

SMB/BJG

29th November, 1993.

Dear Colleague,

**C.D.S. USERS GROUP**

**NEWSLETTER NO. 6**

This newsletter contains the handout to participants at the CDS Workshop in Perth held in September 1993. There were about 80 participants at the workshop including a number to whom the meeting was an introduction to the CDS method. Ten new members joined the CDS Users Group which now has 340 members on its mailing list. The opportunity was taken at the workshop to reiterate the steps in the CDS method (pages 2 & 3), emphasize some important points on quality assurance (page 4), revisit the CDS-QANTAS checklist (page 5) and draw attention to some exceptions to the standard interpretation of results (page 6). Disc approximation tests were illustrated (page 7) and earlier tables on calibrations and surrogate disc testing were consolidated and updated (pages 8-12). To complete the set, the table containing relevant information on the reference strains used in the CDS method was included without amendment on pages 14 & 15.

We hope that this brief report of the workshop will be of interest and value to those who were unable to attend.

Yours sincerely,

**S.M. BELL,**  
Department of Microbiology.

CDS USERS GROUP NEWSLETTER No.6

Report on the

**CDS USERS GROUP WORKSHOP**

**ANNUAL SCIENTIFIC MEETING**

**ASM**

**PERTH 1993**

**Table of Contents**

	Page
The CDS method:- description	2-3
Points on quality assurance	4
The CDS-QANTAS Check List	5
Exceptions to the standard interpretation of results	6
Disc approximation tests demonstrated	7
Calibrations:- Consolidated and updated table	8-10
Surrogate Disc Testing	11-13
Acceptable zone sizes with the reference strains	14-15

*The Antibiotics Laboratory  
S.M. Bell, B.J. Gatus, J.N. Pham, A.S. Jimenez & M.J. Hardy.*

## SUSCEPTIBILITY TESTING BY THE CDS METHOD

### Materials and method

#### Materials

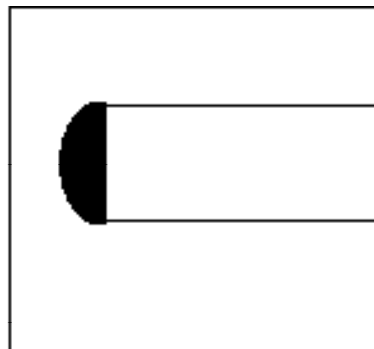
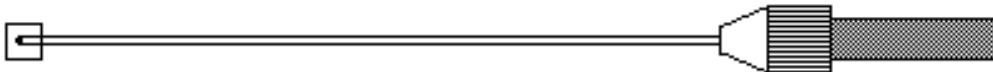
1. Dehydrated media
  - Sensitest Agar (Oxoid CM409)
  - Columbia Agar Base (Oxoid CM331, BBL or Lab M)
2. Defibrinated horse blood.
3. 90 mm diameter plastic Petri dishes.
4. 2.5 ml of sterile isotonic saline in 13 mm x 100 mm test tubes.
5. 10 cm of 0.56 mm diameter nichrome wire in a loop holder.  
Available from: Australia Electrical Electronics, 342-350 Parramatta Road, Petersham, NSW 2049. Tel: (02) 568 3888, Fax: (02) 568 3144
6. Pasteur pipettes.
7. 6 mm diameter antibiotic discs supplied only by Oxoid, BBL or Mast.
8. Disc dispenser (maximum of 6 discs) available from Oxoid and BBL suppliers.
9. Max/min thermometers.
10. Clear plastic mm ruler.

#### Agar plates

1. Dehydrated media must be prepared strictly according to the manufacturer's instructions.
  - 5% Horse blood is added to Sensitest Agar to prepare blood Sensitest Agar.
  - 8% Horse blood is added to Columbia Agar Base and heated to 80°C until chocolated (approximately 30 min. for 2 litres) to prepare "chocolate agar".
2. 20 ml of agar is dispensed into Petri dishes.
3. Agar plates are stored at 4°C for a maximum of 14 days.
4. Plates are dried face down with the lid removed in an incubator at 35°C. This will usually take 1 hour in a fan-forced incubator or 2 hours in an ordinary incubator.
5. Dried plates may be stored in the refrigerator for a maximum of 2 days.

#### The inoculum

1. Use an overnight culture preferably on blood agar to prepare the inoculum.
2. The standard method to obtain the inoculum is to stab 1 colony which should result in bacterial material being visible on the tip of the straight wire as shown in the diagram below.



3. If bacterial material is not visible using the standard method then one of the following methods may be used:
  - Stab 3-5 colonies (suitable for small colonies).
  - Tease the colony apart and pick up bacterial material (suitable for sticky colonies).
  - Stab the edge of confluent growth (this may be necessary with *Strep. milleri* and *Strep. pneumoniae*). This is the least preferable method since there is a possibility that the inoculum may not be pure.

- Inoculate the saline by rotating the straight wire at least 10 times with the tip in contact with the bottom of the tube.
- Mix up and down at least 10 times using a Pasteur pipette.

#### ***Inoculation of plates***

- Flood agar plate and remove excess. If 2 plates are to be flooded then half the suspension can be used for each plate.
- Remove the lid and place the plate next to a bunsen burner to dry. This will usually take 5 - 10 min. Plates must **NOT** be left longer than 45 min.
- Apply antibiotic discs.

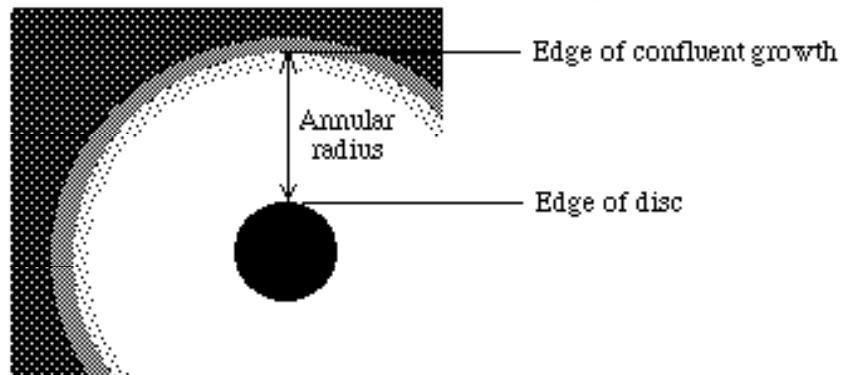
#### ***Incubation of plates***

Most organisms are incubated at 35°C overnight in air but there are a few exceptions which are:

- *B. catarrhalis* 35°C in 5% CO<sub>2</sub>.
- *Campylobacter spp.* 42°C in microaerophilic conditions.
- *N. meningitidis* 35°C in 5% CO<sub>2</sub>.
- *Y. enterocolitica* 30°C in air.

#### ***Reading the zones***

- Measure the zones from the back of the plate where possible.
- Measure the annular radius which is the shortest distance from the edge of the disc to the edge of confluent growth. The edge of confluent growth usually corresponds to the sharpest edge of the zone.



#### ***Interpretation***

For most organism/antibiotic combinations the interpretation is:

Annular radius  $\geq$  6 mm = SUSCEPTIBLE  
 $<$  6 mm = RESISTANT

There are some exceptions (see Table 1) eg *Pseudomonas aeruginosa* with aminoglycosides where the interpretation is:

Annular radius  $\geq$  4 mm = SUSCEPTIBLE  
 $<$  4 mm = RESISTANT

#### ***Notes on organisms with special growth requirements***

- Cysteine or thymidine requiring strains of Enterobacteriaceae* can be tested by adding 5 drops of a sterile aqueous solution containing either cysteine (2000 mg/l) or thymidine (5000 mg/l) to 2.5 ml of saline before inoculation. Sulphafurazole and trimethoprim cannot be tested in the presence of thymidine.
- CO<sub>2</sub> dependent staphylococci and streptococci* can be incubated in 5% CO<sub>2</sub> at 35°C. The effect of CO<sub>2</sub> on the zone sizes does not appear to alter the susceptibility results.

## Quality Assurance

1. Antibiotic susceptibility tests with the relevant reference strains should be performed on the same day that isolates are tested. In those laboratories where antibiotic susceptibility tests are performed infrequently, all discs "in use" should be tested with the relevant reference strain at least once a week.
2. The actual measurement of the annular radius of each zone of inhibition should be recorded every time a reference strain is tested so as to expose any problems which may arise during the performance of the test.

The following table is an example of the method of recording used at the Prince of Wales Hospital which may be of use to some laboratories.

<i>Staphylococcus aureus</i> NCTC 6571.						
	Annular radii (mm)					
Date	P 0.5 8.7 - 13.5	MET 5 8.8 - 12.0	E 5 7.1 - 10.7	TE 30 10.6 - 16.2	C 30 7.8 - 11.4	CIP 2.5 9.2 - 12.4
9.6.92	12	10.5	11	12.5	9.5	10.5
16.6.92	11	11	9.5	14	10	11

Please circle zone sizes which are not within the acceptable limits.

3. If any problems arise when performing a susceptibility test e.g. you fail to obtain acceptable zone sizes with a reference strain then refer to the CDS-QANTAS check list. This should enable you to define any problem which has occurred.
4. The following reference strains are used with the CDS method:

<i>Staphylococcus aureus</i>	NCTC 6571.
<i>Escherichia coli</i>	NCTC 10418.
<i>Escherichia coli</i>	NCTC 11560.
<i>Pseudomonas aeruginosa</i>	NCTC 10662.
<i>Haemophilus influenzae</i>	NCTC 4560.
<i>Yersinia enterocolitica</i>	I P 22273.

These strains may be obtained from:

Antibiotics Laboratory,  
Department of Microbiology,  
The Prince of Wales Hospital,  
Randwick, NSW 2031,  
Australia.  
Tel: (02) 399-4053.  
Fax: (02) 399-1120.

## CDS-QANTAS Check List

### Quality Assurance Notations when Testing Antimicrobial Susceptibility

Organism tested: ..... [ √ ] or [ X ]

<b>Medium</b>	Appropriate medium used	[ ]
	90 mm Petri dish used	[ ]
	Dehydrated media used within expiry date	[ ]
	Manufacturer's instructions followed	[ ]
	20 ml of medium in Petri dish	[ ]
	4 mm depth of medium in Petri dish	[ ]
	Poured plate with lid weighs approx. 35 g	[ ]
	Poured plates are stored at 4°C	[ ]
	Plates used within 2 weeks of pouring	[ ]
<b>Inoculum</b>	0.56 mm diameter wire used	[ ]
	Colony sampled less than 36 hours old	[ ]
	Material visible on tip of wire	[ ]
	Tip of wire not pointed	[ ]
	Tip of wire not corroded	[ ]
	Wire allowed to cool before stabbing colony	[ ]
	Homogeneous suspension	[ ]
	Suspension turbidity visible	[ ]
	Whole plate flooded	[ ]
	Excess suspension removed	[ ]
Flooded plate dry within 15 min	[ ]	
<b>Antibiotic discs</b>	Stock discs stored at -20°C	[ ]
	Discs in use stored at 4°C	[ ]
	Packaging of discs not damaged	[ ]
	Discs used within expiry date	[ ]
	Dispenser at room temperature before opening	[ ]
	Desiccant in dispenser active	[ ]
	Positions in dispensers not shared	[ ]
	Correct disc potencies	[ ]
	No more than 6 discs on plate	[ ]
	Antibiotic discs applied within 45 min of flooding	[ ]
Discs flat on medium	[ ]	
<b>Incubation conditions</b>	Correct incubation temperature	[ ]
	Correct atmosphere of incubation	[ ]
	Incubated overnight (min. 16 hours)	[ ]
	No more than 5 plates per stack	[ ]
<b>Measuring zones of inhibition</b>	Homogeneous lawn of growth	[ ]
	Satisfactory growth of organism	[ ]
	Measured from edge of disc	[ ]
	Measured to edge of confluent growth	[ ]
	Measured from back of plate (where possible)	[ ]
	Not measured adjacent to another antibiotic disc	[ ]
	Check antibiotics with 4mm cut-off	[ ]

## Exceptions to the standard interpretation

### *Staphylococci and methicillin*

If colonies are seen within the zone of inhibition around a methicillin 5 µg disc, the isolate should be reported resistant to methicillin irrespective of the zone size.

### *Enterobacteriaceae and colonies within the zone of inhibition around β-lactam discs*

If colonies are seen within the zone of inhibition around any β-lactam disc, either an inducible chromosomal β-lactamase or a cefotaximase (an extended-spectrum β-lactamase or ESB) may be present. These β-lactamases can be detected by disc approximation tests described on page 6.

### *Enterobacteriaceae with inducible chromosomal β-lactamases*

The following species produce an inducible chromosomal β-lactamase:

*Citrobacter freundii*  
*Enterobacter aerogenes*  
*Enterobacter cloacae*  
*Morganella morganii*  
*Proteus penneri*  
*Proteus vulgaris*  
*Providencia rettgeri*  
*Providencia stuartii*  
*Serratia marcescens*

These should be reported resistant to all β-lactams except imipenem which can be tested.

NB: The presence of an inducible chromosomal β-lactamase in an unspiciated isolate indicates that it may belong to one of the species mentioned above.

### *Enterobacteriaceae with cefotaximases*

Some isolates of the Enterobacteriaceae may produce a cefotaximase and should be reported resistant to all β-lactams except Augmentin, cefotetan, cefoxitin and imipenem. The susceptibility to the latter 4 antibiotics can be tested.

### *Aeromonas spp. and β-lactams*

1. All strains of *Aeromonas spp.* should be reported resistant to amoxicillin, ampicillin, Augmentin, azlocillin, piperacillin, ticarcillin and Timentin.
2. If induction of chromosomal β-lactamase is detected then the isolate should be reported resistant to all β-lactams except imipenem which can be tested. The phenomenon of chromosomal β-lactamase induction can be demonstrated by a disc approximation test described on page 6.
3. If colonies are seen within the zone of inhibition around an imipenem 10 µg disc then the isolate should be reported resistant to imipenem.

NB: If colonies are seen within the zone of inhibition around discs containing any cephalosporin, cephamycin or aztreonam then the isolate should be suspected of producing an inducible chromosomal β-lactamase.

### *Yersinia enterocolitica and β-lactams*

All strains of *Yersinia enterocolitica* should be reported resistant to amoxicillin, ampicillin, azlocillin and piperacillin because they all produce β-lactamase.

### *Xanthomonas maltophilia*

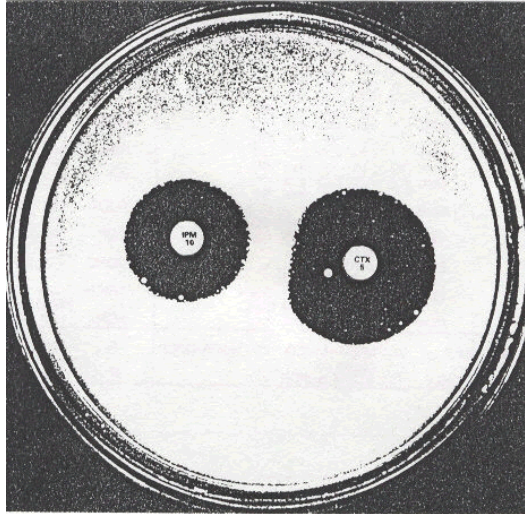
1. All strains should be reported resistant to gentamicin, tobramycin, netilmicin and amikacin.
2. All strains should be reported resistant to all β-lactams including imipenem.
3. All strains should be reported resistant to ciprofloxacin and norfloxacin.

## Disc approximation tests

### *Detection of inducible chromosomal $\beta$ -lactamase*

Place an imipenem 10  $\mu$ g disc approximately 20 mm from a cefotaxime 5  $\mu$ g disc or in adjacent positions in a dispenser when performing the CDS method.

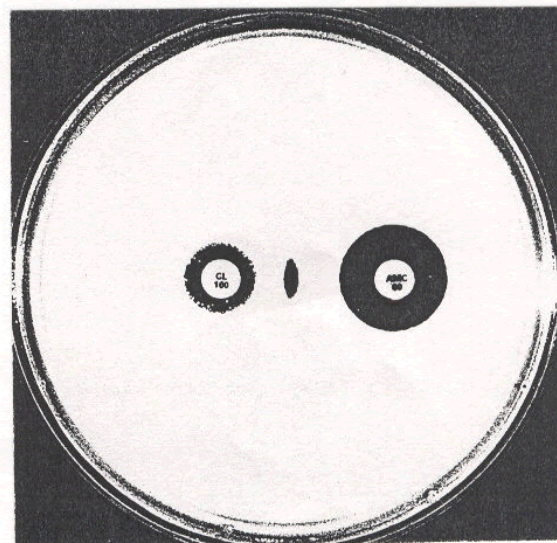
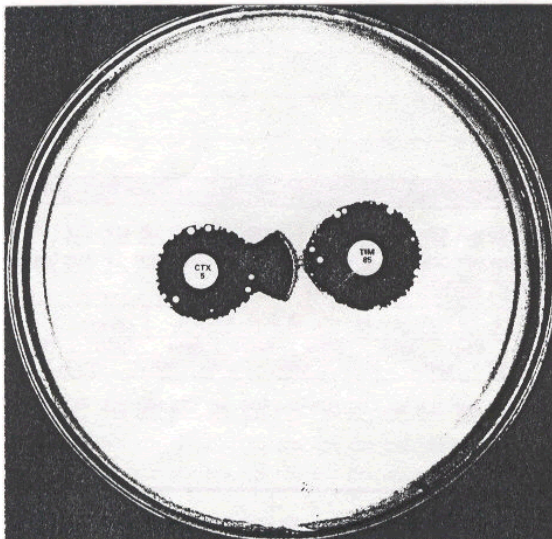
A flattening of the zone of inhibition around the cefotaxime disc adjacent to that of imipenem indicates the presence of an inducible chromosomal  $\beta$ -lactamase.



### *Detection of cefotaximases*

Place an Augmentin 60  $\mu$ g or a Timentin 85  $\mu$ g disc approximately 20 mm from a cefotaxime 5  $\mu$ g disc or a cephalixin 100  $\mu$ g disc (adjacent positions in a dispenser) when performing the CDS method.

A "key hole" effect or a clear elliptical area between the antibiotic discs indicates the presence of a cefotaximase.





**Table 1a. Calibrations:** Antibiotic disc potency, the MIC for susceptible strains, media and incubation conditions used.

Antibiotic reported	Disc potency (µg)		MIC for susceptible strains
<b><i>Acinetobacter spp.</i></b> (Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin *	60		≤ 8.0/4.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Netilmicin	30		≤ 2.0
Norfloxacin *	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Ticarcillin	75		≤ 32.0
Tobramycin	10		≤ 1.0
<b><i>Aeromonas spp.</i></b> (Sensitest, air, 35°C)			
Amikacin	30	4 mm†	≤ 4.0
Aztreonam	10		≤ 4.0
Cefotaxime	5		≤ 1.0
Cefotetan	10		≤ 4.0
Cefoxitin	30		≤ 8.0
Cephalexin *	100		≤ 16.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10	4 mm†	≤ 1.0
Imipenem	10		≤ 4.0
Netilmicin	30	4 mm†	≤ 2.0
Tetracycline	30		≤ 4.0
Tobramycin	10	4 mm†	≤ 1.0
<b><i>Branhamella catarrhalis</i></b> (Blood Sensitest, CO <sub>2</sub> , 35°C)			
Benzylopicillin	0.5 u		≤ 0.125
Cefaclor	30		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Tetracycline	30		≤ 4.0
<b><i>Campylobacter spp.</i></b> (Blood Sensitest, microaerophilic, 42°C)			
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5	4 mm†	≤ 0.5
Gentamicin	10		≤ 1.0
Tetracycline	30		≤ 4.0
<b><i>Enterobacteriaceae</i></b> (Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin *	60		≤ 8.0/4.0
Aztreonam	10		≤ 4.0
Cefotaxime	5		≤ 1.0
Cefotetan	10		≤ 4.0
Cefoxitin	30		≤ 8.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cephalexin*	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Nalidixic acid *	30		≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin *	200		≤ 32.0
Norfloxacin *	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Tetracycline	30		≤ 4.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0

\* ONLY for isolates from urine

† The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm

**Table 1b. Calibrations (continued):** Antibiotic disc potency, the MIC for susceptible strains, media and incubation conditions used.

Antibiotic reported	Disc potency (µg)		MIC for susceptible strains
<b>Enterococci</b>			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 8.0
Gentamicin	200	4 mm†	≤ 512
Nitrofurantoin *	200		≤ 32.0
Vancomycin	60	4 mm†	≤ 2.0
<b>Haemophilus influenzae</b>			
(Chocolate agar, air, 35°C)			
Ampicillin	2		≤ 1.0
Cefaclor +	30		≤ 4.0
Cefotaxime	5		≤ 0.25
Chloramphenicol	10		≤ 2.0
Ciprofloxacin +	2.5		≤ 1.0
Oxytetracycline +	30		≤ 4.0
<b>Listeria monocytogenes</b>			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 2.0 c
Gentamicin	10		≤ 1.0
<b>Neisseria meningitidis</b>			
(Blood Sensitest, CO <sub>2</sub> , 35°C)			
Benzylpenicillin	0.5 u	4 mm†	≤ 0.25
Cefotaxime	5		≤ 0.25
Chloramphenicol	10		≤ 2.0
<b>Pasteurella multocida</b>			
(Blood Sensitest, air, 35°C)			
Amoxicillin	10		≤ 0.5
Ciprofloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
<b>Pseudomonas aeruginosa, Burkholderia cepacia &amp; other pseudomonads</b>			
(Sensitest, air, 35°C)			
Amikacin	30	4 mm†	≤ 16.0
Aztreonam	30		≤ 8.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin	2.5		≤ 2.0
Gentamicin	10	4 mm†	≤ 4.0
Imipenem	10		≤ 4.0
Netilmicin	30	4 mm†	≤ 8.0
Norfloxacin *	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm†	≤ 1.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm†	≤ 4.0
<b>Staphylococci</b>			
(Sensitest, air, 35°C)			
Amoxicillin §	10		≤ 0.5
Benzylpenicillin #	0.5 u		≤ 0.06
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Gentamicin	10		≤ 1.0
Kanamycin	50		≤ 8.0
Methicillin #	5		≤ 4.0
Nitrofurantoin *	200		≤ 32.0
Rifampicin	1		≤ 0.5
Sulphafurazole *	300		≤ 64.0
Tetracycline	30		≤ 2.0
Trimethoprim *	5		≤ 2.0
Vancomycin	60	4 mm†	≤ 2.0

\* ONLY for isolates from urine

† The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm

§ ONLY for testing isolates of *S. saprophyticus*. # Not for testing *S. saprophyticus*. + ONLY for non-encapsulated strains.

**Table 1c. Calibrations (continued):** Antibiotic disc potency, the MIC for susceptible strains, media and incubation conditions used.

Antibiotic reported	Disc potency ( $\mu\text{g}$ )	MIC for susceptible strains
<b>Streptococci</b>		
(Blood Sensitest, $\text{CO}_2$ , $35^\circ\text{C}$ )		
Benzylpenicillin	0.5 u	$\leq 0.25$
Chloramphenicol	30	$\leq 8.0$
Erythromycin	5	$\leq 0.5$
Gentamicin	10	$\leq 1.0$
Kanamycin	50	$\leq 8.0$
Rifampicin	1	$\leq 0.5$
Tetracycline	30	$\leq 2.0$
Vancomycin	60	$\leq 2.0$
		4 mm <sup>†</sup>
<b><i>Xanthomonas maltophilia</i></b>		
(Sensitest, air, $35^\circ\text{C}$ )		
Sulphafurazole	300	$\leq 64.0$
<b><i>Yersinia enterocolitica</i></b>		
(Sensitest, air, $30^\circ\text{C}$ )		
Amikacin	30	$\leq 4.0$
Augmentin	3	$\leq 2.0/1.0$
Aztreonam	10	$\leq 4.0$
Cefotaxime	5	$\leq 1.0$
Chloramphenicol	30	$\leq 8.0$
Ciprofloxacin	2.5	$\leq 1.0$
Gentamicin	10	$\leq 1.0$
Imipenem	10	$\leq 4.0$
Netilmicin	30	$\leq 2.0$
Sulphafurazole	300	$\leq 64.0$
Tetracycline	30	$\leq 4.0$
Timentin	85	$\leq 32.0/2.0$
Tobramycin	10	$\leq 1.0$
Trimethoprim	5	$\leq 2.0$

<sup>†</sup> The annular radius of the zone of inhibition for susceptible strains is  $\geq 4$  mm

## SURROGATE DISC TESTING

Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

**Table 2a. Antibiotics of therapeutic relevance**

Antibiotic reported	Surrogate disc used	Disc potency (µg)
<b><i>Acinetobacter</i> spp.</b>		
Amoxicillin	Ampicillin	25
Co-trimoxazole <sup>♠</sup>	Sulphafurazole	300
Sulphonamides	Sulphafurazole	300
Timentin	Ticarcillin	75
<b><i>Aeromonas</i> spp.</b>		
Ceftazidime	Cefotaxime	5
Ceftriaxone	Cefotaxime	5
Tetracyclines	Tetracycline	30
<b><i>Branhamella catarrhalis</i></b>		
Azithromycin	Erythromycin	5
Amoxicillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefaclor	30
Cephalexin	Cefaclor	30
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<b><i>Campylobacter</i> spp.</b>		
Azithromycin	Erythromycin	5
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<b><i>Enterobacteriaceae</i></b>		
Amoxicillin	Ampicillin	25
Cefaclor *	Cephalexin	100
Cefotaxime	Ceftriaxone	5
Ceftriaxone	Cefotaxime	5
Cephalothin	Ampicillin	25
Cephazolin	Ampicillin	25
Co-trimoxazole <sup>♠</sup>	Sulphafurazole	300
Co-trimoxazole <sup>♠</sup>	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<b><i>Enterococci</i></b>		
Amoxicillin	Ampicillin	25
Benzylpenicillin	Ampicillin	25
<b><i>Haemophilus influenzae</i></b>		
Amoxicillin	Ampicillin	2
Augmentin <sup>+</sup>	Cefaclor	30
Ceftriaxone	Cefotaxime	5
Cephalexin <sup>+</sup>	Cefaclor	30
Tetracyclines <sup>+</sup>	Oxytetracycline	30
<b><i>Listeria monocytogenes</i></b>		
Amoxicillin	Ampicillin	25
Benzylpenicillin	Ampicillin	25
<b><i>Neisseria meningitidis</i></b>		
Ampicillin	Benzylpenicillin	0.5 u
Ceftriaxone	Cefotaxime	5
<b><i>Pasteurella multocida</i></b>		
Ampicillin	Amoxicillin	10
Benzylpenicillin	Amoxicillin	10
Tetracyclines	Tetracycline	30
<b><i>Pseudomonas aeruginosa</i></b>		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u

\* ONLY for isolates from urine.

<sup>+</sup> Only for non-encapsulated strains.

<sup>♠</sup> Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

Table 2a (continued). Antibiotics of therapeutic relevance

Antibiotic Reported	Surrogate disc used	Disc potency (µg)
<b>Staphylococci (except <i>S. saprophyticus</i>)</b>		
Amoxicillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Azithromycin	Erythromycin	5
Cefaclor	Methicillin	5
Cephalexin	Methicillin	5
Cephalothin	Methicillin	5
Cephazolin	Methicillin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole * <sup>‡</sup>	Sulphafurazole	300
Co-trimoxazole * <sup>‡</sup>	Trimethoprim	5
Dicloxacillin	Methicillin	5
Flucloxacillin	Methicillin	5
Lincomycin	Erythromycin	5
Norfloxacin *	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5
Roxithromycin	Erythromycin	5
Sulphonamides *	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<b><i>Staphylococcus saprophyticus</i> from urine</b>		
Ampicillin	Amoxicillin	10
Augmentin	Amoxicillin	10
Benzylpenicillin	Amoxicillin	10
Cefaclor	Amoxicillin	10
Cephalexin	Amoxicillin	10
Cephalothin	Amoxicillin	10
Cephazolin	Amoxicillin	10
Cloxacillin	Amoxicillin	10
Co-trimoxazole * <sup>‡</sup>	Sulphafurazole	300
Co-trimoxazole * <sup>‡</sup>	Trimethoprim	5
Flucloxacillin	Amoxicillin	10
Norfloxacin *	Ciprofloxacin	2.5
Penicillin V	Amoxicillin	10
Sulphonamides *	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<b>Streptococci</b>		
Amoxicillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Cefaclor	Benzylpenicillin	0.5 u
Cephalexin	Benzylpenicillin	0.5 u
Cephalothin	Benzylpenicillin	0.5 u
Cephazolin	Benzylpenicillin	0.5 u
Clindamycin	Erythromycin	5
Cloxacillin	Benzylpenicillin	0.5 u
Flucloxacillin	Benzylpenicillin	0.5 u
Lincomycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<b><i>Xanthomonas maltophilia</i></b>		
Co-trimoxazole	Sulphafurazole	300
<b><i>Yersinia enterocolitica</i></b>		
Ceftazidime	Cefotaxime	5
Ceftriaxone	Cefotaxime	5
Co-trimoxazole <sup>‡</sup>	Sulphafurazole	300
Co-trimoxazole <sup>‡</sup>	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30

\* ONLY for isolates from urine.

<sup>‡</sup> Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.<sup>+</sup> Only for non-encapsulated strains

Table 2b. Antibiotics of questionable therapeutic relevance

Antibiotic reported	Surrogate disc used	Disc potency ( $\mu\text{g}$ )
<b><i>Acinetobacter</i> spp.</b>		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
<b><i>Branhamella catarrhalis</i></b>		
Azlocillin	Benzylpenicillin	0.5 u
Piperacillin	Benzylpenicillin	0.5 u
<b><i>Enterobacteriaceae</i></b>		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
Ticarcillin	Ampicillin	25
<b>Enterococci</b>		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
<b><i>Haemophilus influenzae</i></b>		
Azlocillin	Ampicillin	2
Piperacillin	Ampicillin	2
Ticarcillin	Ampicillin	2
<b>Staphylococci (except <i>S. saprophyticus</i>)</b>		
Azlocillin	Benzylpenicillin	0.5 u
Imipenem	Methicillin	5
Piperacillin	Benzylpenicillin	0.5 u
Ticarcillin	Benzylpenicillin	0.5 u
<b>Streptococci</b>		
Azlocillin	Benzylpenicillin	0.5 u
Imipenem	Benzylpenicillin	0.5 u
Piperacillin	Benzylpenicillin	0.5 u
Ticarcillin	Benzylpenicillin	0.5 u

**Table 3a. Reference strains:** Antibiotic disc content and the acceptable range (mm) of the annular radii of inhibition with the reference strains used in the CDS method.

Antibiotic and	Disc content ( $\mu\text{g}$ )	Acceptable range* (mm)		
<b><i>Staphylococcus aureus</i> NCTC 6571</b>				
Amoxycillin	10	11.5	-	15.9
Benzylpenicillin	0.5 u	8.7	-	13.5
Chloramphenicol	30	7.8	-	11.4
Ciprofloxacin	2.5	9.2	-	12.4
Erythromycin	5	7.1	-	10.7
Fusidic acid	2.5	8.6	-	12.6
Gentamicin	10	6.6	-	9.4
Kanamycin	50	5.9	-	8.7
Methicillin	5	8.8	-	12.0
Nitrofurantoin	200	6.7	-	10.3
Rifampicin	1	9.3	-	12.5
Sulphafurazole	300	9.3	-	13.7
Tetracycline	30	10.6	-	16.2
Trimethoprim	5	7.3	-	10.1
Vancomycin	60	5.4	-	7.8
<b><i>Haemophilus influenzae</i> NCTC 4560</b>				
Ampicillin	2	6.0	-	9.2
Chloramphenicol	10	7.7	-	10.9
Cefaclor	30	7.3	-	10.9
Cefotaxime	5	8.9	-	14.1
Ciprofloxacin	2.5	9.7	-	14.9
Oxytetracycline	30	6.6	-	9.0
<b><i>Yersinia enterocolitica</i> IP 22273</b>				
Amikacin	30	6.0	-	8.4
Aztreonam	10	10.1	-	13.7
Augmentin	3	6.4	-	8.4
Chloramphenicol	30	6.7	-	11.9
Ciprofloxacin	2.5	12.1	-	16.1
Gentamicin	10	6.0	-	8.0
Imipenem	10	11.1	-	15.1
Netilmicin	30	8.1	-	10.5
Sulphafurazole	300	8.9	-	13.1
Tetracycline	30	9.9	-	13.9
Timentin	85	10.6	-	15.4
Tobramycin	10	6.1	-	8.1
Trimethoprim	5	9.6	-	13.2

\* The acceptable range (95% confidence limits) is the mean  $\pm$  2 standard deviations.

**Table 3b. Reference strains (continued):** Antibiotic disc content and the acceptable range (mm) of the annular radii of inhibition with the reference strains used in the CDS method.

Antibiotic and	Disc content ( $\mu\text{g}$ )	Acceptable range* (mm)	
<b><i>Escherichia coli</i> NCTC 10418</b>			
Amikacin	30	6.7	- 10.3
Ampicillin	25	7.5	- 10.7
Aztreonam	10	11.8	- 14.2
Cefotaxime	5	9.7	- 13.7
Cefotetan	10	11.6	- 13.6
Cefoxitin	30	9.8	- 13.0
Ceftazidime	10	8.7	- 11.9
Ceftriaxone	5	10.5	- 14.3
Cephalexin	100	6.9	- 10.9
Chloramphenicol	30	8.7	- 11.9
Ciprofloxacin	2.5	12.4	- 15.8
Gentamicin	10	6.2	- 9.4
Imipenem	10	10.3	- 13.5
Kanamycin	50	6.2	- 11.8
Nalidixic acid	30	8.9	- 12.1
Netilmicin	30	7.7	- 11.3
Nitrofurantoin	200	6.3	- 9.5
Norfloxacin	10	10.4	- 16.4
Sulphafurazole	300	5.0	- 9.4
Tetracycline	30	5.8	- 11.0
Tobramycin	10	6.4	- 8.4
Trimethoprim	5	8.7	- 11.1
<b><i>Escherichia coli</i> NCTC 11560</b>			
Augmentin	60	6.4	- 9.6
Timentin	85	6.0	- 8.4
<b><i>Pseudomonas aeruginosa</i> NCTC 10662</b>			
Amikacin	30	7.4	- 10.6
Aztreonam	30	8.3	- 13.1
Ceftazidime	10	7.5	- 11.9
Ciprofloxacin	2.5	8.9	- 14.5
Gentamicin	10	5.5	- 9.5
Imipenem	10	7.9	- 10.3
Netilmicin	30	6.4	- 10.4
Piperacillin	50	8.1	- 12.9
Polymyxin	300 u	5.2	- 7.2
Ticarcillin	75	7.3	- 12.1
Tobramycin	10	7.0	- 10.6

\* The acceptable range (95% confidence limits) is the mean  $\pm$  2 standard deviations.