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Dear Colleague,

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

### *Newsletter No. 8*

This newsletter contains important additions and modifications to the CDS test. The Haemophilus Test Medium (HTM) as modified by us finally is being introduced. There have been some important changes in the disc potency of ampicillin and vancomycin. We also have introduced a co-trimoxazole disc for the testing of *Haemophilus influenzae* and *Streptococcus pneumoniae*. Testing of teicoplanin and meropenem is introduced in this newsletter and we now have calibrated cefazolin for *Enterobacteriaceae* and related species, rifampicin and ciprofloxacin for *Neisseria meningitidis*. Please replace existing Tables 1, 2, 3 and 4 headed respectively "Calibrations", "Surrogate disc testing", "Reference strains" and "A Guide to Testing and Reporting of  $\beta$ -lactam Antibiotics" with the updated Tables. Note that there have been some important changes in the potency of some antibiotic discs in these Tables.

The new potencies are ampicillin 5  $\mu$ g for all organisms other than *Enterobacteriaceae*, *Vibrionaceae* and *Acinetobacter* spp and vancomycin 5  $\mu$ g for gram-positive cocci.

There will be a workshop conducted at the ASM Annual Meeting in Adelaide. Please find a questionnaire attached. I look forward to seeing you there.

Yours faithfully,

**S. M. BELL,**  
For the Antibiotics Laboratory.  
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## CDS USERS GROUP NEWSLETTER No. 8

Additions, modifications to the CDS method and a report of the  
 CDS Users Group Workshop  
 Annual Scientific Meeting ASM & NZMS  
 Christchurch - New Zealand

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*The Antibiotic Reference Laboratory*

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## I. ADDITIONS AND MODIFICATIONS TO THE CDS METHOD.

A number of recalibrations were undertaken for newer antibiotics, modified disc potencies or changed growth conditions.

### 1. The testing of *Haemophilus influenzae* on Haemophilus Test Medium and incubation in 5 % CO<sub>2</sub>.

Haemophilus Test Medium or HTM (Oxoid CM898) supplemented with haematin and NAD prepared freshly from haemin and NAD pure substances, both at the concentration of 15 mg/L in agar, was found satisfactory for the testing of *Haemophilus influenzae*. All antibiotics were calibrated on this medium at 35°C, in an atmosphere of 5% CO<sub>2</sub>. The antibiotic disc potencies and the MICs for susceptible strains are incorporated in Table 1. Calibrations, 1997.

#### Important changes.

- i. Co-trimoxazole 25 µg was calibrated at the request of CDS Users.
- ii. Ampicillin 5 µg replaces the ampicillin 2 µg disc used previously.
- iii. Augmentin 3 µg (amoxycillin 2 µg/clavulanate 1 µg) was calibrated with an annular radius of the zone of inhibition for susceptible strains  $\geq 4$  mm.
- iv. Tetracycline 30 µg replaces oxytetracycline 30 µg.
- v. *H. influenzae* type b strains may be tested against all antibiotics calibrated.
- vi. Other *Haemophilus* species may be tested on the new medium provided the growth is satisfactory.

### 2. Co-trimoxazole 25 µg for the testing of *Streptococcus pneumoniae*.

Co-trimoxazole 25 µg was calibrated for the testing of *Strep. pneumoniae*. The MIC breakpoint of co-trimoxazole for susceptible strains is  $\leq 1$  mg/L (1mg/L of trimethoprim and 19 mg/L of sulphamethoxazole) and the annular radius of the zone of inhibition is  $\geq 6$  mm.

### 3. Cephalexin 100 µg for the testing of *Staphylococcus saprophyticus* from URINE ONLY.

Cephalexin 100 µg was calibrated for the testing of *Staph. saprophyticus* from urine only. The MIC breakpoint of cephalexin for susceptible strains is  $\leq 16$  mg/L and the annular radius of the zone of inhibition is  $\geq 6$ mm (see Table 1. Calibrations, 1997).

### 4. Vancomycin 5 µg for the testing of *Enterococcus* species, *Staphylococcus* species and *Streptococcus* species.

The raised MIC of vancomycin observed with some strains of enterococci is of concern. However, worldwide, the MIC "breakpoint" of vancomycin that separates susceptible from resistant strains has not been established. As an interim measure, we have chosen a vancomycin MIC of  $\leq 4.0$  mg/L as indicative of susceptibility. We found that a vancomycin 5 µg disc was suitable for testing these strains and all yielded annular radii of the zone of inhibition  $\geq 2$ mm (see Table 1. Calibrations, 1997). Testing of susceptibility to vancomycin by disc methods is difficult but the CDS test is reproducible if the method is carefully performed. Interpretation of the results with the three genera is listed below:

#### a. Enterococci.

Three patterns of zones of inhibition around a vancomycin 5 µg disc were observed:

1. Clear zone of inhibition with an annular radius  $\geq 2$  mm and a sharp edge: MIC of vancomycin  $\leq 4$  mg/L (Figure 1a).
2. Hazy growth up to the disc and the zone of hazy growth has an annular radius of approximately 2 mm: MIC of vancomycin 8 to 32 mg/L (Figure 1b).
3. No zone of inhibition: MIC of vancomycin  $\geq 128$  mg/L (Figure 1c).

#### b. Staphylococci.

The MIC of vancomycin recorded with all strains of staphylococci ranged from 0.5 to 2 mg/L.

**Note:** Colonies may be present near the edge of the zone of inhibition when testing staphylococci. In our experience, when these colonies were tested, the MIC of vancomycin was found to be similar to that of the parent strain. If the annular radius of the zone of inhibition is  $\geq 2$  mm, the isolate is reported as susceptible to vancomycin irrespective of the presence of colonies within the inhibitory zone.

#### c. Streptococci.

The MIC of vancomycin for a variety of *Streptococcus* species was 0.5 mg/L and the annular radius of the zone of inhibition was generally  $> 4$  mm.

### 5. Teichoplanin 15 µg.

Teichoplanin 15 µg was calibrated for the testing of staphylococci, streptococci and enterococci. The MIC breakpoint of teichoplanin for susceptible strains is  $\leq 8$  mg/L and the annular radius of the zone of inhibition is

≥ 2 mm (see Table 1. Calibrations, 1997).

#### 6. Cefotaxime 0.5 µg and ceftriaxone 0.5 µg for the testing of *Streptococcus* species.

Cefotaxime 0.5 µg and ceftriaxone 0.5 µg were calibrated for the testing of *Streptococcus* species including *Strep. pneumoniae* (see Table 1. Calibrations, 1997).

#### 7. Nitrofurantoin 200 µg for the testing of Groups B & D streptococci from urine.

Nitrofurantoin 200 µg was calibrated for the testing of Groups B and D streptococci from urine only. The MIC breakpoint of nitrofurantoin for susceptible strains is ≤ 32 mg/L and the annular radius of the zone of inhibition is ≥ 6mm.

#### 8. *Acinetobacter* species.

*Acinetobacter* species are now included in the table of calibrations with the *Enterobacteriaceae*. Users are reminded that for antibiotic susceptibility testing, *Acinetobacter* spp. can be separated "broadly" into two groups, the ampicillin-susceptible *A. lwoffii* / *A. lwoffii*-like group and the ampicillin-resistant *A. baumannii* / *A. baumannii*-like group which produces a non-inducible cephalosporinase. Both groups have a degree of intrinsic resistance to cephalosporins indicated by a zone of inhibition < 6mm around a cefotaxime 5 µg disc. This is often observed even with β-lactamase-negative *A. lwoffii* which have a large zone around an ampicillin 25 µg disc.

#### 9. *Aeromonas* species.

*Aeromonas* species are now included in the table of calibrations with the *Enterobacteriaceae*.

For details, see section III in this Newsletter concerning "Gram-negative bacteria: Patterns which indicate the presence of inducible β-lactamases and ESBLs."

#### 10. *Yersinia enterocolitica* and other *Yersinia* species.

*Yersinia enterocolitica* and other *Yersinia* species are now included in the table of calibrations with the *Enterobacteriaceae* but are tested on Sensitest Agar at 30°C.

**Note:** Augmentin 3 µg is not in the new list of calibrations for the *Enterobacteriaceae*, *Vibrionaceae* and *Acinetobacter* species. This disc potency was included previously as an aid in the identification of the virulent biotype 4, serotype O:3 *Y. enterocolitica*. Characteristically, strains of this bio-serotype isolated in Australia gave an annular radius of the zone of inhibition > 6mm with an Augmentin 3 µg disc whilst those isolated in Europe and Asia have a reduced zone. The lack of enzyme B in *Y. enterocolitica* 4/0:3 isolated in Australia explains the high susceptibility of these strains to Augmentin.

#### 11. Augmentin 60 µg.

Augmentin 60 µg may be tested against members of the *Enterobacteriaceae* including *Yersinia* species, the *Vibrionaceae* and *Acinetobacter* species isolated not only from urine but other body sites. A guide to testing and reporting is provided (Table 4, 1997) for species known to produce inducible cephalosporinases, inducible penicillinase/carbapenemase or extended spectrum β-lactamases (ESBL).

**Note:** When an ESBL is present, report Augmentin for isolates from URINE only. Mutants which produce high levels of an ESBL may be present in a bacterial population and in systemic infections, these can be selected by therapy with Augmentin. However, the high concentrations of amoxycillin/clavulanate in the urine should be sufficient to eliminate such mutants.

#### 12. Ampicillin 5 µg discs for the testing of *Staphylococcus saprophyticus*, *Pasteurella multocida*, *Haemophilus influenzae*, *Listeria monocytogenes* and *Enterococcus* species.

CDS Users reported difficulties with using amoxycillin 10 µg discs before their expiry date because of the infrequent need to test *Staph. saprophyticus* and *Past. multocida*. As a consequence, the discs expired before all could be used. The testing of *Staph. saprophyticus*, *H. influenzae*, *L. monocytogenes*, *P. multocida* and *Enterococcus* species against ampicillin was re-examined. An ampicillin 5 µg disc was found appropriate for the testing of all those species including β-lactamase producing enterococci.

Criteria when using ampicillin 5 µg discs are:

<i>Staphylococcus saprophyticus</i>	Ampicillin	MIC ≤ 0.5 mg/L	Annular radius of inhibition ≥ 6 mm.
<i>Haemophilus influenzae</i>	Ampicillin	MIC ≤ 1 mg/L	Annular radius of inhibition ≥ 6 mm.
<i>Listeria monocytogenes</i>	Ampicillin	MIC ≤ 1 mg/L	Annular radius of inhibition ≥ 6 mm.
<i>Pasteurella multocida</i>	Penicillin	MIC ≤ 1 mg/L	Annular radius of inhibition ≥ 6 mm.
<i>Enterococcus</i> species	Ampicillin	MIC ≤ 2 mg/L	Annular radius of inhibition ≥ 4 mm.

#### 13. Meropenem 5 µg.

Meropenem 5 µg was calibrated for the testing of the *Enterobacteriaceae*, the *Vibrionaceae*, *Acinetobacter* species and *Pseudomonas* / *Burkholderia* species. The MIC breakpoint of meropenem for susceptible strains is 2 mg/L and

the annular radius of the zone of inhibition is  $\geq 6$ mm.

**Note:** *Aeromonas* species and *Stenotrophomonas maltophilia* are considered RESISTANT to both meropenem and imipenem because these species possess a metallo-enzyme type of  $\beta$ -lactamase which hydrolyses the carbapenem antibiotics (See Table 4, Newsletter No 7).

**14. Cefazolin 30  $\mu$ g for the testing of the *Enterobacteriaceae*, *Vibrionaceae* and *Acinetobacter* species.**

Cefazolin 30  $\mu$ g was calibrated for the testing of the *Enterobacteriaceae*, *Vibrionaceae* and *Acinetobacter* species. The MIC breakpoint of cefazolin for susceptible strains is  $\leq 16$  mg/L and the annular radius of the zone of inhibition is  $\geq 6$ mm.

**15. Rifampicin 1  $\mu$ g for the testing of *Neisseria meningitidis*.**

Rifampicin 1  $\mu$ g was calibrated for the testing of *N. meningitidis*. The MIC breakpoint of rifampicin for susceptible strains is  $\leq 0.5$  mg/L and the annular radius of the zone of inhibition is  $\geq 6$ mm. The rifampicin 1 $\mu$ g disc and the low MIC breakpoint may be used for epidemiological "screening" purposes. Perform a formal MIC if the annular radius is  $< 6$ mm and do not report to a Public Health as being resistant. Invasive isolates of meningococci should be sent to a reference centre for testing.

**16. Ciprofloxacin 2.5  $\mu$ g for the testing of *Neisseria meningitidis*.**

Ciprofloxacin 2.5  $\mu$ g was calibrated for the testing of *N. meningitidis*. The MIC breakpoint of ciprofloxacin for susceptible strains is  $\leq 1$  mg/L and the annular radius of the zone of inhibition is  $\geq 6$ mm.

**17. Mast Diagnostics and CDS discs.**

Mast Diagnostics now supplies antibiotics discs which we found to be satisfactory for use with the CDS method. These discs were tested in parallel with Oxoid discs and yielded equivalent results.

**18. CDS Representatives (updated).**

The CDS Representatives who are able to supply reference strains for Quality Control of the CDS method are:

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## II. TOPICS RAISED BY MEMBERS OF THE CDS USERS GROUP AND PRESENTED AT THE CHRISTCHURCH MEETING.

### i. Group B streptococci and cephalosporins.

In Newsletter No. 7, we stated that the treatment of choice for Group B streptococcal infections was penicillin, ampicillin or amoxycillin with or without an aminoglycoside. Serious Group B streptococcal infections occur predominantly in neonates and the antibiotics stated above are those of choice for the therapy of such infections. For serious infections where the patient is allergic to penicillin or for a minor infection such as in the urine or a wound, a cephalosporin antibiotic may be substituted. The susceptibility of streptococci including Group B streptococci to a variety of penicillins and cephalosporins is shown in Table A.

**Note:** In general, Group B streptococci are less susceptible not only to cephalosporins but also to penicillin when compared with the pyogenic streptococci of Lancefield Groups A, C and G.

Table A. The MIC (mg/L) of benzylpenicillin, ampicillin, amoxycillin and a variety of cephalosporins recorded with different groups of streptococci.

$\beta$ -Lactams	Groups A, C & G streptococci	Group B streptococci
Benzylpenicillin	0.008	0.03-0.06
Ampicillin	0.03	0.06
Amoxycillin	0.008	0.015
Cefaclor	0.25-0.5	2.0-4.0
Cefpodoxime	0.008-0.015	0.015-0.03
Cephalexin	0.5	2.0-4.0
Cephalothin	0.06-0.12	0.12
Cefotaxime	0.015	0.03-0.06
Ceftriaxone	0.015-0.03	0.06

### ii. Testing staphylococci for susceptibility to methicillin.

An incubating temperature of 35°C is the optimum temperature for the demonstration of resistance to methicillin especially with coagulase-negative staphylococci. Although testing on Sensitest Agar at 30°C or on Mannitol Salt Agar at 35°C may be useful confirmatory tests with some strains, this is rarely necessary.

Some strains with heterogeneous resistance may show a zone of inhibition > 6mm but generally show individual colonies within the zone. If there is any doubt, reincubate the Sensitest Agar plate for a further 24 hours.

### iii. *Staphylococcus aureus* resistant to penicillin and methicillin only.

Some CDS Users encountered isolates of *Staph. aureus* which were resistant to penicillin and methicillin (the annular radius of the zones of inhibition < 6 mm) but otherwise were susceptible to the majority of other antibiotics which were tested. By definition, these isolates are "methicillin-resistant" and should be reported as such. The susceptibility to other agents is reported according to conventional CDS criteria (see Table 1. Calibrations, 1997).

### iv. Methicillin-resistant *Staphylococcus aureus* and the testing of rifampicin and fusidate.

Colonies may be observed within the zones of inhibition around rifampicin 1 µg and fusidate 2.5 µg discs when testing staphylococci including MRSA. These are mutants that arise at a high frequency and are resistant to the respective antibiotic. If the zones of inhibition around rifampicin and fusidate are ≥ 6 mm, the isolates are reported as susceptible to the individual antibiotics. However, it is advisable that a warning such as "rifampicin and fusidate must be given in combination for resistance will develop rapidly to either agent if used alone" be issued when reporting the susceptibility of these two antibiotics.

### v. Enterococci and trimethoprim.

Enterococci can utilise exogenous dihydrofolate, folic acid, tetrahydrofolate and thymine that may be present in urine. These compounds may antagonise the antibacterial activity of co-trimoxazole or trimethoprim and result not only in the failure of therapy of enterococcal urinary infections but the development of bacteraemia with enterococci despite the patient receiving these antibiotics (Murray, B.E. 1990. The Life and Times of the Enterococcus. *Clinical Microbiology Reviews*. 46-65).

#### vi. *Stenotrophomonas maltophilia* and sulphonamides.

Some CDS Users experienced problems when testing *Sten. maltophilia* against sulphafurazole because of a haze of growth within the zone of inhibition around the disc. The fine growth, due to the presence of sulphonamide-antagonist in the medium, should be ignored. If the isolate is susceptible to sulphafurazole, there is a visible a "comet-tail" zone of inhibition is between sulphafurazole 300 µg and an adjacent trimethoprim 5 µg disc (Fig. 2a). If there is any doubt, make sure that the inoculum is not too heavy ( $1 \times 10^7$  cfu/ml).

**Note:** If the isolate is resistant to sulphafurazole, test the isolate against Timentin 85 µg, aztreonam 30 µg and ciprofloxacin 2.5 µg using the criteria set out for *Pseudomonas* species along with a warning "A combination of antibiotics (to which the isolate is susceptible) may be necessary for therapy. Please contact the laboratories for further advice". Figure 2b shows resistant mutants arising at a high frequency justifying the need for combined therapy.

#### vii. The testing of organisms not listed in Table 1. Calibrations, 1997.

When antibiotic susceptibility is required for a species not listed in Table 1. Calibrations, 1997, Users can extrapolate testing from that of a species which has been calibrated provided that the conditions of incubation and growth requirements are similar. For example:

*Moraxella* species can be extrapolated from *Branhamella catarrhalis*.

Test: Benzylpenicillin, cefaclor, cefpodoxime, ciprofloxacin, erythromycin, tetracycline.

Conditions: Blood Sensitest Agar, CO<sub>2</sub>, 35°C

*Alcaligenes* species and *Flavobacterium* species from *Pseudomonas/Burkholderia* species.

Test: Any antibiotics calibrated for *Pseudomonas* spp. and *Burkholderia* spp.

Conditions: Sensitest Agar, air, 35°C

*Corynebacterium* species from streptococci.

Test: Any antibiotics calibrated for streptococci.

Conditions: Blood Sensitest Agar, air, 35°C

#### viii. The testing of organisms which require CO<sub>2</sub> for growth.

Occasionally, clinical isolates of *Staph. aureus* or streptococci require the addition of 5% CO<sub>2</sub> to grow. To accommodate this requirement, Sensitest Agar (for *Staph. aureus*) or Blood Sensitest Agar (for streptococci) can be incubated in 5% CO<sub>2</sub>. Parallel testing with these species indicated that the addition of CO<sub>2</sub> does not alter the results significantly.

#### ix. The testing of organisms with special nutrient requirements.

Some isolates of the *Enterobacteriaceae* may grow poorly or not at all on Sensitest Agar whilst streptococci or enterococci may grow poorly or not at all on Blood Sensitest Agar because they may require cysteine, thymidine, pyridoxal, glutamine or a combination of these substances. Testing can be performed after the addition of 5 drops from a Pasteur pipette (125 µL) of one or more of the sterile stock solutions to 2.5 ml saline prior to preparation of the inoculum.

<u>Nutrient stock solution</u>	<u>Final concentration in saline</u>
Cysteine 2000 mg/L	250 mg/L
Thymidine 5000 mg/L	100 mg/L
Pyridoxal 1000 mg/L	50 mg/L
Glutamine 1000 mg/L	50 mg/L

**Hint:** The more common requirements for *Enterobacteriaceae* include thymidine, cysteine and glutamine. Nutritionally requiring enterococci from urine specimens are usually thymidine dependent. Streptococci may show a requirement for pyridoxal.

#### x. β-Lactam antibiotics for the testing of *Staphylococcus saprophyticus* from urine ONLY.

The MICs of benzylpenicillin and methicillin observed with *Staph. saprophyticus* were relatively high when compared with other staphylococci. For this reason, the annular radius of the zone of inhibition around penicillin 0.5 u and methicillin 5 µg discs recorded with *Staph. saprophyticus* was < 6mm. Higher potency discs in the form of **ampicillin 5 µg** and **cephalexin 100µg** were chosen to test isolates of this species from URINE only (see Table 1. Calibrations, 1997).

**Note:** Isolates of coagulase-negative staphylococci from urine which are resistant to novobiocin (annular radius of the zone of inhibition < 6 mm around a novobiocin 5 µg disc) are identified presumptively as *Staph. saprophyticus*.



### III. GRAM-NEGATIVE BACTERIA: PATTERNS THAT INDICATE THE PRESENCE OF INDUCIBLE $\beta$ -LACTAMASES and ESBLs.

#### i. *Aeromonas* species.

*Aeromonas* species are now included in the table of calibrations with the *Enterobacteriaceae*.

**Note:** *Aeromonas* spp. are considered resistant to penicillins, imipenem and meropenem since they produce the penicillinase/carbapenemase A2 which hydrolyses these antibiotics. This enzyme is not inhibited by clavulanic acid.

*A. hydrophila* and *A. caviae* in addition, produce the inducible cephalosporinase A1 which is indicated by a flattened zone of inhibition between cefotaxime 5  $\mu$ g and imipenem 10  $\mu$ g discs (Fig. 3a). These species are considered resistant to cephalosporins and cephamycins. However, aztreonam can be tested as in all the strains we have examined so far, we have not observed derepressed mutants arise on exposure to this  $\beta$ -lactam antibiotic (see Table 4, 1997).

*A. sobria* usually lacks the inducible cephalosporinase A1 as indicated by the absence of a flattened zone of inhibition between cefotaxime 5  $\mu$ g and imipenem 10  $\mu$ g discs (Fig. 3b). This species can be tested for the susceptibility to cephalosporins, cephamycins and aztreonam (see Table 4, 1997).

#### ii. *Enterobacteriaceae* known to produce inducible cephalosporinases.

CDS Users are reminded that the species listed below are known to produce inducible chromosomal cephalosporinases (see Table 4, p.19, Newsletter No 7 for details on the classification of  $\beta$ -lactamases).

Class 1: *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*.

Class 1': *Providencia rettgeri*, *Providencia stuartii*, *Morganella morganii*.

Class 2e: *Proteus vulgaris*, *Proteus penneri*.

These species are considered resistant to most  $\beta$ -lactam agents except imipenem, meropenem and a few specific  $\beta$ -lactam antibiotics depending on the nature of the cephalosporinase. See Table 4, 1997: A guide to the testing and reporting of susceptibility to  $\beta$ -lactam antibiotics for the *Enterobacteriaceae* and *Aeromonas* species.

#### iii. The detection of ESBLs during routine antibiotic susceptibility testing by the CDS method.

The extended spectrum  $\beta$ -lactamases (ESBLs) produced by *Klebsiella pneumoniae* or other species of the *Enterobacteriaceae* can be detected whilst performing routine antibiotic susceptibility tests using the CDS method.

When **either** an Augmentin 60  $\mu$ g **or** a Timentin 85  $\mu$ g disc is placed next to a cefotaxime 5  $\mu$ g disc in a disc dispenser, the presence of an ESBL is indicated by a clear, elliptical area (Fig. 4a) or the "key-hole" effect between the two discs (Fig. 4b, 4c).

**Note:** Alternatively, cephalexin 100  $\mu$ g may be used instead of cefotaxime 5  $\mu$ g (Fig. 4d) for isolates from urine where cephalexin might be used for routine testing. However, the combination cephalexin 100  $\mu$ g/Augmentin 60  $\mu$ g may not be as sensitive as the combination cefotaxime 5  $\mu$ g/Augmentin 60  $\mu$ g to demonstrate the presence of ESBLs.

#### iv. The detection of ESBLs and inducible $\beta$ -lactamases.

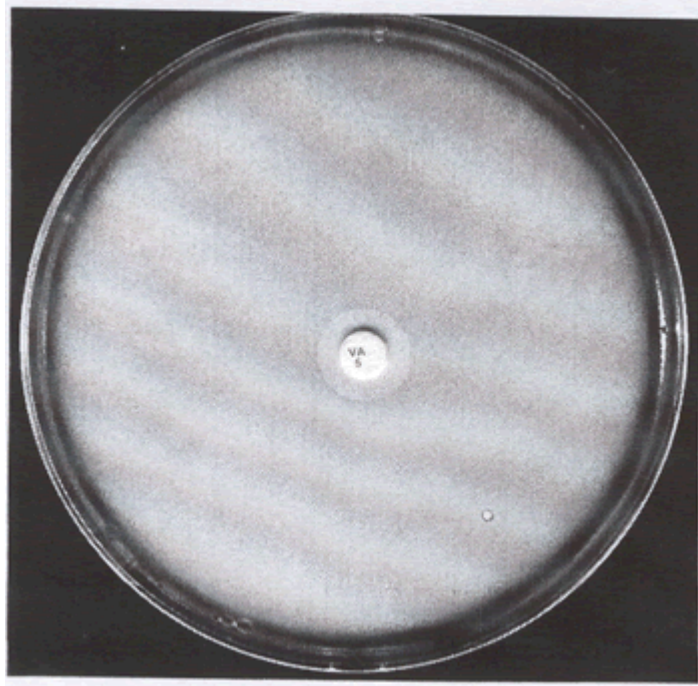
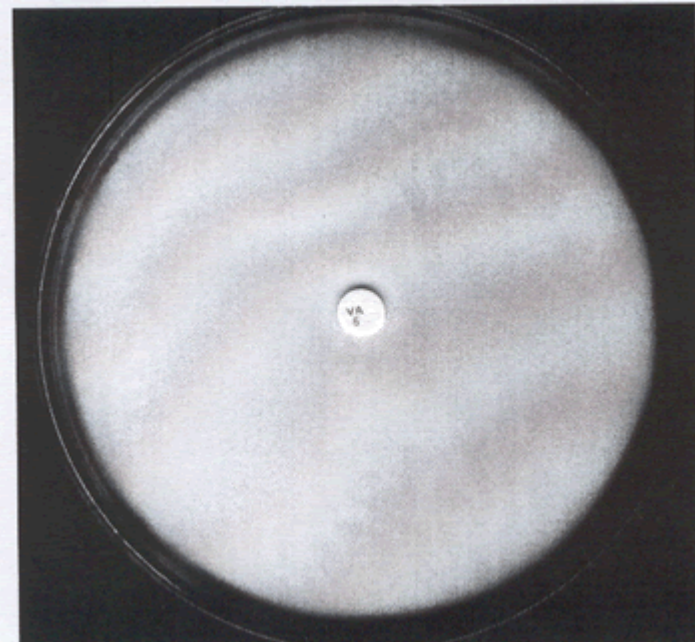
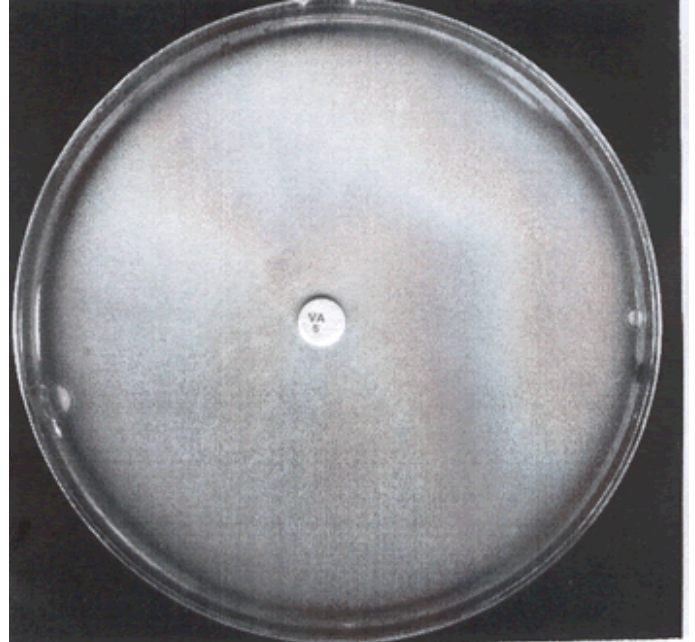
When **either** an Augmentin 60  $\mu$ g **or** a Timentin 85  $\mu$ g disc is placed adjacent to a cefotaxime 5  $\mu$ g disc and an imipenem 10  $\mu$ g disc is placed adjacent to the cefotaxime 5  $\mu$ g disc in a disc dispenser, ESBLs (Fig. 4a, 4b, 4c) and inducible  $\beta$ -lactamases may be detected (Figs. 5a, 5b, 5c, 5d).

**Note:** For urinary isolates, some laboratories may use cephalexin instead of cefotaxime and may not include imipenem for testing. Resistance to cephalexin may indicate the presence of an inducible cephalosporinase belonging to Classes 1, 1', 2e or A1 (*Aeromonas hydrophila* & *caviae*)  $\beta$ -lactamases or an ESBL or that the organism may belong to the *Acinetobacter baumannii*-like group.

**ILLUSTRATIONS****Figures 1a, 1b, 1c.**

Three patterns of the zone of inhibition around a vancomycin 5 µg (VA 5) disc observed with enterococci:

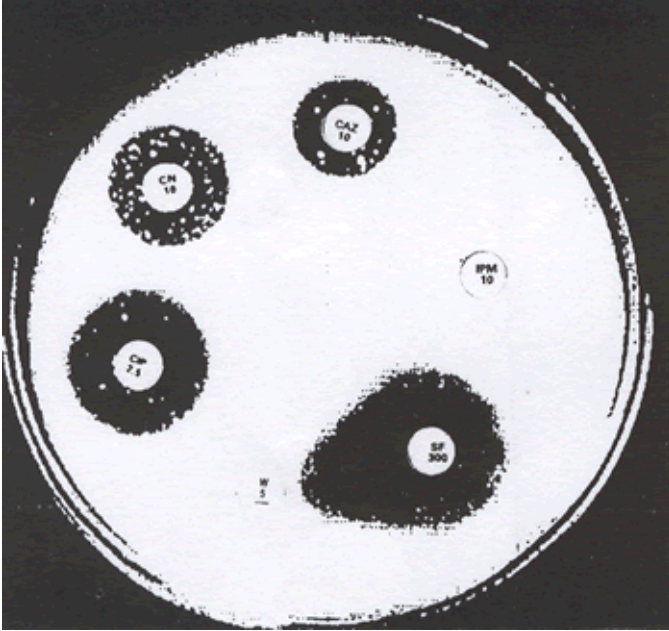
1. Clear zone of inhibition with an annular radius  $\geq 2$  mm and a sharp edge: MIC of vancomycin  $\leq 8$  mg/L (Figure 1a).
2. Hazy growth within the zone of inhibition with an annular radius of approximately 2 mm and a hazy edge: MIC of vancomycin 16 mg/L or 32 mg/L (Figure 1b).
3. No zone of inhibition: MIC of vancomycin  $\geq 128$  mg/L (Figure 1c).

**Figure 1a****Figure 1b****Figure 1c**

**Figures 2a, 2b.**

A susceptibility pattern typical of *Steno. maltophilia* with no zone around an imipenem 10 µg disc (IPM 10) and synergy (comet-tail effect) between the adjacent sulphafurazole 300 µg (SF 300) and trimethoprim 5 µg (W 5) discs. This unique pattern helps CDS Users to identify *Steno. maltophilia* (Fig. 2a).

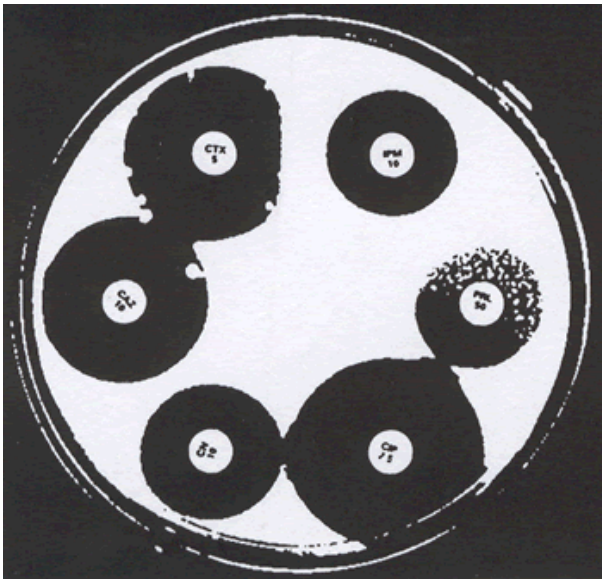
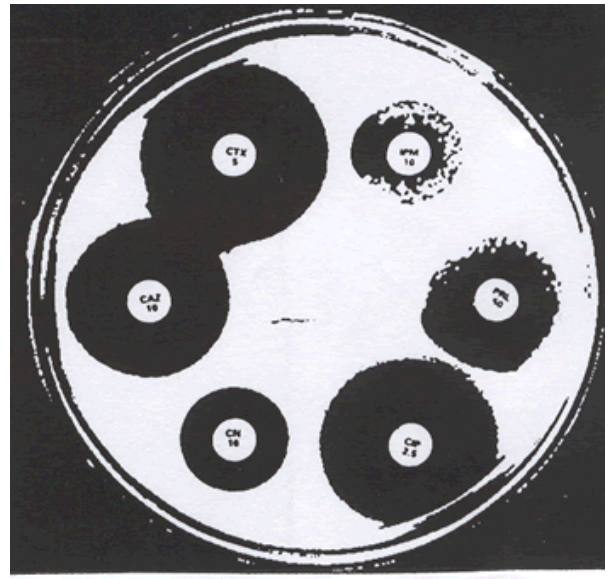
*Steno. maltophilia* showing resistant mutants to Timentin (Fig. 2b).

**Figure 2a****Figure 2b****Figures 3a, 3b.**

*A. hydrophila* and *A. caviae* produce the inducible cephalosporinase A1 indicated by a flattened zone of inhibition between cefotaxime 5 µg (CTX 5) and imipenem 10 µg (IPM 10) discs (Figure 3a).

*A. sobria* often lack the inducible cephalosporinase A1 indicated by the absence of a flattened zone between cefotaxime 5 µg (CTX 5) and imipenem 10 µg (IPM 10) discs (Figure 3b).

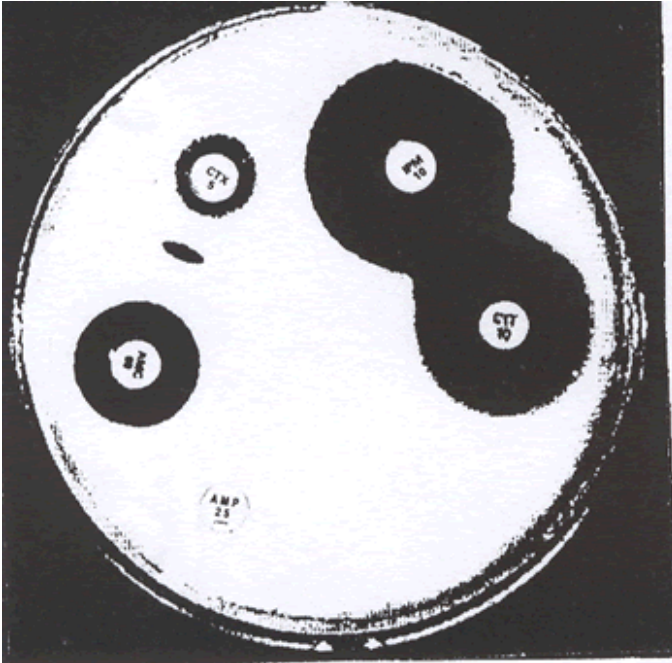
**Note:** All *Aeromonas* species are considered resistant to imipenem and meropenem due to the presence of the penicillinase/carbapenemase A2 β-lactamase (Fig. 3b). Mutants which hyperproduce this enzyme are not always visible in the zone of inhibition around the imipenem disc (Fig. 3a).

**Figure 3a****Figure 3b****Figures 4a, 4b, 4c, 4d.**

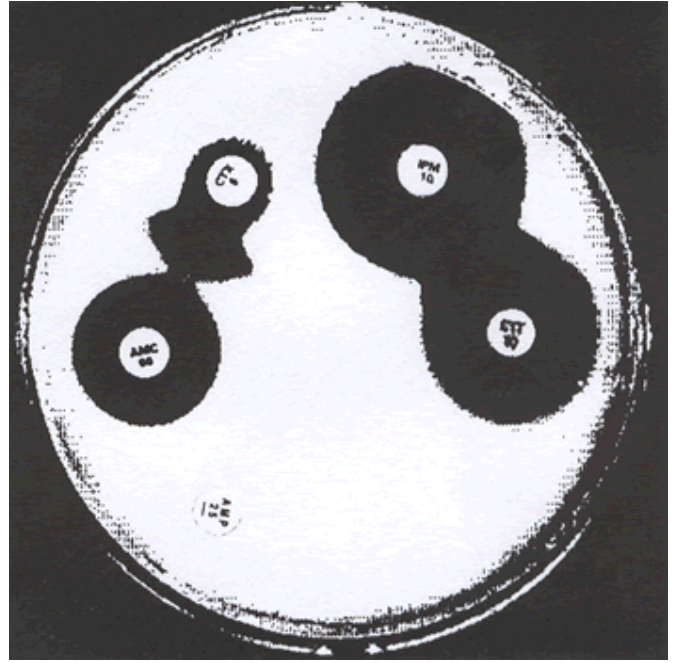


The presence of an ESBL is indicated by a clear, elliptical area (Fig. 4a) or the "key-hole" effect (Figs. 4b & 4c) between cefotaxime 5 µg (CTX 5) and Augmentin 60 µg (AMC 60) or cefotaxime 5 µg (CTX 5) and Timentin 85 µg (TIM 85) which are placed in adjacent positions in a disc dispenser. Alternatively, for isolates from urine, cephalixin 100 µg (CL 100) can be used instead of cefotaxime 5 µg (Fig. 4d) although the combination of cephalixin 100 µg/Augmentin 60 µg may not be as sensitive as the combination cefotaxime 5µg/Augmentin 60 µg to demonstrate the presence of ESBLs.

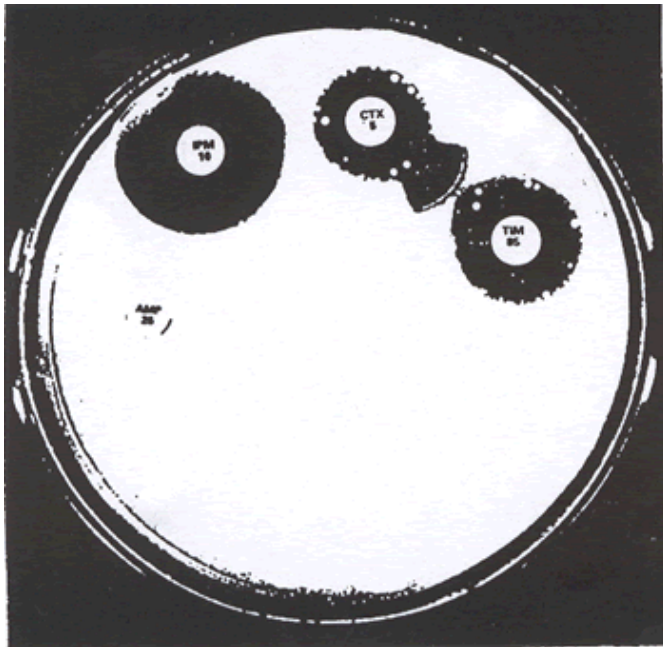
**Figure 4a**



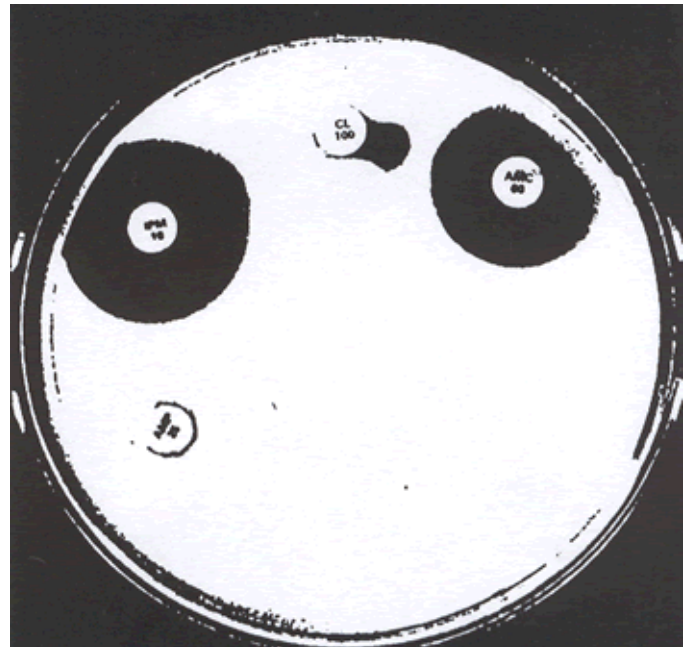
**Figure 4b**



**Figure 4c**

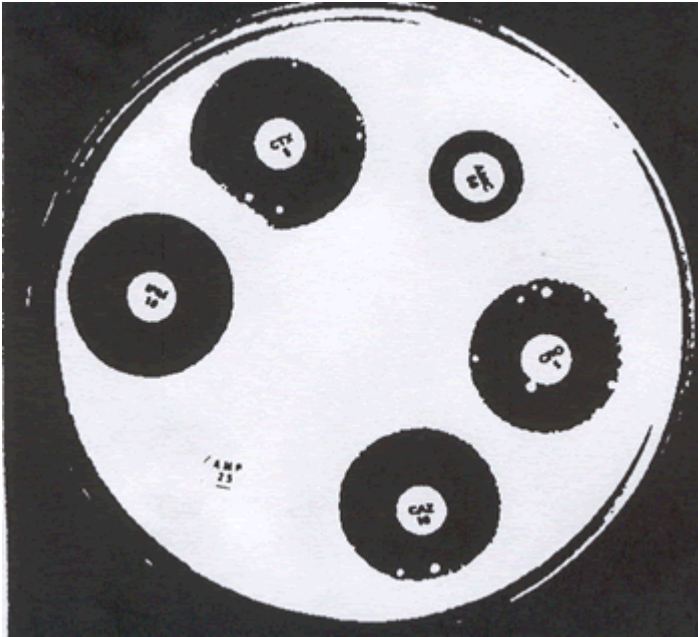
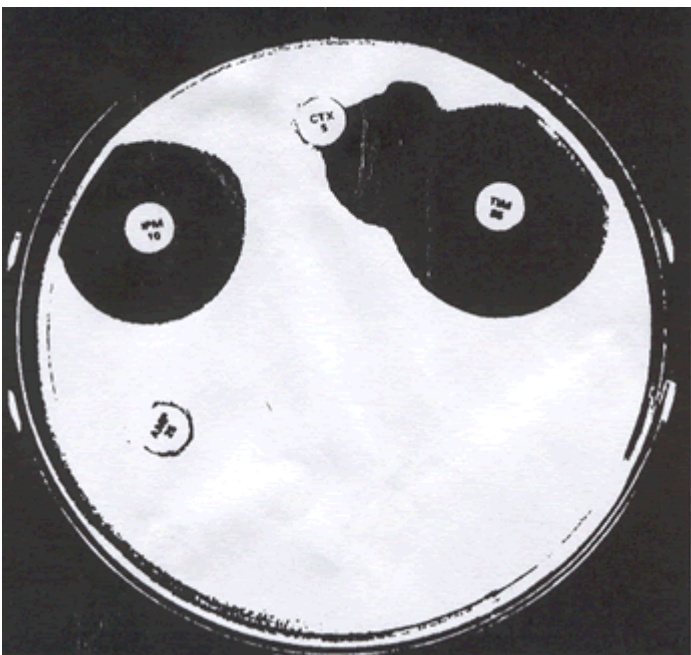
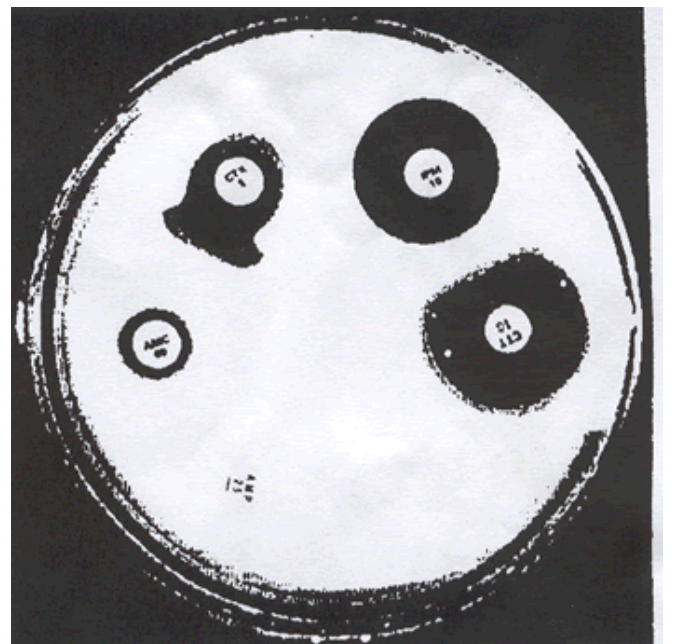


**Figure 4d**



**Figures 5a, 5b, 5c, 5d.**

The detection of various types of  $\beta$ -lactamases based on induction of the enzymes by imipenem and inhibition by clavulanic acid. Figure 5a indicates a Class 1 inducible cephalosporinase (not inhibited by clavulanic acid) produced by *Enterobacter cloacae*. Figure 5b illustrates the induction of a Class 2e inducible cephalosporinase which is inhibited by clavulanic acid, found in *Proteus penneri* (indole-negative) or *Proteus vulgaris* (indole-positive). Note the characteristic flattened zone of inhibition around cefotaxime 5  $\mu$ g (CTX 5) adjacent to imipenem 10  $\mu$ g (IPM 10), numerous resistant colonies around CTX 5 and a zone > 6mm around Augmentin (AMC 60). Figure 5c illustrates *Proteus penneri* or *Proteus vulgaris* which hyperproduce  $\beta$ -lactamase with no zone around cefotaxime 5  $\mu$ g (CTX 5) and a large zone around Timentin 85  $\mu$ g (TIM 85). Note the "key-hole" effect which may be mistaken as an indication of the presence of an ESBL. Figure 5d shows the co-production of an ESBL and an inducible Class 1 cephalosporinase in *E. cloacae*.

**Figure 5a****Figure 5b****Figure 5c****Figure 5d**

**Table 1a. Calibrations, 1997:** Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

Antibiotic	Disc potency ( $\mu\text{g}$ )		MIC for susceptible strains (mg/L)
<b><i>Branhamella catarrhalis</i></b>			
(Blood Sensitest, CO <sub>2</sub> , 35°C)			
Benzylpenicillin	0.5 u		$\leq 0.25$
Cefaclor	30		$\leq 4.0$
Cefpodoxime	10		$\leq 2.0$
Ciprofloxacin	2.5		$\leq 1.0$
Erythromycin	5		$\leq 0.5$
Tetracycline	30		$\leq 4.0$
<b><i>Campylobacter spp.</i></b>			
(Blood Sensitest, microaerophilic, 42°C)			
Ciprofloxacin	2.5		$\leq 1.0$
Erythromycin	5	4 mm †	$\leq 0.5$
Gentamicin	10		$\leq 1.0$
Tetracycline	30		$\leq 4.0$
<b><i>Enterobacteriaceae, Vibrionaceae, &amp; Acinetobacter spp.</i></b>			
(Sensitest, air, 35°C)*			
Amikacin	30		$\leq 4.0$
Ampicillin	25		$\leq 8.0$
Augmentin °	60		$\leq 16.0/8.0$
Aztreonam	30		$\leq 8.0$
Cefazolin	30		$\leq 16.0$
Cefotaxime	5		$\leq 1.0$
Cefotetan	10		$\leq 4.0$
Cefoxitin	30		$\leq 8.0$
Cefpirome	10		$\leq 2.0$
Cefpodoxime	10		$\leq 2.0$
Ceftazidime	10		$\leq 4.0$
Ceftriaxone	5		$\leq 1.0$
Cephalexin +	100		$\leq 16.0$
Chloramphenicol	30		$\leq 8.0$
Ciprofloxacin	2.5		$\leq 1.0$
Enoxacin	10		$\leq 4.0$
Gentamicin	10		$\leq 1.0$
Imipenem	10		$\leq 4.0$
Kanamycin	50		$\leq 8.0$
Meropenem	5		$\leq 2.0$
Nalidixic acid +	30		$\leq 4.0$
Netilmicin	30		$\leq 2.0$
Nitrofurantoin +	200		$\leq 32.0$
Norfloxacin +	10		$\leq 4.0$
Sulphafurazole	300		$\leq 64.0$
Tazocin	55		$\leq 16.0/2.0$
Tetracycline	30		$\leq 4.0$
Timentin	85		$\leq 32.0/2.0$
Tobramycin	10		$\leq 1.0$
Trimethoprim	5		$\leq 2.0$
<b>Enterococci</b>			
(Blood Sensitest, air, 35°C)			
Ampicillin	5	4 mm †	$\leq 2.0$
Chloramphenicol	30	4 mm †	$\leq 8.0$
Gentamicin	200	4 mm †	$\leq 512$
Nitrofurantoin +	200	4 mm †	$\leq 64.0$
Teichoplanin	15	2 mm £	$\leq 8.0$
Vancomycin	5	2 mm £	$\leq 4.0$

† The annular radius of the zone of inhibition for susceptible strains is  $\geq 4$  mm.

£ The annular radius of the zone of inhibition for susceptible strains is  $\geq 2$  mm. \* *Yersinia enterocolitica* is incubated in air at 30°C.

o If an ESBL is present, report Augmentin for isolates from URINE ONLY. + ONLY for isolates from urine

**Table 1b. Calibrations, 1997:** Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

Antibiotic	Disc potency ( $\mu\text{g}$ )		MIC for susceptible strains (mg/L)
<b><i>Haemophilus influenzae</i>/<i>Haemophilus</i> spp</b>			
(HTM <sup>@</sup> agar, CO <sub>2</sub> , 35°C)			
Ampicillin	5		$\leq 1.0$
Augmentin	3	4 mm †	$\leq 2.0/1.0$
Cefaclor	30		$\leq 4.0$
Cefotaxime	0.5		$\leq 0.25$
Cefpodoxime	10		$\leq 2.0$
Ceftriaxone	0.5		$\leq 0.25$
Chloramphenicol	10		$\leq 2.0$
Ciprofloxacin	2.5		$\leq 1.0$
Co-trimoxazole	25		$\leq 1.0/19.0$
Tetracycline	30		$\leq 4.0$
<b><i>Listeria monocytogenes</i></b>			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		$\leq 1.0$
Gentamicin	10		$\leq 1.0$
<b><i>Neisseria meningitidis</i></b>			
(Blood Sensitest, CO <sub>2</sub> , 35°C)			
Benzylpenicillin	0.5 u	4 mm †	$\leq 0.25$
Cefotaxime	0.5		$\leq 0.25$
Ceftriaxone	0.5		$\leq 0.25$
Chloramphenicol	10		$\leq 2.0$
Ciprofloxacin	2.5		$\leq 1.0$
Rifampicin	1		$\leq 0.5$
<b><i>Pasteurella multocida</i></b>			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		$\leq 1.0$
Ciprofloxacin	2.5		$\leq 1.0$
Tetracycline	30		$\leq 4.0$
<b><i>Pseudomonas</i> spp. &amp; <i>Burkholderia</i> spp.</b>			
(Sensitest, air, 35°C)			
Amikacin	30	4 mm †	$\leq 16.0$
Aztreonam	30		$\leq 8.0$
Cefpirome	10		$\leq 2.0$
Ceftazidime	10		$\leq 4.0$
Ciprofloxacin	2.5		$\leq 2.0$
Gentamicin	10	4 mm †	$\leq 4.0$
Imipenem	10		$\leq 4.0$
Meropenem	5		$\leq 2.0$
Netilmicin	30	4 mm †	$\leq 8.0$
Norfloxacin +	10		$\leq 4.0$
Piperacillin	50		$\leq 16.0$
Polymyxin	300 u	4 mm †	$\leq 1.0$
Tazocin	55		$\leq 16.0/2.0$
Ticarcillin	75		$\leq 32.0$
Timentin	85		$\leq 32.0/2.0$
Tobramycin	10	4 mm †	$\leq 4.0$
Trimethoprim	5		$\leq 2.0$

† The annular radius of the zone of inhibition for susceptible strains is  $\geq 4$  mm.

+ ONLY for isolates from urine

@ Haemophilus Test Medium containing 15 mg/L freshly prepared Haematin and NAD.

**Table 1c. Calibrations, 1997(continued):** Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

Antibiotic	Disc potency ( $\mu\text{g}$ )		MIC for susceptible strains (mg/L)
<b>Staphylococci</b> (Sensitest, air, 35°C)			
Ampicillin §	5		$\leq 0.5$
Benzylpenicillin #	0.5 u		$\leq 0.06$
Cephalexin §	100		$\leq 16.0$
Chloramphenicol	30		$\leq 8.0$
Ciprofloxacin	2.5		$\leq 1.0$
Erythromycin	5		$\leq 0.5$
Fusidic acid	2.5		$\leq 0.5$
Gentamicin	10		$\leq 1.0$
Kanamycin	50		$\leq 8.0$
Methicillin #	5		$\leq 4.0$
Nitrofurantoin +	200		$\leq 32.0$
Rifampicin	1		$\leq 0.5$
Sulphafurazole	300		$\leq 64.0$
Teichoplanin	15	2 mm £	$\leq 8.0$
Tetracycline	30		$\leq 4.0$
Trimethoprim	5		$\leq 2.0$
Vancomycin	5	2.mm £	$\leq 4.0$
<b>Streptococci</b> (Blood Sensitest, air, 35°C) Ω			
Benzylpenicillin	0.5 u		$\leq 0.25$
Cefotaxime	0.5		$\leq 0.25$
Ceftriaxone	0.5		$\leq 0.25$
Chloramphenicol	30		$\leq 8.0$
Co-trimoxazole &	25		$\leq 0.5/9.5$
Erythromycin	5		$\leq 0.5$
Nitrofurantoin +	200		$\leq 32.0$
Rifampicin	1		$\leq 0.5$
Teichoplanin	15	2mm £	$\leq 8.0$
Tetracycline	30		$\leq 4.0$
Vancomycin	5	2.mm £	$\leq 4.0$
<b><i>Stenotrophomonas maltophilia</i></b> (Sensitest, air, 35°C)			
Sulphafurazole	300		$\leq 64.0$

£ The annular radius of the zone of inhibition for susceptible strains is  $\geq 2$  mm.

§ ONLY for testing isolates of *S. saprophyticus*. # Not for testing *S. saprophyticus*. + ONLY for isolates from urine.

Ω *S. pneumoniae* and *S. anginosus* are incubated in 5% CO<sub>2</sub> & For *Streptococcus pneumoniae* only.



**Table 2a. . Surrogate disc testing (1997).** Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc potency (µg)
<b><i>Branhamella catarrhalis</i></b>		
Azithromycin	Erythromycin	5
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefaclor	30
Cephalosporins	Cefaclor	30
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<b><i>Campylobacter spp.</i></b>		
Tetracyclines	Tetracycline	30
<b><i>Enterobacteriaceae &amp; Vibrionaceae &amp; Acinetobacter spp.</i></b>		
Amoxycillin	Ampicillin	25
Cefotaxime	Ceftriaxone	5
Ceftriaxone	Cefotaxime	5
Cephalothin	Ampicillin	25
Co-trimoxazole <sup>φ</sup>	Sulphafurazole	300
Co-trimoxazole <sup>φ</sup>	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<b>Enterococci</b>		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
<b><i>Haemophilus influenzae/Haemophilus spp.</i></b>		
Amoxycillin	Ampicillin	5
Cefpirome	Cefotaxime/ceftriaxone	0.5
Ceftazidime	Cefotaxime/ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
Cefotaxime	Ceftriaxone	0.5
Cephalexin	Cefaclor	30
Tetracyclines	Tetracycline	30
<b><i>Listeria monocytogenes.</i></b>		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
<b><i>Neisseria meningitidis</i></b>		
Ampicillin	Benzylpenicillin	0.5 u
Amoxycillin	Benzylpenicillin	0.5 u
Ceftriaxone	Cefotaxime	0.5
Cefotaxime	Ceftriaxone	0.5
<b><i>Pasteurella multocida</i></b>		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
Tetracyclines	Tetracycline	30
<b><i>Pseudomonas spp &amp; Burkholderia spp.</i></b>		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole <sup>φ</sup>	Trimethoprim	5

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

**Table 2a. Surrogate disc testing (1997) continued.** Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

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
Cephalosporins	Methicillin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole <sup>φ</sup>	Sulphafurazole	300
Co-trimoxazole <sup>φ</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	5
Augmentin	Cephalexin	100
Benzylpenicillin	Ampicillin	5
Cefaclor	Cephalexin	100
Cephalothin	Cephalexin	100
Cefazolin	Cephalexin	100
Cloxacillin	Cephalexin	100
Co-trimoxazole <sup>φ</sup>	Sulphafurazole	300
Co-trimoxazole <sup>φ</sup>	Trimethoprim	5
Dicloxacillin	Cephalexin	100
Flucloxacillin	Cephalexin	100
Norfloxacin	Ciprofloxacin	2.5
Penicillin V	Ampicillin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<b>Streptococci *</b>		
Amoxicillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Cephalosporins	Cefotaxime/Ceftriaxone	0.5
Clindamycin	Erythromycin	5
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

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

\* For streptococci groups A, B, C, G and *Strep. anginosus*, the susceptibility to penicillin and cephalosporin antibiotics is extrapolated from the testing of benzylpenicillin 0.5 u.

**Table 3a. Reference strains, 1997:** 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)
<b><i>Staphylococcus aureus</i> NCTC 6571</b>		
(Sensitest, air 35°C)		
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
Teichoplanin	15	3.4 - 6.1
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	7.3 - 10.1
Vancomycin	5	2.8 - 4.9
<b><i>Haemophilus influenzae</i> NCTC 4560</b>		
(HTM <sup>@</sup> agar, CO <sub>2</sub> , 35°C)		
Ampicillin	5	7.0 - 11.1
Chloramphenicol	10	9.8 - 12.6
Cefaclor	30	8.1 - 12.1
Cefotaxime	0.5	9.2 - 12.8
Cefpodoxime	10	10.9 - 14.1
Ceftriaxone	0.5	9.1 - 12.9
Ciprofloxacin	2.5	11.1 - 15.9
Co-trimoxazole	25	9.0 - 12.5
Tetracycline	30	9.9 - 13.3
<b><i>Haemophilus influenzae</i> NCTC 11315</b>		
(HTM <sup>@</sup> agar, CO <sub>2</sub> , 35°C)		
Augmentin	3	3.6 - 6.6
<b><i>Pseudomonas aeruginosa</i> NCTC 10662 #</b>		
(Sensitest, air 35°C)		
Amikacin	30	7.4 - 10.6
Aztreonam	30	8.3 - 13.1
Ceftazidime	10	7.5 - 11.9
Cefpirome	10	8.1 - 10.6
Ciprofloxacin	2.5	8.9 - 14.5
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
Meropenem	5	9.7 - 14.8
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. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

@ Haemophilus Test Medium containing 15 mg/L freshly prepared Haematin and NAD.

# If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 or *Yersinia enterocolitica* IP 22273 as well and vice versa.

**NOTE: Additional testing with reference strains must be performed when:**

- A new batch of medium is used.
- A new batch of discs is used.
- Once a week if the batches of both media and discs have been tested previously.
- If there is an infrequent requirement for testing either an antibiotic or an organism, then the appropriate reference strain must be tested at the same time as the clinical isolate.

**Table 3b. Reference strains, 1997 (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)
<b><i>Escherichia coli</i> NCTC 10418 #</b>		
(Sensitest, air, 35°C)		
Amikacin	30	6.7 - 10.3
Ampicillin	25	7.5 - 10.7
Aztreonam	30	13.7 - 15.9
Cefazolin	30	6.7 - 12.7
Cefotaxime	5	9.7 - 13.7
Cefotetan	10	11.6 - 13.6
Cefoxitin	30	9.8 - 13.0
Cefpirome	10	11.9 - 14.6
Cefpodoxime	10	10.3 - 12.7
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
Enoxacin	10	9.7 - 15.7
Gentamicin	10	6.2 - 9.4
Imipenem	10	10.3 - 13.5
Kanamycin	50	6.2 - 11.8
Meropenem	5	11.0 - 14.4
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>		
(Sensitest, air 35°C)		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
Tazocin	55	7.4 - 9.2
<b><i>Streptococcus pneumoniae</i> ARL 10582</b>		
(Blood Sensitest, 5% CO <sub>2</sub> , 35°C)		
Benzylpenicillin	0.5u	8.3 - 14.8
Cefotaxime	0.5	9.3 - 14.8
Ceftriaxone	0.5	9.1 - 14.3
Chloramphenicol	30	8.0 - 13.2
Co-trimoxazole	25	7.0 - 9.2
Erythromycin	5	7.1 - 12.9
Rifampicin	1	7.5 - 10.8
Tetracycline	30	9.2 - 14.5
Teichoplanin	15	5.1 - 8.0
Vancomycin	5	5.1 - 8.6
<b><i>Enterococcus faecalis</i> POW 1994</b>		
(Blood Sensitest, air 35°C)		
Ampicillin	5	5.9 - 9.2
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Teichoplanin	15	3.1 - 5.3
Vancomycin	5	2.4 - 3.7

\* The acceptable range (95% confidence limits) is the mean  $\pm$  2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

# If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 or *Yersinia enterocolitica* IP 22273 as well and vice versa.

**NOTE: Additional testing with reference strains must be performed when:**

- a. A new batch of medium is used.
- b. A new batch of discs is used.
- c. Once a week if the batches of both media and discs have been tested previously.
- d. If there is an infrequent requirement for testing either an antibiotic or an organism, then the appropriate reference strain must be tested at the same time as the clinical isolate.

**Table 4. 1997: A guide to the testing / reporting of  $\beta$ -lactam antibiotics for the *Enterobacteriaceae* and *Aeromonas* species.**

**R** = the organism is resistant to the antibiotic because it possesses a mechanism of resistance which may not be demonstrated by disc testing.

**T** = can be tested.

Organism/ Species	AMP	AMC	ATM	Antibiotic CAZ	CL	CPD	CPO	CRO
<b>Inducible <math>\beta</math>-lactamases present</b>								
<i>Ent. cloacae</i> / <i>Ent. aerogenes</i>	R	R	R	R	R	R	R	R
<i>Cit. freundii</i>	R	R	R	R	R	R	R	R
<i>Ser. marcescens</i>	R	R	R	R	R	R	R	R
<i>Prov. stuartii</i> / <i>Prov. rettgeri</i>	R	R	<b>T</b>	R	R	R	R	R
<i>Morg. morgani</i>	R	R	<b>T</b>	R	R	R	R	R
<i>Prot. vulgaris</i> / <i>Prot. penneri</i> <sup>1</sup>	R	<b>T</b>	R	R	R	R	R	R
<i>Aeromonas</i> /A2 (most <i>A. sobria</i> )	R	R	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>
<i>Aeromonas</i> /A1 & A2	R	R	<b>T</b>	R	R	R	R	R
<b>ESBL present alone</b>								
<i>Enterobacteriaceae</i> with ESBL	R	<b>T<sup>2</sup></b>	R	R	R	R	R	R
<b>Inducible <math>\beta</math>-lactamases and ESBL present</b>								
<i>Enterobacteriaceae</i> with ESBL	R	R	R	R	R	R	R	R
<i>Aeromonas</i> /A2 with ESBL	R	R	R	R	R	R	R	R
<i>Aeromonas</i> /A1 & A2 with ESBL	R	R	R	R	R	R	R	R

AMP=ampicillin, AMC=Augmentin, ATM=aztreonam, CAZ=ceftazidime, CL=cephalexin, CPD=cefepodoxime, CPO=cefpirome, CRO=ceftriaxone

**1.** Isolates with high  $\beta$ -lactamase activity may give no zone around CTX 5 but show a "key-hole" effect which may be mistaken as an indication of the presence of an ESBL.

**2.** Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.

**Table 4. 1997 (continued): A guide to the testing / reporting of  $\beta$ -lactam antibiotics for the*****Enterobacteriaceae* and *Aeromonas* species.**

**R** = the organism is resistant to the antibiotic because it possesses a mechanism of resistance which may not be demonstrated by disc testing.

**T** = can be tested.

Organism/ Species	Antibiotic							
	CTT	CTX	FOX	KZ	IPM	MEM	TIM	TZP
<b>Inducible <math>\beta</math>-lactamases present</b>								
<i>Ent. cloacae</i> / <i>Ent. aerogenes</i>	R	R	R	R	<b>T</b>	<b>T</b>	R	R
<i>Cit. freundii</i>	R	R	R	R	<b>T</b>	<b>T</b>	R	R
<i>Ser. marcescens</i>	R	R	R	R	<b>T</b>	<b>T</b>	R	R
<i>Prov. stuartii</i> / <i>Prov. rettgeri</i>	R	R	R	R	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>
<i>Morg. morgani</i>	R	R	R	R	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>
<i>Prot. vulgaris</i> / <i>Prot. penneri</i> <sup>1</sup>	<b>T</b>	R	<b>T</b>	R	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>
<i>Aeromonas</i> /A2 (most <i>A. sobria</i> )	<b>T</b>	<b>T</b>	<b>T</b>	<b>T</b>	R	R	R	R
<i>Aeromonas</i> /A1 & A2	R	R	R	R	R	R	R	R
<b>ESBL present alone</b>								
<i>Enterobacteriaceae</i> with ESBL	<b>T</b>	R	R	R	<b>T</b>	<b>T</b>	R	R
<b>Inducible <math>\beta</math>-lactamases and ESBL present</b>								
<i>Enterobacteriaceae</i> with ESBL	R	R	R	R	<b>T</b>	<b>T</b>	R	R
<i>Aeromonas</i> /A2 with ESBL	<b>T</b>	R	R	R	R	R	R	R
<i>Aeromonas</i> /A1 & A2 with ESBL	R	R	R	R	R	R	R	R

CTT=cefotetan, CTX=cefotaxime, FOX=cefoxitin, KZ=cephazolin, IPM=imipenem, MEM=meropenem, TIM=Timentin, TZP=Tazocin.

1. Isolates with high  $\beta$ -lactamase activity may give no zone around CTX 5 but show a "key-hole" effect which may be mistaken as an indication of the presence of an ESBL.

2. Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.