for α-haemolytic streptococci

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (µg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Amoxicillin	1	–	1	2	19	–	20
Cefotaxime	1	–	1	5	20	–	21
Clindamycin	0.5	–	0.5	2	19	–	20
Erythromycin	0.5	–	0.5	5	19	–	20
Linezolid	2	–	2	10	19	–	20
Penicillin	0.12	–	0.12	1 U	21	–	22
Teicoplanin	4	–	4	30	15	–	16
Tetracycline	1	–	1	10	23	–	24
Vancomycin	4	–	4	5	13	–	14

5 Antimicrobial discs

5.1 Disc contents are given in Tables 6–22.
5.2 *Storage and handling of discs*
Loss of potency from discs will result in reduced zones of inhibition. To avoid loss of potency as a result of improper handling the following procedures are essential.
5.2.1 Store discs in sealed containers with a desiccant and protected from light (this is particularly important for some light-susceptible agents such as metronidazole, chloramphenicol and the quinolones).
5.2.2 Store stocks at –20°C except for drugs known to be unstable at this temperature (refer to the disc manufacturer's instructions on disc storage). If this is not possible, store discs at <8°C.
5.2.3 Store working supplies of discs at <8°C.
5.2.4 To prevent condensation, allow discs to warm to room temperature before opening containers.
5.2.5. Store disc dispensers in sealed containers with an indicating desiccant.
5.2.6. Discard any discs on the expiry date shown on the side of the container.

5.3 Application of discs

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

6 Incubation

6.1 If the 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.

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Table 12. MIC and zone diameter breakpoints for β-haemolytic streptococci

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg unless stated)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Azithromycin	1	–	1	15	19	–	20
Cefadroxil	1	–	1	30	24	–	25
Cefixime	1	–	1	5	19	–	20
Cefotaxime	1	–	1	5	27	–	28
Cefalexin	2	–	2	30	24	–	25
Cefalotin	1	–	1	30	28	–	29
Clarithromycin	0.5	–	0.5	2	19	–	20
Co-trimoxazole[a]	32	–	32	25	16	–	17
Ertapenem	2	–	2	10	34	–	35
Erythromycin	0.5	–	0.5	5	19	–	20
Linezolid	4	4	2	10	19	–	20
Penicillin	0.12	–	0.12	1 U	19	–	20
Telithromycin	0.5	–	0.5	15	25	–	26
Tetracycline	1	–	1	10	19	–	20

[a]For advice on testing susceptibility to co-trimoxazole see Appendix 1. **MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.**

Table 13. MIC and zone diameter breakpoints for *Moraxella catarrhalis*

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Ampicillin[a]	1	–	1	2	29	–	30
Cefaclor	1	–	1	30	22	–	23
Cefuroxime	1	–	1	5	19	–	20
Chloramphenicol	2	–	2	10	22	–	23
Ciprofloxacin[b]	0.5	–	0.5	1	17	–	18
Clarithromycin	0.5	–	0.5	2	19	–	20
Co-amoxiclav	1	–	1	2/1	18	–	19
Co-trimoxazole[c]	32		32	25	11	–	12
Ertapenem	2	–	2	10	34	–	35
Erythromycin	0.5	–	0.5	5	27	–	28
Gatifloxacin[b]	1	–	1	2	19	–	20
Gemifloxacin[b]	0.25	–	0.25	1	19	–	20
Levofloxacin	1	–	1	1	19	–	20
Linezolid	4	–	4	10	19	–	20
Moxifloxacin[b]	0.5	–	0.5	1	17	–	18
Ofloxacin[b]	0.5	–	0.5	5	34	–	35
Telithromycin	0.5	–	0.5	15	29	–	30
Tetracycline	1	–	1	10	21	–	22

[a]Test for β-lactamase. β-Lactamase-positive isolates of *Moraxella 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 (see manufacturer's instructions for use).
[b]Quinolone resistance is most reliably detected with nalidixic acid discs. Isolates with reduced susceptibility to fluoroquinolones show no zone of inhibition with nalidixic acid.
[c]For advice on testing susceptibility to co-trimoxazole, see Appendix 1. **MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.**

6.2 Conditions of incubation

Conditions of incubation for different organisms are summarized in Table 5. Stacking plates too high in the incubator may affect results owing to uneven heating of plates. The efficiency of heating of plates depends on the incubator and the racking system used. Control of incubation, including height of plate stacking, should therefore be part of the laboratory's Quality Assurance programme.

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Table 14. MIC and zone diameter breakpoints for *Neisseria gonorrhoeae*

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (µg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Azithromycin	1	–	1	15	27	–	28
Cefixime[a]	1	–	1	5	29	–	30
Cefotaxime[a]	1	–	1	5	29	–	30
Ceftriaxone[a]	0.25	–	0.25	5	34	–	35
Cefuroxime	1	–	1	5	19	–	20
Ciprofloxacin[b,c]	0.06	0.06	0.03	1	28	–	29
Erythromycin	0.5	–	0.5	5	11	–	12
Nalidixic acid[b]	–	–	–	30	*6*	*7–31*	*32*
Penicillin[d]	1	0.12–1	0.06	1 U	17	18–25	26
Rifampicin	1	–	1	2	20	–	21
Spectinomycin	64	–	64	25	13	–	14
Tetracycline[e]	1	–	1	10	13		27

The information in italics is tentative. Breakpoints will remain tentative for 1 year from when first published.

[a]Resistance to ceftriaxone, cefotaxime and cefixime has not been described. Isolates with chromosomally encoded reduced susceptibility to penicillin have slightly reduced zones of inhibition with these agents but they remain susceptible. Results for isolates with reduced zones around ceftriaxone, cefotaxime and cefixime discs should be confirmed by MIC determinations.

[b]Quinolone resistance is generally reliably detected with nalidixic acid, however there have been a few isolates that are resistant to ciprofloxacin yet susceptible to nalidixic acid in disc diffusion tests. The mechanism of resistance and the prevalence of these isolates in the UK are still under investigation. Isolates with reduced susceptibility to fluoroquinolones normally have no zone of inhibition with a 30 µg nalidixic acid disc. For organisms with nalidixic acid zone diameters 7–31 mm a ciprofloxacin MIC should be determined if the patient is to be treated with this agent.

[c]The MIC breakpoint has been lowered to ensure that isolates with reduced susceptibility to ciprofloxacin are detected.

[d]Test for β-lactamase.

[e]Use the tetracycline result to infer susceptibility to doxycycline. For epidemiological purposes, isolates with plasmid-mediated resistance to tetracycline may be distinguished from those with chromosomal resistance on the basis of zone diameters; isolates with plasmid-mediated resistance have no zones of inhibition and those with low-level chromosomal resistance have zone diameters 14–26 mm.

Table 15. MIC and zone diameter breakpoints for *Neisseria meningitidis*

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (µg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Cefotaxime	1	–	1	5	29	–	30
Chloramphenicol	2	–	2	10	19	–	20
Ciprofloxacin[a]	0.06	0.06	0.03	1	31	–	32
Erythromycin	0.5	–	0.5	5	26	–	27
Penicillin	0.06	–	0.06	1 U	24	–	25
Rifampicin	1	–	1	2	29	–	30
Tetracycline	1	–	1	10	21	–	22

Note. *Neisseria meningitidis* is a category 2 pathogen, but should be regarded as a category 3 pathogen when heavy suspensions are used (DoH Hazard 29 January 1993). Consequently suspension and dilution of organisms and inoculation of plates for susceptibility tests must be carried out in a class 1 safety cabinet.

[a]Quinolone resistance is most reliably detected with nalidixic acid. Isolates with reduced susceptibility to fluoroquinolones have no zone of inhibition with nalidixic acid discs.

7 Measuring zones and interpretation

7.1 Acceptable inoculum density

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

7.2 Measuring zones

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

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

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

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Table 16. MIC and zone diameter breakpoints for *Haemophilus influenzae*

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg unless stated)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Amoxicillin[a]	1	–	1	2	16	–	17
Ampicillin[a]	1	–	1	2	17	–	18
Azithromycin[b]	4	0.5–4	0.25	15	19	20–34	35
Cefaclor[c]	1	–	1	30	36	–	37
Cefotaxime	1	–	1	5	24	–	25
Ceftazidime	2	–	2	30	29	–	30
Ceftriaxone	1	–	1	30	34	–	35
Cefuroxime	1	–	1	5	16	–	17
Chloramphenicol	2	–	2	10	24	–	25
Ciprofloxacin[d]	0.5	–	0.5	1	27	–	28
Clarithromycin	16	1–16	0.5	5	9	10–24	25
Co-amoxiclav	1	–	1	2/1	16	–	17
Co-trimoxazole[e]	32		32	25	21	–	22
Ertapenem	2	–	2	10	29	–	30
Erythromycin	8	1–8	0.5	5	14	15–27	28
Gatifloxacin[d]	1	–	1	2	19	–	20
Gemifloxacin[d]	0.25	–	0.25	1	19	–	20
Imipenem	4	–	4	10	19	–	20
Levofloxacin[d]	1	–	1	1	19	–	20
Meropenem	4	–	4	10	27	–	28
Moxifloxacin[d]	0.5	–	0.5	1	17	–	18
Nalidixic acid[d]	–	–	–	30	–	–	–
Ofloxacin[d]	0.5	–	0.5	5	36	–	37
Telithromycin[f]	2	1–2	0.5	15	15	16–19	20
Tetracycline	1	–	1	10	21	–	22
Trimethoprim	0.5	–	0.5	2.5	20	–	21

[a]Test for β-lactamase.
[b]No resistant strains yet described.
[c]See Appendix 2.
[d]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.
[e]For advice on testing susceptibility to co-trimoxazole see Appendix 1. MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.
[f]The mode telithromycin MIC for these organisms is 1 mg/L; therefore, the majority of isolates will be interpreted as having intermediate susceptibility.

Table 17. MIC and zone diameter breakpoints for *Pasteurella multocida*

Antibiotic	MIC breakpoint (mg/L)			Disc content (μg unless stated)	Interpretation of zone diameters (mm)		
	R>	I	S≤		R≤	I	S≥
Ampicillin	1	–	1	10	29	–	30
Cefotaxime	1	–	1	5	33	–	34
Ciprofloxacin[a]	1	–	1	1	28	–	29
Nalidixic acid	–	–	–	30	27	–	28
Penicillin	0.12	–	0.12	1 U	21	–	22
Tetracycline	1	–	1	10	25	–	26

[a]Quinolone resistance is most reliably detected with nalidixic acid discs.

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Table 18. MIC and zone diameter breakpoints for *Campylobacter* spp.

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (μg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Erythromycin	0.5	–	0.5	5	19	–	20
Ciprofloxacin[a]	1	–	0.5	1	17	–	18

[a]Quinolone resistance is most reliably detected with nalidixic acid discs.

Table 19. MIC and zone diameter breakpoints for Coryneform organisms

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (μg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Ciprofloxacin	1	–	0.5	1	*11*	*12–16*	*17*
Penicillin	0.12	–	0.12	1 U	*19*	–	*20*
Vancomycin	8	–	4	5	*19*	–	*20*

The information in italics is tentative. Breakpoints will remain tentative for 1 year from when first published.

Table 20. MIC and zone diameter breakpoints for *Bacteroides fragilis*, *Bacteroides thetaiotaomicron* and *Clostridium perfringens*

Antibiotic	MIC breakpoint (mg/L) R>	I	S≤	Disc content (μg unless stated)	Interpretation of zone diameters (mm) R≤	I	S≥
Metronidazole	*8*	–	*8*	5	*17*	–	*18*

The information in italics is tentative. Breakpoints will remain tentative for 1 year from when first published.

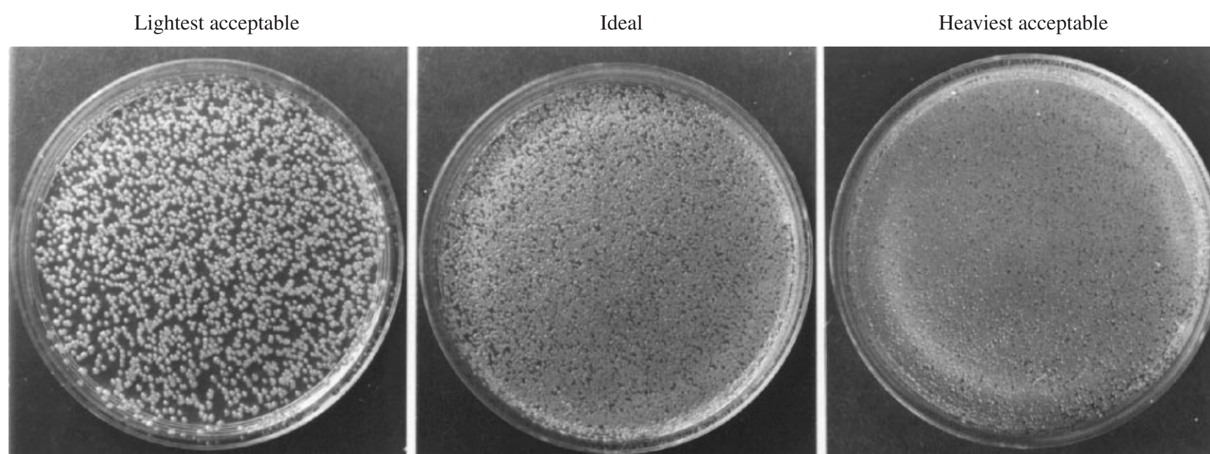

Figure 1. Acceptable inoculum density range for a Gram-negative rod.

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Table 21. MIC and zone diameter breakpoints for Gram-negative rods isolated from urinary tract infections[a–d]

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)								
					coliforms			*Escherichia coli*			*Proteus mirabilis*		
Antibiotic	R>	I	S≤	Disc content (μg)	R≤	I	S≥	R≤	I	S≥	R≤	I	S≥
Amoxicillin[e]	32	–	32	25	11	–	12	11	–	12	11	–	12
Ampicillin[e]	32	–	32	25	11	–	12	11	–	12	11	–	12
Cefalexin[f]	32	–	32	30	–	–	–	15	–	16	11	–	12
Ciprofloxacin	4	–	4	1	19	–	20	19	–	20	19	–	20
Co-amoxiclav[e]	32	–	32	20/10	11	–	12	11	–	12	11	–	12
Fosfomycin[g,h]	128	–	128	200/50	–	–	–	19	–	20	33	–	34
Mecillinam[i]	8	–	8	10	–	–	–	13	–	14	13	–	14
Nalidixic acid	16	–	16	30	17	–	18	17	–	18	17	–	18
Nitrofurantoin	32	–	32	200	–	–	–	19	–	20	–	–	–
Norfloxacin	4	–	4	2	15	–	16	15	–	16	15	–	16
Trimethoprim	2	–	2	2.5	16	–	17	16	–	17	16	–	17

Note. These recommendations are for organisms associated with uncomplicated urinary tract infections. For complicated infections systemic recommendations should be used.
[a]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 (e.g. if the blood isolate is resistant and the urine isolate susceptible, both should be reported resistant irrespective of the results obtained using interpretative criteria for urine isolates).
[b]For agents not listed criteria given for systemic isolates may be used for urinary tract isolates (see Tables 6 and 7).
[c]Direct susceptibility tests on urine samples may be performed as long as the inoculum gives semi-confluent growth.
[d]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.
[e]These interpretative standards apply only to *Escherichia coli* and *Proteus mirabilis* and not to species that have chromosomal penicillinases (*Klebsiella* spp.) or those that typically have inducible AmpC enzymes (e.g. *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp.). The identification of Enterobacteriaceae to the species level is essential before applying expert rules for the interpretation of susceptibility.
[f]Cefalexin results may be used to report susceptibility to cefadroxil.
[g]Fosfomycin/glucose-6-phosphate.
[h]Fosfomycin—the susceptibility of *Proteus spp.* that swarm up to the disc can be difficult to interpret.
[i]Isolates of *Escherichia coli* and *Klebsiella spp.* that produce ESBLs often appear susceptible to mecillinam *in vitro* but clinical efficacy against these organisms is unproven.

Table 22. MIC and zone diameter breakpoints for Gram-positive cocci isolated from urinary tract infections[a,b]

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)								
					Enterococci			*Staphylococcus saprophyticus*			group B streptococci		
Antibiotic	R>	I	S≤	Disc content (μg)	R≤	I	S≥	R≤	I	S≥	R≤	I	S≥
Ampicillin	32	–	32	25	19	–	20	25	–	26	25	–	26
Cefalexin[c]	32	–	32	30	–	–	–	–	–	–	23	–	24
Ciprofloxacin	4	–	4	1	11	–	12	17	–	18	12	–	13
Ciprofloxacin	4	–	4	5	15	–	16	–	–	–	18	–	19
Co-amoxiclav	32	–	32	20/10	20	–	21	27	–	28	27	–	28
Fosfomycin[d]	128	–	128	200/50	19	–	20	19	–	20	–	–	–
Mecillinam	64	–	64	50	–	–	–	9	–	10	–	–	–
Nalidixic acid	16	–	16	30	17	–	18	–	–	–	–	–	–
Nitrofurantoin	32	–	32	200	14	–	15	19	–	20	19	–	20
Norfloxacin	4	–	4	2	15	–	16	–	–	–	–	–	–
Trimethoprim[e]	2	–	2	2.5	21	–	22	14	–	15	15	–	16

Note. These recommendations are for organisms associated with uncomplicated urinary tract infections. For complicated infections and infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are associated with more serious infections, systemic recommendations should be used.
[a]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 (e.g. if the blood isolate is resistant and the urine isolate susceptible, both should be reported resistant irrespective of the results obtained using interpretative criteria for urine isolates).
[b]Direct susceptibility tests on urine samples may be performed as long as the inoculum gives semi-confluent growth.
[c]Cefalexin results may be used to report susceptibility to cefadroxil.
[d]Fosfomycin/glucose-6-phosphate.
[e]There is some doubt about the clinical relevance of testing the susceptibility of Enterococci to trimethoprim.

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Table 23. Acceptable zone diameter ranges for control strains on Iso-Sensitest agar, plates incubated at 35–37°C in air for 18–20 h

Antimicrobial agent	Disc content (μg unless stated)	*Escherichia coli*			*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Enterococcus faecalis*
		NCTC 10418	ATCC 25922	NCTC 11560[a]	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
Ampicillin	25	24–30	21–28	–	–	–	–	–	–
Aztreonam	30	39–44	36–40	–	27–30	26–30	–	–	–
Azithromycin	15	–	–	–	–	–	–	–	15–21
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	–	–	–	–	–	–
Cefalexin	30	21–28	16–21	–	–	–	–	–	–
Cefradine	30	19–25	16–22	–	–	–	–	–	–
Chloramphenicol	10	21–27	20–29	–	–	–	20–26	19–27	–
Ciprofloxacin	1	31–40	31–37	–	21–28	24–30	25–32	17–22	14–19
Ciprofloxacin	5	–	–	–	29–37	31–37	–	–	21–27
Clindamycin	2	–	–	–	–	–	30–35	26–33	no zone
Co-amoxiclav	3	–	–	–	–	–	–	27–32	–
Co-amoxiclav	30	18–31	20–26	12–18	–	–	–	–	–
Colistin	25	15–19	16–20	–	17–20	16–20	–	–	–
Ertapenem	10	35–41	35–39	–	–	–	–	–	–
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	–
Gentamicin	200	–	–	–	–	–	–	–	22–27
Imipenem	10	32–37	33–37	–	20–27	23–28	–	–	28–32
Levofloxacin	1	30–33	28–34	–	–	–	–	–	–
Levofloxacin	5	–	–	–	22–29	23–29	–	–	–
Linezolid	10	–	–	–	–	–	31–35	26–30	24–29
Meropenem	10	38–42	27–39	–	32–39	32–39	–	–	22–28
Mupirocin	5	–	–	–	–	–	26–35	24–34	–
Mupirocin	20	–	–	–	–	–	30–38	27–35	–
Nalidixic acid	30	28–36	26–32	–	–	–	–	–	–
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	2	34–37	32–36	–	–	–	–	–	–
Ofloxacin	5	31–37	31–38	–	18–26	18–25			
Penicillin	1 U	–	–	–	–	–	32–40	29–36	–
Piperacillin	75	30–35	27–32	–	27–35	27–34	–	–	–
Piperacillin/tazobactam	85	30–35	26–31	–	28–35	28–35	–	–	26–32
Quinupristin/dalfopristin	15	–	–	–	–	–	27–31	–	12–19
Rifampicin	2	–	–	–	–	–	27–39	29–36	–
Streptomycin	10	18–24	17–22	–	–	–	–	–	–
Teicoplanin	30	–	–	–	–	–	17–23	16–20	19–25
Tetracycline	10	23–29	22–28	–	–	–	31–40	26–35	–
Ticarcillin	75	32–35	27–30	–	24–28	23–27	–	–	–
Ticarcillin/clavulanic acid	85	33–37	27–31		25–29	24–27	–	–	–
Tobramycin	10	24–27	–	–	23–30	26–32	–	29–35	–
Trimethoprim	2.5	30–37	25–31	–	–	–	25–30	20–28	28–35
	5	–	–	–	–	–	24–34	–	–
Vancomycin	5	–	–	–	–	–	14–20	13–17	13–19

[a]β-Lactamase-producing strain.

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Table 24. Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, plates incubated at 35–37°C in air for 18–20 h

Antimicrobial agent	Disc content (μg unless stated)	*Staphylococcus aureus*	
		NCTC 6571	ATCC 25923
Erythromycin	5	22–29	23–29
Penicillin	1 U	30–41	27–35
Tetracycline	10	30–38	28–36

7.2.4 When using cefoxitin for the detection of methicillin/oxacillin/cefoxitin resistance in *S. aureus*, measure the obvious zone, taking care to examine zones carefully in good light to detect minute colonies that may be present within the zone of inhibition **(see Figure 2).**

7.2.5 Confirm that the zone of inhibition for the control strain falls within the acceptable ranges in Tables 23–27 before interpreting the test.

7.3 A template can also be used for interpreting zone diameters (Figure 3). A program for preparing templates is available from the BSAC (http://www.bsac.org.uk). The test plate is placed over the template and the zones of inhibition are examined in relationship to the template zones. If the zone

Table 25. Acceptable zone diameter ranges for control strains for detection of methicillin/oxacillin/cefoxitin resistance in staphylococci

Antimicrobial agent	Medium	Disc content (μg)	*Staphylococcus aureus*		
			NCTC 6571	ATCC 25923	NCTC 12493[a]
Methicillin	Columbia/Mueller–Hinton agar + 2% NaCl	5	18–30	18–28	no zone
Oxacillin	Columbia/Mueller–Hinton agar + 2% NaCl	1	19–30	19–29	no zone
Cefoxitin	ISA	10	**26–31**	**24–29**	**13–19**

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

Table 26. Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood and NAD, plates incubated at 35–37°C in 10% CO_2/10% H_2/80% N_2 for 18–20 h

Antimicrobial agent	**Disc content (μg unless stated)**	***Bacteroides fragilis* NCTC 9343**	***Bacteroides thetaiotaomicron* ATCC 29741**	***Clostridium perfringens* NCTC 8359**
Metronidazole	**5**	**34–43**	**26–40**	**11–23**

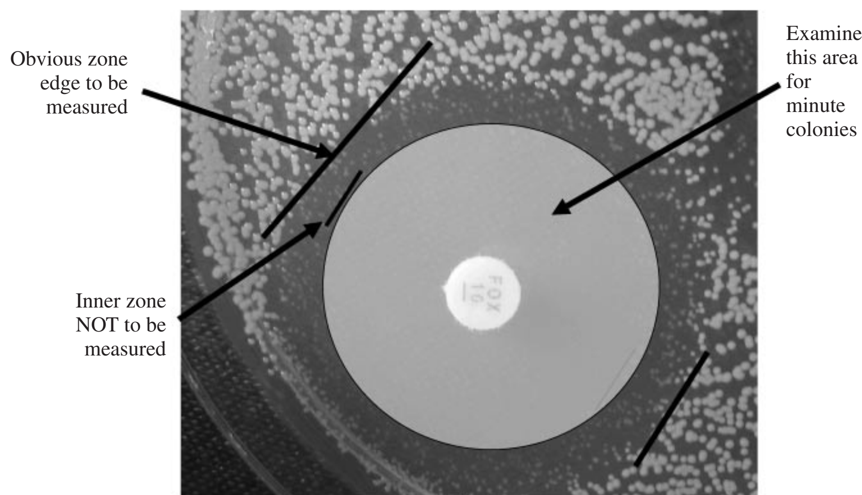

Figure 2. Reading cefoxitin zones of inhibition with *Staphylococcus aureus*.

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Table 27. Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood with or without the addition of NAD, plates incubated at 35–37°C in 4–6% CO_2 for 18–20 h

		Staphylococcus aureus		*Haemophilus influenzae* (with NAD)		*Streptococcus pneumoniae*	*Pasteurella multocida*
Antimicrobial agent	Disc content (μg unless stated)	NCTC 6571	ATCC 25923	NCTC 11931	ATCC 49247	ATCC 49619	NCTC 8489
Amoxicillin	2	29–34	–	–	–	–	–
Ampicillin	2	–	–	22–30	6–13	–	–
Ampicillin	10	–	–	–	–	–	32–37
Azithromycin	15	–	–	24–36	20–30	–	–
Cefotaxime	5	26–32	–	33–45	27–38	–	35–41
Cefuroxime	5	22–29	24–29	22–28	6–16	–	–
Chloramphenicol	10	21–26	–	30–40	30–38	21–29	–
Ciprofloxacin	1	22–29	18–23	32–40	33–44	14–21	31–37
Clindamycin	2	21–25	–	–	–	–	–
Co-amoxiclav	3	–	–	20–27	10–20	–	–
Ertapenem	10	–	–	30–38	25–34	35–40	–
Erythromycin	5	25–29	–	12–23	9–16	23–36	–
Linezolid	10	22–26	–	–	–	–	–
Nalidixic acid	30	9–17	9–17	33–38	–	–	–
Oxacillin	1	–	–	–	–	8–16	–
Penicillin	1 U	37–44	29–36	–	–	–	24–28
Quinupristin/dalfopristin	15	–	–	–		21–29	–
Rifampicin	2	32–37	–	–	–	–	–
Rifampicin	5	–	–	–	–	28–35	–
Teicoplanin	30	14–19	–	–	–	–	–
Tetracycline	10	33–40	27–34	27–35	9–14	26–36	29–34
Trimethoprim	2.5	–	–	30–40	28–36	–	–
Vancomycin	5	12–16	–	–	–	–	–

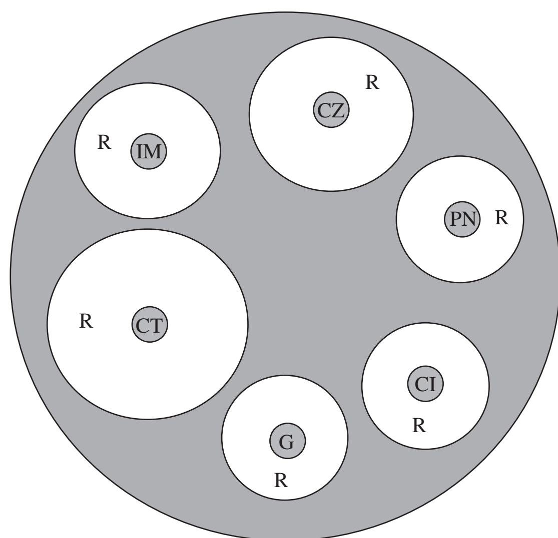

Figure 3. Template for interpreting susceptibility.

of inhibition of the test strain is within the area marked with an 'R' the organism is resistant. If the zone of inhibition is equal to or larger than the marked area the organism is susceptible.

Transparency declarations

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Appendix 1. Testing antimicrobial susceptibility to co-trimoxazole

Breakpoints for testing susceptibility to co-trimoxazole are provided. However, the following recommendations from the UK Committee on the Safety of Medicines (CSM) should be noted.

'Co-trimoxazole should be limited to the role of drug of choice in *Pneumocystis 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.'

Appendix 2. Efficacy of cefaclor in the treatment of respiratory infections caused by *Haemophilus influenzae*

Concerns have been expressed, particularly by laboratories moving from Stokes' method to the BSAC disc diffusion method, about the interpretation of susceptibility of *Haemophilus influenzae* to cefaclor. When using Stokes' method the majority of isolates appeared susceptible; but with the BSAC disc diffusion method most isolates are now reported resistant. The following comments explain the BSAC rationale for the interpretation of cefaclor susceptibility.

Cefaclor pharmacokinetics

Cefaclor is dosed at 250–500 mg orally three times daily: 250 mg three times daily is probably the most common dose but data are not available to confirm this. The expected C_{max} for 250 mg is 5–10 mg/L and 10–20 mg/L for 500 mg; the half life is 1 h; drug concentration in blood is <1 mg/L at 4 h and the protein binding is 25–50%. Tissue penetration is similar to other β-lactams.

Cefaclor potency against Haemophilus influenzae

Data from the BSAC surveillance programme 2003–2004 (n = 899) indicate that the cefaclor MIC range is 0.12–128 mg/L; MIC_{50} 2 mg/L; MIC_{90} 8 mg/L.

Pharmacodynamics

An average patient with a *Haemophilus influenzae* infection will have a free drug time > MIC of 25% with 250 mg dosing and 37% with 500 mg dosing. A conservative time > MIC target for cephalosporins in community practice is 40–50%, but this is not achieved with cefaclor. Therefore, it is likely that cefaclor will have at best borderline activity against *Haemophilus influenzae*.

Conclusion

The pharmacodynamic data indicate that cefaclor has borderline activity against *Haemophilus influenzae*, even for community use. The outcome of infection will be difficult to predict and susceptibility testing is likely to be of limited value.

Appendix 3.

1. Susceptibility testing of Helicobacter pylori

Disc diffusion methods are not suitable for testing *Helicobacter 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).

Suspend colonies from a 2–3 day culture on a blood agar plate in sterile distilled water and adjust the density to equal a McFarland 3 standard.

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.

Allow the plate to dry and apply Etest strip.

Incubate at 35°C in microaerophilic conditions for 3–5 days.

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 Table A1.

2. Susceptibility testing of Brucella species

***Brucella* spp. are Hazard Group 3 pathogens and all work must be performed 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, susceptibility testing in routine laboratories is not recommended.**

Table A1. Tentative MIC breakpoints for *Helicobacter pylori*

	MIC breakpoint (mg/L)	
Antimicrobial agent	R>	S≤
Amoxicillin	1	1
Clarithromycin	1	1
Tetracycline	2	2
Metronidazole	4	4

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3. Susceptibility testing of Legionella species

Legionella **spp. are slow growing and have particular growth requirements. Disc diffusion methods for susceptibility testing 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 the range of the normal wild-type distribution, given in Table A2, clinical susceptibility may be assumed.**

Table A2. MIC ranges for wild-type *Legionella* spp.

Antimicrobial agent	MIC range for wild-type *Legionella* spp. (mg/L)
Erythromycin	0.06–0.5
Clarithromycin	0.004–0.06
Rifampicin	0.004–0.06
Ciprofloxacin	0.016–0.06

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