

THE PRINCE OF WALES HOSPITAL

SMB/VAE

The Prince of Wales Hospital,
High Street,
Randwick. N.S.W. 2031.

15th April, 1993.

Dear Colleague,

CDS USERS GROUP

Newsletter No. 5

Please find enclosed Newsletter No. 5. This newsletter summarises some of the proceedings of the CDS Users Group Workshop at the 1992 Sydney ASM meeting and responds to questions raised by users since the last newsletter. We have included revisions of the calibration tables and the reference strains data. Two new tables also have been added. The first we've called "Surrogate Disc Testing" and it lists those antibiotics the susceptibilities of which can be reported on the basis of the results observed with similar or related antibiotics. We would appreciate comments or criticisms of the Surrogate Disc Testing table.

We named the final table the "CDS-QANTAS" check list because it is loosely based on part of an actual pre-flight checklist completed by QANTAS staff of a Boeing 747-400. Some liberty was taken with the QANTAS acronym in the hope that this would remind laboratories of the need to approach the maintenance of quality of testing in a systematic fashion.

The staff of the Antibiotics Laboratory are keen to hold a workshop at this years ASM meeting in Perth in September. At this stage we need to get some idea of the numbers who will be attending. Please communicate your intentions and any ideas for the workshop by mail, facsimile 02-3991120 or telephone 02-3994053.

Yours faithfully,

S. M. BELL,
Department of Microbiology.

CDS USERS GROUP NEWSLETTER No.5

This newsletter reports some matters which were raised at the CDS Users Group workshop at the 1992 Sydney ASM meeting and also responds to questions raised by the CDS users since the last newsletter.

1. FINDING IMPORTANT INFORMATION

In response to queries raised by several members of the CDS Users Group, the following are the sources of three items of important information

- i.* Information on the preparation of media for use with the CDS method is found on the first 9 pages of the Oxoid Manual (6th Ed. 1990).
- ii.* Details of the CDS method can be found on pages 7 - 10 of the CDS Users Group Newsletter No.3.
- iii.* The cost of sending reference strains will increase dramatically in the near future for this reason it is important that CDS users maintain their cultures of reference strains. Details of maintenance of cultures are contained on page 10 of the CDS Users Group Newsletter No. 3.

2. INTERNAL QUALITY ASSURANCE

- i. Frequency of testing reference strains:* Antibiotic susceptibility tests of the relevant reference strains should be performed on the same day that the isolates are tested. In those laboratories where antibiotic susceptibility tests are performed infrequently, each reference strain should be tested at least once a week.
- ii. Recording zone sizes of reference strain tests:* The actual measurement of the annular radius of each antibiotic zone should be recorded each time a reference strain is tested. It is not sufficient to record simply as susceptible or resistant as an accurate record of the measurements is required to help define problems which may arise in the test. (See 3)

3. THE CDS-QANTAS CHECK LIST

At the ASM workshop we introduced the CDS-QANTAS (Quality Assurance Notations when Testing Antimicrobial Susceptibility) check list (page 8) as one way of reinforcing the need to adopt a "trouble shooting" approach to defining problems revealed by the reference strain test results (2 ii).

If any problems arise when performing susceptibility tests e.g. you fail to obtain acceptable zone sizes with the reference strains, refer to the check list carefully and you should be able to define the problem which has occurred.

4. SUSCEPTIBILITY TESTING WITH A SURROGATE DISC

At the ASM meeting we introduced a table entitled "Surrogate Disc Testing" (table 4, page 7) showing antibiotic susceptibilities which can be reported on the basis of a susceptibility test result obtained using a closely related, that is, a "surrogate" antibiotic disc. The use of a surrogate disc is simply an extension of the use of a group representative disc which has always been a part of the CDS method. Testing with a surrogate disc is based on well established patterns of cross resistance and/or the spectrum of activity of antibiotic inactivating enzymes elaborated by a particular species.

An example: If the isolate is *Staphylococcus aureus* and the susceptibility to cephalothin is requested then the surrogate disc is methicillin (5 µg). Where susceptibility to methicillin is demonstrated the organism can be reported as susceptible to cephalothin although this antibiotic was not tested.

5. EXTERNAL QUALITY ASSURANCE

The Royal College of Pathologists of Australasia Quality Assurance Programme.

At the ASM meeting discussions were held with the coordinators of the RCPA Quality Assurance Programme (QAP) concerning differences in their results and those obtained by the CDS method. It was agreed that in future accurate susceptibilities reported according to the CDS criteria by participants who indicate that they are using the CDS method would be acceptable in the QAP. Users of the CDS method are reminded to follow the guide-lines listed below when participating in the QAP.

- a. The identity of the organism should be ascertained even though this may not be requested. The reason for this is that the identity will dictate the final susceptibility report. eg. *Enterobacter cloacae* should be reported as resistant to most β -lactam antibiotics irrespective of the zone sizes obtained (pages 5-6 of CDS Users Group Newsletter No.3).
- b. Do not test antibiotics or use discs which have not been calibrated for use in the CDS method.
- c. Report the result as either **S** (susceptible) or **R** (resistant) and also record the zone size (annular radius).
- d. If the susceptibility to an antibiotic which is not actually tested is required then look up the "Surrogate Disc Testing" table and report **S** or **R** accordingly.
- e. Do not report the susceptibility of any antibiotic which is not calibrated or is not on the "Surrogate Disc Testing" table.

6. TESTING *H. INFLUENZAE* AND *S. SAPROPHYTICUS*

We have been asked by several members of the CDS Users Group to clarify the testing and reporting of antimicrobial susceptibilities with *Haemophilus influenzae* in table 1 (page 4) and *Staphylococcus saprophyticus* in table 2 (page 5). At the Prince of Wales Hospital we report selectively antimicrobial susceptibilities for reasons given below.

i. Haemophilus influenzae

Haemophilus influenzae type b is usually associated with serious systemic infections in children such as septicaemia, meningitis and epiglottitis whereas non capsulate strains of *H. influenzae* are often isolated from sputum.

At present there are only 4 antibiotics regarded as appropriate agents for treatment of serious systemic infections with *H. influenzae*. In such cases report only the following antibiotic susceptibilities:

Ampicillin	
Cefotaxime	
Ceftriaxone	(by surrogate disc)
Chloramphenicol	

For *H. influenzae* isolated from sputum the following antibiotic susceptibilities may be reported:

Amoxicillin	(by surrogate disc)
Ampicillin	
Augmentin	(by surrogate disc)
Cefaclor	
Ciprofloxacin	
Tetracyclines	(by surrogate disc)

ii. *Staphylococcus saprophyticus* (novobiocin-resistant coagulase-negative staph.)

For *Staphylococcus saprophyticus* isolated from urine the following antibiotic susceptibilities may be reported depending on the type of practice your laboratory services:

Amoxicillin		Lincomycin	(by surrogate disc)
Ampicillin	(by surrogate disc)	Nitrofurantoin	
Benzylpenicillin	(by surrogate disc)	Norfloxacin	(by surrogate disc)
Cephalexin	(by surrogate disc)	Penicillin V	(by surrogate disc)
Ciprofloxacin		Sulphonamides	(by surrogate disc)
Clindamycin	(by surrogate disc)	Tetracyclines	(by surrogate disc)
Erythromycin		Trimethoprim	

7. DETECTION OF EXTENDED SPECTRUM β -LACTAMASE (ESB) IN *KLEBSIELLA* SPECIES

Klebsiella with an extended spectrum β -lactamase are being isolated more frequently in larger hospitals. The following information is provided in response to several requests on how best to detect these strains.

i. Any strain of Klebsiella which gives rise to colonies inside the zone of inhibition around a 5 μ g cefotaxime disc should be suspected of producing an ESB.

ii. To detect an ESB place a 60 μ g Augmentin disc (or a 85 μ g Timentin disc) and a 5 μ g cefotaxime disc with the adjacent edges 15 - 20 mm apart (or in adjacent positions in a dispenser) when performing the CDS method. Either a "key hole" effect (see page 13 of Newsletter No. 3) or a clear elliptical area between the antibiotic discs will be observed in the presence of an ESB.

8. METHICILLIN SUSCEPTIBILITY OF STAPHYLOCOCCI

It is essential that susceptibility tests with methicillin of staphylococci (both *Staph. aureus* and coagulase-negative staphylococci) are performed at 35°C (NOT AT 37°C). An incubating temperature of 35°C, as prescribed in the CDS method, is the optimum temperature for the demonstration of resistance to methicillin. It is generally unnecessary to perform confirmatory tests to indicate the susceptibility to methicillin eg. incubation at 30°C or testing on Mannitol Salt Agar.

9. ADDITIONAL CALIBRATIONS

The following additional calibrations have been performed and are now included in table 2 (page 5).

i. *Enterococci.*

Nitrofurantoin 200 μ g. Calibrated for testing enterococci from **urine** only.

ii. *Pasteurella multocida.*

Susceptibility testing is performed on blood Sensitest Agar incubated at 35°C in air.

Amoxicillin 10 μ g. Also used as a surrogate disc for benzylpenicillin and ampicillin.

Tetracycline 30 μ g. Also used as a surrogate disc for ALL tetracyclines.

Ciprofloxacin 2.5 μ g.

10. AMENDED TABLES

The following tables have been amended :- "Established Calibrations", "Provisional Calibrations" and "Reference Strains". Please replace the older tables with the updated versions included in this newsletter and listed as tables 1, 2 & 3.

The Antibiotics Laboratory

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Table 1. Established calibrations: Disc potencies and MIC of susceptible strains for common pathogens, all tested in air, at 35°C using Sensitest Agar unless indicated.

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
Staphylococci, streptococci¹ and pneumococci¹			
Benzylpenicillin	0.5 u		≤ 0.06
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5	(<i>Staph. only</i>)	≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Gentamicin	10		≤ 1.0
Kanamycin	50		≤ 4.0
Methicillin	5		≤ 4.0
Rifampicin	1		≤ 0.5
Tetracycline	30		≤ 2.0
Vancomycin	60	4 mm ³	≤ 2.0
Enterobacteriaceae			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin	60	(<i>Urine only</i>)	≤ 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		≤ 2.0
Cephalexin	100	(<i>Urine only</i>)	≤ 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	(<i>Urine only</i>)	≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin	200	(<i>Urine only</i>)	≤ 32.0
Norfloxacin	10	(<i>Urine only</i>)	≤ 4.0
Sulphafurazole	300		≤ 64.0
Tetracycline	30		≤ 4.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
Pseudomonas aeruginosa			
Amikacin	30	4 mm ³	≤ 16.0
Aztreonam	30		≤ 8.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin	2.5		≤ 2.0
Gentamicin	10	4 mm ³	≤ 4.0
Imipenem	10		≤ 4.0
Netilmicin	30	4 mm ³	≤ 8.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm ³	≤ 1.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm ³	≤ 4.0
Haemophilus influenzae²			
Ampicillin	2		≤ 1.0
Cefaclor	30	(<i>Sputum only</i>)	≤ 4.0
Cefotaxime	5		≤ 0.12
Chloramphenicol	10		≤ 2.0
Ciprofloxacin	2.5	(<i>Sputum only</i>)	≤ 1.0
Oxytetracycline	30	(<i>Sputum only</i>)	≤ 4.0

1. Test on blood Sensitest Agar.

2. Test on chocolate blood agar (Columbia Agar Base).

3. The annular radius of the zone of inhibition for sensitive strains is ≥ 4 mm.

Table 2a. Provisional calibrations: Disc potencies, MIC of susceptible strains, media used and incubation conditions for uncommon pathogens.

Antibiotic	Disc potency (μg)		MIC for susceptible strains (mg/L)
<i>Acinetobacter species</i>			
(Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
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
Tobramycin	10		≤ 1.0
<i>Branhamella catarrhalis</i>			
(Blood Sensitest, CO ₂ , 35°C)			
Benzylpenicillin	0.5 u		≤ 0.06
Cefaclor	30		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Tetracycline	30		≤ 4.0
Enterococci			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 8.0
Gentamicin	200	4 mm ¹	≤ 512
Nitrofurantoin	200		≤ 32.0
Vancomycin	60	4 mm ¹	≤ 2.0
<i>Listeria monocytogenes</i>			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 8.0
Gentamicin	10		≤ 1.0
<i>Neisseria meningitidis</i>			
(Blood Sensitest, CO ₂ , 35°C)			
Benzylpenicillin	0.5 u	4 mm ¹	≤ 0.06
Cefotaxime	5		≤ 0.12
Chloramphenicol	10		≤ 2.0

1. The annular radius of the zone of inhibition for sensitive strains is ≥ 4 mm.

Table 2b. Provisional calibrations (continued): Disc potencies, MIC of susceptible strains, media used and incubation conditions for uncommon pathogens.

Antibiotic	Disc potency (μg)	MIC for susceptible strains (mg/L)
Coagulase-negative staphylococci from URINE ONLY		
(Sensitest, air, 35°C)		
Amoxicillin (<i>Staph. sapro. only</i>)	10	≤ 0.5
Benzylpenicillin ²	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	≤ 4.0
Methicillin ²	5	≤ 4.0
Rifampicin	1	≤ 0.5
Tetracycline	30	≤ 2.0
Vancomycin	60	4 mm ¹ ≤ 2.0
<i>Pasteurella multocida</i>		
(Blood Sensitest, air, 35°C)		
Amoxicillin	10	≤ 0.5
Ciprofloxacin	2.5	≤ 1.0
Tetracycline	30	≤ 4.0
<i>Xanthomonas maltophilia</i>		
(Sensitest, air, 35°C)		
Sulphafurazole	300	≤ 64.0
<i>Yersinia enterocolitica</i>		
(Sensitest, air, 30°C)		
Amikacin	30	≤ 4.0
Augmentin	3	$\leq 2.0/1.0$
Aztreonam	10	≤ 4.0
Chloramphenicol	30	≤ 8.0
Ciprofloxacin	2.5	≤ 1.0
Gentamicin	10	≤ 1.0
Imipenem	10	≤ 4.0
Netilmicin	30	≤ 2.0
Sulphafurazole	300	≤ 64.0
Tetracycline	30	≤ 4.0
Timentin	85	$\leq 32.0/2.0$
Tobramycin	10	≤ 1.0
Trimethoprim	5	≤ 2.0

1. The annular radius of the zone of inhibition for sensitive strains is ≥ 4 mm.

2. Benzylpenicillin and methicillin are not used for testing *S. saprophiticus*.

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	Disc content (μg)	Acceptable range* (mm)
<i>Staphylococcus aureus</i> NCTC 6571		
Amoxicillin	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
<i>Haemophilus influenzae</i> NCTC 4560		
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
<i>Yersinia enterocolitica</i> IP 22273		
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 strain (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	Disc content (μg)	Acceptable range* (mm)
<i>Escherichia coli</i> NCTC 10418		
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
<i>Escherichia coli</i> NCTC 11560		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
<i>Pseudomonas aeruginosa</i> NCTC 10662		
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.

Table 4. Surrogate Disc Testing: Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Organism	Antibiotic reported	Surrogate disc used	Disc content	
<i>Acinetobacter</i> spp.	Amoxicillin	Ampicillin	25 µg	
	Piperacillin	Ampicillin	25 µg	
<i>Branhamella catarrhalis</i>	Amoxicillin	Benzylpenicillin	0.5 u	
	Ampicillin	Benzylpenicillin	0.5 u	
	Augmentin	Cefaclor	30 µg	
	Clindamycin	Erythromycin	5 µg	
	Lincomycin	Erythromycin	5 µg	
	Penicillin V	Benzylpenicillin	0.5 u	
	Piperacillin	Benzylpenicillin	0.5 u	
	Roxithromycin	Erythromycin	5 µg	
	Tetracyclines	Tetracycline	30 µg	
	Enterococci	Amoxicillin	Ampicillin	25 µg
		Benzylpenicillin	Ampicillin	25 µg
		Piperacillin	Ampicillin	25 µg
Enterobacteriaceae	Amoxicillin	Ampicillin	25 µg	
	Ceftazidime	Cefotaxime	5 µg	
	Ceftriaxone	Cefotaxime	5 µg	
	Cephalothin	Ampicillin	25 µg	
	Piperacillin	Ampicillin	25 µg	
	Sulphonamides	Sulphafurazole	300 µg	
	Tetracyclines	Tetracycline	30 µg	
	Ticarcillin	Ampicillin	25 µg	
	<i>H. influenzae</i>	Amoxicillin	Ampicillin	2 µg
		Augmentin	Cefaclor	30 µg
Ceftriaxone		Cefotaxime	5 µg	
Tetracyclines		Oxytetracycline	30 µg	
<i>Listeria monocytogenes</i>	Amoxicillin	Ampicillin	25 µg	
	Benzylpenicillin	Ampicillin	25 µg	
<i>Pasteurella multocida</i>	Ampicillin	Amoxicillin	10 µg	
	Benzylpenicillin	Amoxicillin	10 µg	
<i>Ps. aeruginosa</i>	Tetracyclines	Tetracycline	30 µg	
	Colistin	Polymyxin	300 u	
<i>Staph. aureus</i>	Norfloxacin	Ciprofloxacin	2.5 µg	
	Amoxicillin	Benzylpenicillin	0.5 u	
	Ampicillin	Benzylpenicillin	0.5 u	
	Augmentin	Methicillin	5 µg	
	Cephalexin	Methicillin	5 µg	
	Cephalothin	Methicillin	5 µg	
	Clindamycin	Erythromycin	5 µg	
	Flucloxacillin	Methicillin	5 µg	
	Imipenem	Methicillin	5 µg	
	Lincomycin	Erythromycin	5 µg	
	Norfloxacin	Ciprofloxacin	2.5 µg	
	Penicillin V	Benzylpenicillin	0.5 u	
	Piperacillin	Benzylpenicillin	0.5 u	
	Roxithromycin	Erythromycin	5 µg	
	Tetracyclines	Tetracycline	30 µg	
	Staphylococci coag. (-) ve.	Amoxicillin	Benzylpenicillin	0.5 u
		Ampicillin	Benzylpenicillin	0.5 u
		Augmentin	Methicillin	5 µg
		Cephalexin	Methicillin	5 µg
Cephalothin		Methicillin	5 µg	
Clindamycin		Erythromycin	5 µg	
Flucloxacillin		Methicillin	5 µg	
Imipenem		Methicillin	5 µg	
Lincomycin		Erythromycin	5 µg	
Norfloxacin		Ciprofloxacin	2.5 µg	
Penicillin V		Benzylpenicillin	0.5 u	
Sulphonamides		Sulphafurazole	300 µg	
Tetracyclines		Tetracycline	30 µg	
<i>Staph. saprophyticus</i>		Ampicillin	Amoxicillin	10 µg
		Benzylpenicillin	Amoxicillin	10 µg
		Cephalexin	Amoxicillin	10 µg
		Clindamycin	Erythromycin	5 µg
	Lincomycin	Erythromycin	5 µg	
	Norfloxacin	Ciprofloxacin	2.5 µg	
	Penicillin V	Amoxicillin	10 µg	
	Tetracyclines	Tetracycline	30 µg	
Streptococci	Amoxicillin	Benzylpenicillin	0.5 u	
	Ampicillin	Benzylpenicillin	0.5 u	
	Cefaclor	Benzylpenicillin	0.5 u	
	Cephalexin	Benzylpenicillin	0.5 u	
	Cephalothin	Benzylpenicillin	0.5 u	
	Clindamycin	Erythromycin	5 µg	
	Lincomycin	Erythromycin	5 µg	
	Penicillin V	Benzylpenicillin	0.5 u	
	Roxithromycin	Erythromycin	5 µg	
	Tetracyclines	Tetracycline	30 µg	

CDS-QANTAS

Check List

Quality Assurance Notations when Testing Antimicrobial Susceptibility

Organism tested:[] or []

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

Newsletter 5 (Addendum)

Susceptibility testing of *Aeromonas spp.*

A. Penicillins

All strains of *Aeromonas spp.* tested so far produce an inducible penicillinase and mutate at a relatively high frequency (10^{-6} to 10^{-7}) to produce this enzyme constitutively resulting in high level resistance to all penicillins. Therefore all isolates should be regarded as resistant to the penicillins i.e amoxycillin, ampicillin, Augmentin, piperacillin, ticarcillin and Timentin irrespective of the size of the inhibitory zone.

Interpretation: • All isolates should be reported as resistant to all penicillins.

B. Imipenem

Most strains (95%) of *Aeromonas spp.* mutate at a high rate and these mutants can be seen within the zone of inhibition around an imipenem 10 µg disc. Strains which produce these mutant colonies should be regarded as resistant to imipenem irrespective of the size of the inhibitory zone.

Interpretation: • Colonies within zone around imipenem 10 µg disc = Resistant to imipenem.
• No colonies within zone around imipenem 10 µg disc & annular radius of inhibition ≥ 6 mm = Susceptible to imipenem.

C. Other β -lactams

Many strains of *Aeromonas spp.* also produce an inducible cephalosporinase and can mutate to produce this enzyme constitutively resulting in high level resistance to all β -lactams except imipenem. Strains demonstrating the presence of an inducible cephalosporinase by the flattening of the inhibitory zone around a cefotaxime 5 µg disc adjacent to an imipenem 10 µg disc or showing resistant mutants which appear as colonies within the zone of inhibition around discs containing any cephalosporin, cephamycin or aztreonam should be regarded as resistant to aztreonam, cefotaxime, cefotetan, ceftaxitin, ceftazidime, ceftriaxone and cephalixin.

Interpretation: • Induction of cephalosporinase demonstrated = Resistant to all cephalosporins, cephamycins and aztreonam.
• Colonies within the zone of inhibition around discs containing any cephalosporin, cephamycins or aztreonam = Resistant to these antibiotics.
• Neither of the above & annular radius of inhibition ≥ 6 mm with the appropriate disc = Susceptible to the antibiotic tested.

D. Calibrated antibiotics and disc potencies

The following antibiotics and disc potencies have been calibrated for use with the CDS method when testing *Aeromonas spp.* Also shown is the annular radius of inhibition for susceptible strains.

Amikacin	(30 µg) ≥ 4 mm = Susceptible
Aztreonam	(10 µg) ≥ 6 mm = Susceptible
Cefotaxime	(5 µg) ≥ 6 mm = Susceptible (also surrogate disc for ceftriaxone, ceftazidime)
Cefotetan	(10 µg) ≥ 6 mm = Susceptible
Ceftaxitin	(30 µg) ≥ 6 mm = Susceptible
Cephalexin	(100 µg) ≥ 6 mm = Susceptible (Urine only)
Ciprofloxacin	(2.5 µg) ≥ 6 mm = Susceptible
Gentamicin	(10 µg) ≥ 4 mm = Susceptible
Imipenem	(10 µg) ≥ 6 mm = Susceptible (see Section B above)
Netilmicin	(30 µg) ≥ 4 mm = Susceptible
Tetracycline	(30 µg) ≥ 6 mm = Susceptible
Tobramycin	(10 µg) ≥ 4 mm = Susceptible

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