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Dear Colleague,

CDS USERS GROUP

Newsletter No. 11

This newsletter contains two sections. Section A is the proceeding of the CDS Users Group Workshop conducted at the Australian Society for Microbiology Annual Meeting in Perth in October 2001. It also includes some additions and modifications introduced to the CDS (See Tables 1a, 1b, 1c of Calibrations 2001 and Table 3a, 3b, 3c References Strains 2001).

Section B reports the CDS Testing of veterinary isolates presented as part of Symposium 56 "Antimicrobial Testing Standardisation for Veterinary Laboratories". This section also includes 3 groups of Tables, Tables 1a and 1b of Calibrations, Table 2 of Surrogate Testing and Tables 3a and 3b of Reference strains. These Tables have been specially prepared for Veterinary Laboratories.

Kind regards,

Syd Bell,
For the CDS Reference Laboratory.
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CDS USERS GROUP NEWSLETTER No. 11

Additions and modifications to the CDS method and a report of the CDS Users Group Workshop and Veterinary Session at the Annual Scientific Meeting ASM Perth

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Section A: CDS Users Group Workshop

I. CDS Web site

The details of the CDS Web site were presented to the workshop and included details of the site's address as shown here <http://www.med.unsw.edu.au/pathology-cds/>. The meeting discussed the various pages in the site and were shown how to access and download documents from the site. The CDS web site contains all the publications relating to the CDS including the Laboratory Manual 1999 and the original Monograph published in PATHOLOGY. All the CDS users Newsletters are contained in the web site and all Newsletters and the Manual are now searchable. Planned additions and modifications to the site were reported to the meeting. The web site also contains instructions on how to join the CDS users e-mail group. It is important that as many members as possible join the e-mail group because it will become the predominant means of communicating information about the CDS, including the Newsletters, in the future.

II. Additions to the CDS method

Gatifloxacin, another new quinolone similar to moxifloxacin has been calibrated for testing by the CDS method. The antibiotic can be tested against *Streptococcus* species including *S. pneumoniae*, *Staphylococcus* spp., *Enterobacteriaceae*, *Vibrionaceae*, *Acinetobacter* spp., *Pseudomonas* spp., *Burkholderia* spp., *Moraxella catarrhalis*, *Haemophilus* spp., and *Pasteurella multocida*. (See Table 1a, 1b, 1c Calibrations 2001).

Mupirocin (topical preparation) and linezolid are being calibrated for testing methicillin-resistant *Staph. aureus* by the CDS method.

III. Results of the Field Trial of the Susceptibility testing of Enterococci to Ampicillin and Vancomycin

Colleagues who were at the previous workshop in Cairns will remember the call for volunteers to participate in a trial of testing 8 strains of enterococci by the CDS method to ampicillin and vancomycin, two difficult areas of susceptibility testing. Eighty three members of the CDS Users Group participated in the trial: 21 were from General Pathology laboratories, 55 from Microbiology laboratories and 7 did not specify. A summary of the results was published in "What's New" on the CDS web site earlier this year. The following analysis and detailed report with each strain were presented at the workshop and are shown below:-

Key: S = susceptible, R = resistant, RS = reduced susceptibility.

- No 1 (Fig. 1).
Amp 5: No zone
Van 5: Hazy edge
E. faecium: R/ Ampicillin (83 reported as R)
R/ Vancomycin, *VanB* (81 reported as R, 2 reported as S)
- No 2 (Fig. 2).
Amp 5: 7.5 mm, Hazy edge
Van 5: Hazy edge
E. faecalis: R/ Ampicillin (83 reported as S)
R/ Vancomycin, *VanB* (83 reported as R)
- No 3 (Fig. 3).
Amp 5: 7 mm, Hazy edge
Van 5: Sharp edge (<2mm)
E. gallinarum: S/ Ampicillin (67 reported as S, 15 as RS, 1 as R)
VanC (20 reported as *VanC*, 58 as S, 2 as VRE)

Note: The organism has natural low level resistance to vancomycin. Only 20 CDS Users recognised the *VanC* type of resistance (17 reported as *E. gallinarum*), 2 reported the organism as VRE. The results show that with the CDS method, one would rarely confuse this natural low level resistance with the acquired *VanB* type of resistance found in *E. faecalis* or *E. faecium*. Such differentiation is critical when screening stool specimens for VRE where the majority of enterococci with a vancomycin MIC > 4 mg/L are *E. gallinarum* or *E. casseliflavus*. In this situation, if other methods of antibiotic susceptibility testing such as broth or agar dilution or E-test are used, one needs to identify the isolate in order to differentiate enterococci with the *VanC* type resistance from *E. faecalis* or *E. faecium* with the *VanB* type of resistance.

- No 4 (Fig. 4).
Amp 5: 8mm, Hazy edge
Van 5: Sharp edge, > 2mm
E. faecalis: S/ Ampicillin (78 reported as S, 5 as RS)
S/ Vancomycin (82 reported as S, 1 as R)

- No 5 (Fig. 5).
Amp 5: 4mm, Sharp edge
Van 5: Sharp edge, > 2mm
E. faecalis: R/ Ampicillin, β -lactamase-positive (82 reported as R, 1 as S)
S/ Vancomycin (82 reported as S, 1 as R)

- No 6 (Fig. 6).
Amp 5: No zone
Van 5: Sharp edge, 5 mm
E. faecium: R/ Ampicillin (83 reported as R)
S/ Vancomycin (82 reported as S, 1 as R)

- No 7 (Fig. 7).
Amp 5: No zone
Van 5: No zone
E. faecium: R/ Ampicillin (83 reported as R)
R/ Vancomycin (82 reported as R, 1 as S)

- No 8 (Fig. 8).
Amp 5: 5mm, Hazy edge
Van 5: Sharp edge, > 2mm
E. faecalis: RS/ Ampicillin (67 reported as RS, 7 as R and 9 as S)
S/ Vancomycin (83 reported as S)

Ampicillin:

Overall the results obtained for the susceptibility testing of ampicillin were very good with an error of 6 %. However, separating *fully susceptible* strains from those with *reduced susceptibility* caused difficulty in the interpretation. From the practical point of view, there is no need to differentiate strains fully susceptible to ampicillin from those with reduced susceptibility. As a result, the annular radius of the zone of inhibition for susceptible strains has been changed from ≥ 6 mm to ≥ 4 mm and the MIC for susceptible strains is now ≤ 4 mg/L. With the revised annular radius of the zone of inhibition for susceptible strains, CDS Users need to be aware that some β -lactamase producing strains of *E. faecalis* may give an inhibitory zone of 5-6 mm in annular radius. Always remember that an ampicillin zone with a sharp edge indicates the presence of β -lactamase (See Table 1a of Calibrations 2001).

Vancomycin:

The results of vancomycin susceptibility testing were very good with the clinically important enterococci *E. faecalis* and *E. faecium* with an error of <1%. CDS Users need to be more aware of the existence of enterococci such as *E. gallinarum* or *E. casseliflavus* possessing *VanC* type resistance.

Hints on Vancomycin interpretation

A number of CDS Users found it difficult to measure an annular radius as small as 2 mm. It is essential to **compare** the test strain with the reference strain *Enterococcus faecalis* POW 1994 that has an inhibitory zone around vancomycin with a sharp edge and an annular radius > 2mm.

Remember it is important also to **examine the edge** of the zone of inhibition around vancomycin.

- If the test strain has a zone similar or larger than that of the reference strain and a **Sharp edge**, it is **Susceptible** to vancomycin.
- If the test strain has a zone with a **Hazy edge** (no matter what the measurement of the annular radius) it is a **VRE** with *VanB* type resistance. **VR** *E. faecium* with low level resistance to vancomycin may have an inhibitory zone up to 4 or 5 mm in annular radius when measured from the edge of confluent growth.
- If there is growth up to the disc and if the organism is resistant to teicoplanin also, it is a **VRE** with *Van A* type resistance.
- An inhibitory zone **smaller** than that of the reference strain with a **Sharp edge** of the zone is typical of enterococci with the natural *VanC* type resistance found in *E. gallinarum* and *E. casseliflavus*.

Modifications to the interpretation of the testing of enterococci against ampicillin and vancomycin

Ampicillin: The annular radius of the zone of inhibition for susceptible strains is **≥4 mm** and the MIC of ampicillin for susceptible enterococci is now **≤4 mg/L** (See Tables 1a Calibrations 2001). Note that β-lactamase-producing isolates have a characteristic zone of inhibition with a **sharp edge** but the annular radius of the zone of inhibition may be > 4 mm. Perform a nitrocefin-based test to confirm the presence of β-lactamase. β-lactamase-producing isolates are reported as “Resistant”.

Vancomycin: Irrespective of the measurement of the annular radius of the zone of inhibition (See Table 1a Calibrations 2001), a **hazy edge** of the zone indicates the presence of an inducible, low level *VanB* type resistance to vancomycin. If in doubt, incubate the plates for a further 24 hours and COMPARE the inhibitory zone with the reference strain *E. faecalis* POW 1994.

IV. Coagulase-negative staphylococci v/s benzylpenicillin and methicillin

Unlike *Staph. aureus*, heterogeneous resistance to methicillin is very common in coagulase-negative staphylococci. A correct CDS inoculum of 10⁷ cfu/ml should demonstrate resistant colonies within the zone of inhibition when highly methicillin-heterogeneous resistant coagulase-negative staphylococci are tested. Sometimes the plate needs to be incubated for a further 24 hours before the resistant colonies are obvious. Clinically, we found that deep seated infections such as those associated with prosthetic devices due to penicillin-resistant, methicillin-susceptible, *MecA*-negative *Staph. epidermidis* failed to have a sustained response to isoxazolyl penicillins or cephalosporins because of the emergence of resistance. In contrast, penicillin-susceptible strains did not develop resistance to penicillin and infections showed a sustained response to amoxycillin.

We recommend that, in reporting the susceptibility of penicillin-resistant, apparently methicillin-susceptible *Staph. epidermidis*, laboratories add a rider to the report that a second non-beta lactam antibiotic (eg ciprofloxacin) be added if the patient is to be treated with either the isoxazolyls (flucloxacillin or dicloxacillin) or cephalosporins (cephalothin or cephalixin).

V. The β-lactamases of members of the *Enterobacteriaceae* -an Update

The resistance to ampicillin in *E. coli*, *Citrobacter diversus* (*koseri*) and *Proteus mirabilis* is due primarily to the production of TEM-1 or TEM-2 β-lactamases and in *Klebsiella pneumoniae* the SHV-1 enzyme. These β-lactamases are inhibited by clavulanic acid and belong to the functional group 2 b (Classification of Bush *et al*, 1995). *Klebsiella oxytoca* produces a chromosomally mediated β-lactamase (K1) that confers resistance not only to ampicillin but also to second generation cephalosporins eg. cefazolin and cephalixin. Furthermore, strains of *K. oxytoca* expressing high levels of this enzyme are resistant to third generation cephalosporins and aztreonam. Therefore these strains are very similar to members of the *Enterobacteriaceae* that express extended spectrum β-lactamases (ESBLs) derived from point mutations in the original TEM and SHV enzymes.

a. Extended spectrum β -lactamases (ESBLs)

ESBLs are derived from mutations in TEM-1, TEM-2 and SHV-1 β -lactamases. These enzymes are capable of hydrolysing the extended-spectrum cephalosporins and aztreonam but not the cefamycins eg. cefotetan and cefoxitin. However, with cefoxitin, intrinsically resistant mutants are selected at a high frequency of 10^{-4} to 10^{-5} . The MIC of the third generation cephalosporins (eg. cefotaxime, ceftriaxone, ceftazidime) recorded with the majority of clinical isolates expressing an ESBL is ≥ 2 mg/L. Therefore, with the CDS method where the MIC of cefotaxime, ceftriaxone and ceftazidime for susceptible strains is ≤ 1 mg/L, most ESBL producing *Enterobacteriaceae* will be recorded as **Resistant** during routine testing. With strategic positioning of an Augmentin 60 μ g disc or a Timentin 85 μ g disc near a cefotaxime 5 μ g disc, CDS Users are able to detect the presence of an ESBL if there is a clear zone of synergy or an elliptical area of clearing between the two discs.

b. Inducible cephalosporinases

Two groups of chromosomally mediated inducible cephalosporinases are expressed by members of the *Enterobacteriaceae*.

The inducible cephalosporinases of functional group 1 (Bush *et al*, 1995), also known as AmpC, are not inhibited by clavulanic acid. They are produced by *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Hafnia alvei*, *Providencia stuartii*, *Providencia rettgeri*, *Morganella morganii* and also by *Aeromonas hydrophila* and *Aeromonas cavia*, the so-called "ESCAPP". Resistance to third generation cephalosporins occurs in all the above species when mutants that arise at a high frequency and produce large amounts of β -lactamase are selected during therapy. With the CDS method, a disc approximation test is used to demonstrate the presence of inducible cephalosporinases during routine antibiotic susceptibility testing. The flattened edge of the inhibitory zone around a cefotaxime 5 μ g disc adjacent to an imipenem 10 μ g disc reveals the presence of an inducible cephalosporinase (Fig. 9) and assists with the identification of the isolate. The reporting of β -lactam antibiotic susceptibility is made simple by following the recommendations of Table 4, Testing/Reporting. CDS Users are reminded that, in order to obtain full marks in the Quality Assurance Program (QAP), the reporting of the "ESCAPP" needs to follow the recommendations in Table 4, Testing/Reporting.

The inducible cephalosporinases of functional group 2e, inhibited by clavulanic acid are produced by *Proteus vulgaris* and *P. penneri*. These inducible cephalosporinases are recognised by the flattened edge of the inhibitory zone around a cefotaxime 5 μ g disc adjacent to an imipenem 10 μ g disc. While organisms producing β -lactamase of functional group 1 are resistant to Augmentin, *Proteus vulgaris* and *P. penneri* are susceptible to Augmentin. Another difference is derepressed mutants of *Proteus vulgaris* and *P. penneri* (hyperproducer of functional group 2e β -lactamase) are susceptible to ceftazidime.

c. *Enterobacteriaceae* expressing an inducible cephalosporinase and an ESBL

Members of the *Enterobacteriaceae* that express an inducible cephalosporinase of functional group 1 not inhibited by clavulanic acid may also possess an ESBL. In strains with low levels of inducible cephalosporinase, there is a typical "key-hole" or clearing area between cefotaxime and Augmentin but the inhibitory zone around Augmentin is reduced to < 6 mm in annular radius because of the presence of an inducible cephalosporinase resistant to clavulanic acid. On the other hand, high levels of inducible cephalosporinase may interfere with the detection of an ESBL. In this case, CDS Users can easily detect the presence of an ESBL by placing a cefepime 10 μ g disc or an aztreonam 30 μ g disc near an Augmentin 60 μ g disc or in the centre of the plate if there is no room on the plate for an extra disc. The synergy between cefepime or aztreonam and Augmentin reveals the presence of an ESBL (Fig. 11).

d. Plasmid mediated cephalosporinases (AmpC β -lactamases)

Plasmid-mediated cephalosporinases have arisen through the transfer of genes coding for the chromosomal AmpC β -lactamase. The substrate profiles of a plasmid-mediated cephalosporinase are similar to those of β -lactamase hyper-producing resistant mutants of *E. cloacae* but the enzyme is not inducible. Amongst the *Enterobacteriaceae*, *E. coli* is the species most commonly found to carry a plasmid mediated AmpC β -lactamase. With the CDS method, isolates producing a plasmid-mediated cephalosporinase are detected easily because of the use of lower potency antibiotic discs (eg. cefotaxime or ceftriaxone 5 μ g discs) and a susceptible MIC breakpoint of 1 mg/L.

e. Inhibitor-Resistant TEM β -lactamases (IRTs)

Recently, resistance to Augmentin in *E. coli* was found to be attributed to the production of mutant forms of TEM β -lactamase. These new TEM β -lactamases are far less susceptible to clavulanic acid than the original TEM enzyme and are called Inhibitor-Resistant TEM β -lactamases or IRTs. IRT producing *E. coli* are resistant to Augmentin but remain susceptible to cephalexin.

VI. Clindamycin v/s *Staph. aureus* and MRSA

The main mechanism of resistance to the macrolides, the methylation of 23S rRNA, may be **constitutive or inductive**. When it is constitutive, the organism is fully resistant to erythromycin and clindamycin. When it is inductive, the organism retains an apparent susceptibility to clindamycin and only shows resistance to this antibiotic in the presence of erythromycin i.e. there is flattening of the inhibitory zone around a clindamycin disc adjacent to that of an erythromycin (Fig. 12). This type of resistance is also known as dissociated or inducible resistance to clindamycin (**ICR**).

MRSA with ICR: The isolates are resistant to erythromycin. Mutants resistant to clindamycin arise at a high frequency of 10^{-5} to 10^{-6} . The MIC of clindamycin with the resistant mutants is **16 mg/L**.

MRSA susceptible to Erythromycin: No clindamycin-resistant mutants were selected when 10^9 cfu were exposed to clindamycin. When exposed to erythromycin, resistant mutants arise at a frequency of 10^{-5} to 10^{-6} .

Summary: With MRSA that are susceptible to erythromycin, it is probably safe to use clindamycin for non-sequestered (superficial and soft tissue) infections. With MRSA that are resistant to erythromycin, it is not safe to use clindamycin. The recommendation found in the CDS Surrogate Testing, Table 2 (CDS Manual 1999) still stands i.e. if the organism is **S to erythromycin**, we report it as **S to clindamycin** and if the organism is **R to erythromycin**, we report it as **R to clindamycin**.

VII. Rifampicin and fusidate v/s staphylococci

Rifampicin and fusidate can be used to treat infections caused by coagulase-positive (MRSA) and coagulase-negative staphylococci. The mutation rate to resistance with each antibiotic is high i.e. in the order 10^{-5} to 10^{-7} . Colonies may be observed within the zones of inhibition around rifampicin 1 μ g and fusidate 2.5 μ g discs when testing staphylococci. If the zones of inhibition around rifampicin and fusidate are ≥ 6 mm report the isolates as susceptible to the individual antibiotics. However, it is advisable that a warning such as "rifampicin and fusidate must be given in combination since resistance will develop rapidly to either agent if used alone" be issued when reporting the susceptibility of these two antibiotics.

VIII. Internal Quality Control

Review of Table 3 for acceptable ranges of reference strains

Streptococcus pneumoniae ARL 10582 and quality control of ampicillin 5 μ g : the acceptable range of the annular radius of the zone of inhibition recorded with ampicillin 5 μ g disc for *S. pneumoniae* ARL 10582, tested on blood Sensitest agar, in 5% CO₂, is now available for quality control: 10.8 – 15.2 (mm).

Over the years, the acceptable ranges of the reference strains recorded with a number of antibiotics have shifted slightly. We have been unable to ascertain why this has occurred, it may be a media effect or a change in the potency of the disks. To accommodate these changes, the acceptable ranges have been adjusted. See Table 3a, 3b and 3c Reference strains, 2001 for the updated ranges.

Frequently asked questions

Q: How often do I subculture the reference strains from the stock culture kept at -70°C?

A: The CDS method recommends that, ideally, the QC is performed whenever a susceptibility test is carried out. Large laboratories usually do the QC every day. Therefore, the reference strains are subcultured on artificial medium up to 30 times a month. Those laboratories need to subculture the reference strains from the stock culture kept at -70°C once a month. ISO 17025 also recommends the monthly subculture of reference strains from the stock culture kept at -70°C to minimise genetic changes.

Laboratories without a -70°C freezer usually perform the QC once a week and this is the minimum requirement. The fresh cultures plates used for QC are kept at 4°C and subcultured again one week later for subsequent testing (CDS Manual 1999, p. 13-14). Unlike storage at room temperature, storage at 4°C results in a decrease in bacterial metabolism and lessens the probability of mutations occurring. Subculture may be repeated up to **30 times** at the end of which the reference strains need to be subcultured from the stock kept at -70°C.

Q: How often should I test the Reference strains and which Reference strains should I use?

A: QC organisms are tested when

- A new batch of medium is used.
- A new batch of discs is used.
- The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly to ensure that all components of the test are in good working condition: eg. with members of the *Enterobacteriaceae*, *Acinetobacter* sp. and *Vibrionaceae* test *Escherichia coli* NCTC 10418 and *Escherichia coli* NCTC 11560 (for Timentin and Augmentin only).

If for example, gentamicin 10 µg or ceftazidime 10 µg discs...etc are tested against *Escherichia coli* NCTC 10418, there is no need to test them against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa. Similarly, if antibiotics such as Penicillin 0.5 u, Erythromycin 5 µg, etc are tested against *Staph. aureus* NCTC 6571, there is no need to test these against *Strep. pneumoniae* ARL 10582.

Q: We use Sensitest Agar plates bought from Metvet Science Pty Ltd. How long should we surface dry our plates?

A: We have been advised by Metvet that the plates have undergone an aeration process prior to packaging. Therefore they only require 30 minutes of surface drying instead of the standard 60 minutes recommended for freshly poured plates.

IX. External Quality Control

The Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program (QAP)

CDS Users are reminded to follow the guidelines listed below when participating in the QAP.

- Do not test antibiotics or use discs that have not been calibrated for use with the CDS method.
 - Look up the "Surrogate Disc Testing" table and report **S** or **R** accordingly if the antibiotic required is not calibrated.
 - Do not report the susceptibility of any antibiotic that is not calibrated or is not on the "Surrogate Disc Testing" table.
 - Read the section relevant to the type of organism or mechanism of resistance in the CDS Concise Manual 1999 when dealing with uncommon mechanisms of resistance ie. not following the standard interpretation. If the organism is shown to produce a functional group 1 inducible β-lactamase see Table 4 of Testing and Reporting.
 - Example: If the organism is a member of the *Enterobacteriaceae* such as *Enterobacter cloacae* or *Serratia marcescens* expressing an inducible β-lactamase (flattened zone between cefotaxime 5ug and imipenem 10ug), it is known that resistant mutants producing large amounts of the enzyme are present at a high frequency. The report should be R for penicillins, penicillin/inhibitor combinations, cephalosporins, cefamycins and monobactams irrespective of the size of the inhibitory zone
- Note:** Test and report ceftazidime, ceftazidime/avopivoxil, imipenem and meropenem, the antibiotics marked as T in Table 4, Testing and Reporting.

X. 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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XI. Illustrations

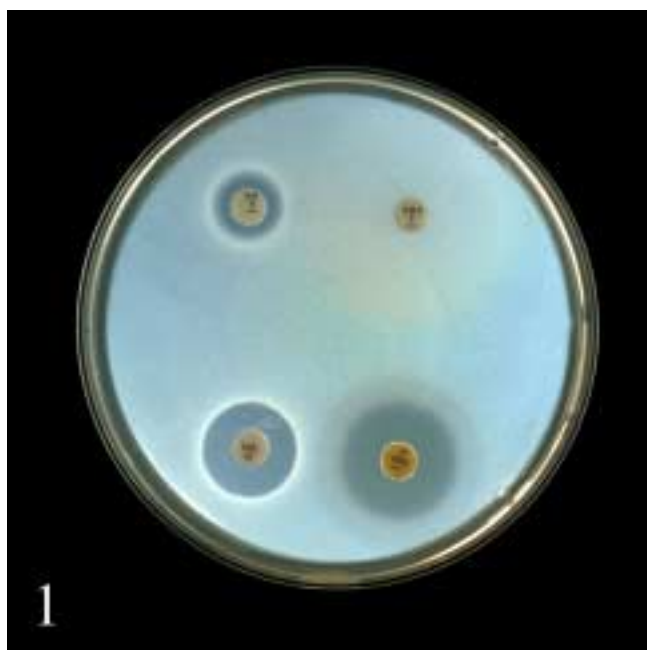


Fig. 1: *E. faecium* resistant to ampicillin and vancomycin (*VanB* type)

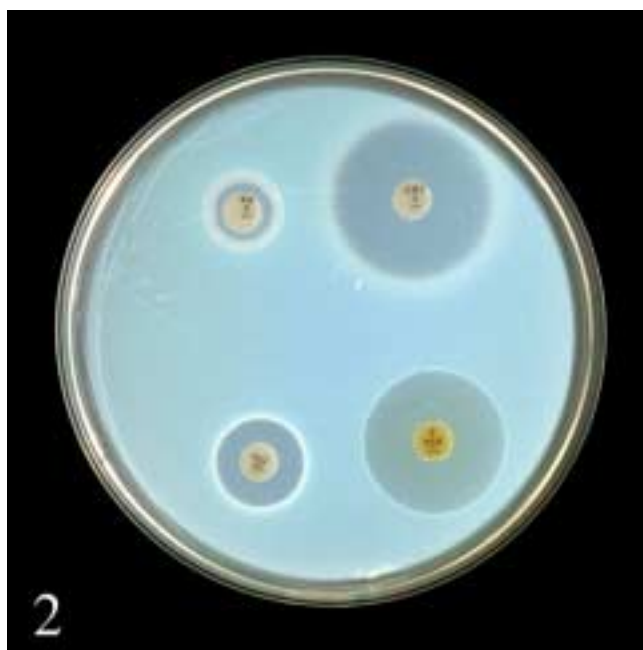


Fig. 2: *E. faecalis* susceptible to ampicillin resistant to vancomycin (*VanB* type)



Fig. 3: *E. gallinarum* susceptible to ampicillin possessing a natural resistance to vancomycin (*VanC* type)



Fig. 4: *E. faecalis* susceptible to ampicillin and vancomycin

XI. Illustrations (continued)



Fig. 5: Beta-lactamase producing *E. faecalis*,
Susceptible to vancomycin

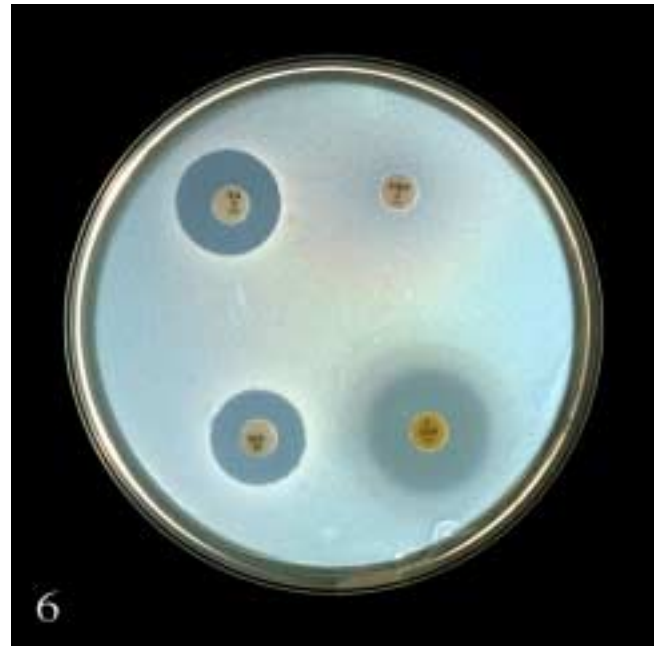


Fig. 6: *E. faecium* resistant to ampicillin
susceptible to vancomycin



Fig. 7: *E. faecium* resistant to ampicillin
Resistant to vancomycin (*VanA* type)

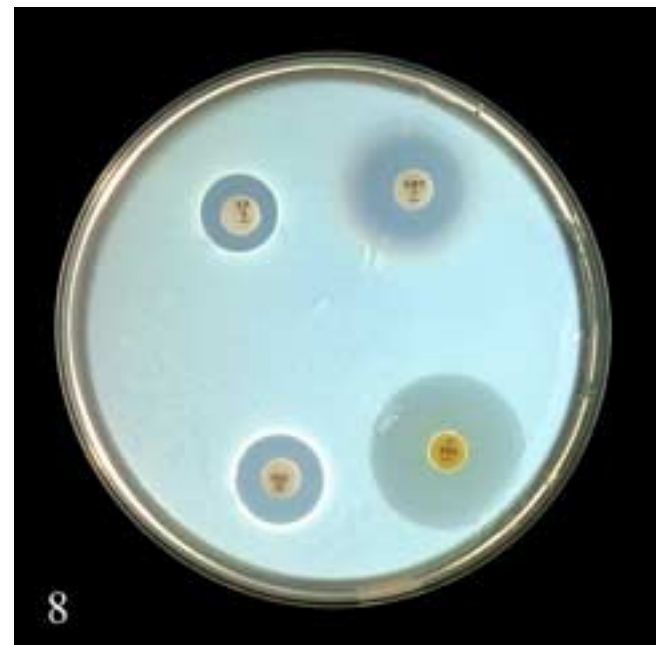


Fig. 8: *E. faecalis* susceptible to ampicillin
and vancomycin

XI. Illustrations (continued)



Fig. 9



Fig. 10

Fig. 9: *E. cloacae* expressing an inducible cephalosporinase (group 1 or AmpC). Note the flattened zone of cefotaxime (CTX 5) next to imipenem (IPM 10), the small zone of < 6 mm around the Augmentin disc (AMC 60) and the small zone < 6 mm around cephalexin (CL 100) with resistant colonies, suggesting the presence of an inducible cephalosporinase not inhibited by clavulanic acid. Referring to Table 4 of Testing and Reporting beta-lactam antibiotics, this isolate is reported resistant to ampicillin, cephalexin, Augmentin, aztreonam, susceptible to imipenem.

Fig. 10: *P. penneri* expressing an inducible cephalosporinase (group 2e). Note the flattened zone of cefotaxime (CTX 5) next to imipenem (IPM 10) and a zone > 6 mm around the Augmentin disc (AMC 60) near cefotaxime disc suggesting the presence of an inducible cephalosporinase inhibited by clavulanic acid.

XI. Illustrations (continued)



Fig. 11: *E. cloacae* expressing an inducible cephalosporinase (functional group 1 or AmpC) and an ESBL. A hyperproducer of group 1 inducible cephalosporinase, the isolate is resistant to cefotetan (CTT 30), susceptible to imipenem (IPM 10) and has a small zone around the Augmentin disc (AMC 60). No synergy was observed with cefotaxime (CTX 5). The synergy between Augmentin (AMC 60) and cefepime (FEP 10) discs demonstrates the presence of an ESBL.



Fig. 12: An MRSA isolate with a dissociated or inducible resistance to clindamycin (**ICR**). The organism has an apparent susceptibility to clindamycin and only shows resistance to this antibiotic in the presence of erythromycin ie. there is flattening of the inhibitory zone around a clindamycin disc (DA 2) adjacent to an erythromycin disc (E 5).

Table 1a. Calibrations, 2001: Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Enterococci			
(Blood Sensitest, air, 35°C)			
Ampicillin	5 ^Φ	4 mm ^Φ	≤ 4.0
Chloramphenicol	30	4 mm	≤ 8.0
Gentamicin	200	4 mm	≤ 512
Nitrofurantoin ⁺	200	4 mm	≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Vancomycin	5	(See foot note) [#]	≤ 4.0
Listeria spp.			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Gentamicin	10		≤ 1.0
Staphylococci			
(Sensitest, air, 35°C)			
Ampicillin [•]	5		≤ 0.5
Benzylpenicillin [§]	0.5 u		≤ 0.06
Cephalexin [•]	100		≤ 16.0
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
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Nitrofurantoin ⁺	200		≤ 32.0
Rifampicin	1		≤ 0.5
Sulphafurazole	300		≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Trimethoprim	5		≤ 2.0
Vancomycin	5	2 mm	≤ 4.0
Streptococci			
(Blood Sensitest, air, 35°C) [@]			
Ampicillin [↓]	5		≤ 2.0
Benzylpenicillin	0.5 u		≤ 0.25
Cefotaxime	0.5		≤ 0.25
Ceftriaxone	0.5		≤ 0.25
Cefotaxime [↓]	5		≤ 2.0
Ceftriaxone [↓]	5		≤ 2.0
Chloramphenicol	30		≤ 8.0
Co-trimoxazole	25		≤ 0.5/9.5
Erythromycin	5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5	4 mm	≤ 1.0
Nitrofurantoin ⁺	200		≤ 32.0
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0

^Φ Perform a nitrocefin based test to detect βlactamase activity if the zone of inhibition has a sharp edge zone and an annular radius > 4 mm.

βlactamase-positive isolates are reported as resistant.

[#] A zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*VanB* type), irrespective of measurement of the annular radius.

⁺ For testing urine isolates only

[@] *Strep. pneumoniae* & *Strep. anginosus (milleri)* are incubated in 5% CO₂.

[§] NOT for testing *Staph. saprophyticus*.

[•] ONLY for testing isolate of *Staph. saprophyticus*.

[↓] NOT for testing *Strep. pneumoniae* from CSF. If *Strep. pneumoniae* or any other *Streptococcus* species from a site other than CSF is resistant to penicillin 0.5u, cefotaxime 0.5µg or ceftriaxone 0.5 µg then test ampicillin 5 µg, cefotaxime 5 µg and ceftriaxone 5 µg.

Table 1b. Calibrations, 2001: Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
<i>Enterobacteriaceae, Vibrionaceae, & Acinetobacter</i> spp.			
(Sensitest, air, 35°C) #			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin •	60		≤ 16.0/8.0
Aztreonam	30		≤ 8.0
Cefazolin	30		≤ 16.0
Cefepime	10		≤ 2.0
Cefotaxime	5		≤ 1.0
Cefotetan	30		≤ 8.0
Cefoxitin	30		≤ 8.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cefuroxime	30		≤ 8.0
Cephalexin	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Enoxacin	10		≤ 4.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Nalidixic acid +	30		≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin +	200		≤ 32.0
Norfloxacin +	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Tazocin •	55		≤ 16.0/2.0
Tetracycline	30		≤ 4.0
Timentin •	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
<i>Pseudomonas</i> spp. & <i>Burkholderia</i> spp.			
(Sensitest, air, 35°C)			
Amikacin	30	4 mm	≤ 16.0
Aztreonam	30		≤ 8.0
Cefepime	10		≤ 2.0
Cefpirome	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10	4 mm	≤ 4.0
Imipenem	10		≤ 4.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Netilmicin	30	4 mm	≤ 8.0
Norfloxacin +	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm	≤ 1.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Ticarillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm	≤ 4.0
Trimethoprim	5		≤ 2.0

Yersinia enterocolitica is incubated in air at 30°C. + For testing urinary isolates only

• If an ESBL is present, report Augmentin, Timentin and Tazocin for isolates from URINE ONLY.

Table 1c. Calibrations, 2001: Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

MISCELLANEOUS GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
<i>Campylobacter spp.</i>			
(Blood Sensitest, microaerophilic, 42°C)			
Ciprofloxacin	2.5	4 mm	≤ 1.0
Erythromycin	5		≤ 0.5
Gentamicin	10		≤ 1.0
Tetracycline	30		≤ 4.0
<i>Haemophilus influenzae/Haemophilus spp</i>			
(HTM[®] agar, 5%CO₂, 35°C)			
Ampicillin	5		≤ 1.0
Augmentin	15		≤ 2.0/1.0
Cefaclor	30		≤ 4.0
Cefotaxime	0.5		≤ 0.25
Cefpodoxime	10		≤ 2.0
Ceftriaxone	0.5		≤ 0.25
Cefuroxime	30		≤ 4.0
Chloramphenicol	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Co-trimoxazole	25		≤ 1.0/19.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
<i>Moraxella catarrhalis</i>			
(Blood Sensitest, 5%CO₂, 35°C)			
Benzylpenicillin	0.5 u		≤ 0.25
Cefaclor	30		≤ 4.0
Cefpodoxime	10		≤ 2.0
Cefuroxime	30		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Co-trimoxazole	25		≤ 1.0/19.0
Erythromycin	5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
<i>Neisseria meningitidis</i>			
(Blood Sensitest, 5% CO₂, 35°C)			
Benzylpenicillin	0.5 u	4 mm	≤ 0.25
Cefotaxime	0.5		≤ 0.25
Ceftriaxone	0.5		≤ 0.25
Chloramphenicol	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Rifampicin	1		≤ 0.5
<i>Pasteurella multocida</i>			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Ciprofloxacin	2.5		≤ 1.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
<i>Stenotrophomonas maltophilia</i>			
(Sensitest, air, 35°C)			
Sulphafurazole	300		≤ 64.0

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

Table 2a. Surrogate disc testing, 2001. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Staphylococci (except <i>S. saprophyticus</i> from urine)		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Azithromycin	Erythromycin	5
Cephalosporins &	Methicillin	5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole +	Sulphafurazole	300
Co-trimoxazole +	Trimethoprim	5
Dicloxacillin	Methicillin	5
Flucloxacillin	Methicillin	5
Lincomycin	Erythromycin	5
Norfloxacin §	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
<i>Staphylococcus saprophyticus</i> from urine		
Amoxycillin	Ampicillin	5
Augmentin	Cephalexin	100
Benzylpenicillin	Ampicillin	5
Cephalosporins &	Cephalexin	100
Cloxacillin	Cephalexin	100
Co-trimoxazole +	Sulphafurazole	300
Co-trimoxazole +	Trimethoprim	5
Dicloxacillin	Cephalexin	100
Flucloxacillin	Cephalexin	100
Norfloxacin §	Ciprofloxacin	2.5
Penicillin V	Ampicillin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Streptococci *		
Amoxycillin	Benzylpenicillin	0.5 u
Amoxycillin	Ampicillin ↓	5
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Benzylpenicillin	Ampicillin ↓	5
Cephalosporins &	Cefotaxime/Ceftriaxone	0.5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Lincomycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<i>Stenotrophomonas maltophilia</i>		
Co-trimoxazole	Sulphafurazole	300

& Ceftazidime is considered inactive against Gram-positive organisms.

+ Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

§ Reporting of norfloxacin is for urine isolates ONLY.

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

↓ NOT for testing *Strep.pneumoniae* from CSF. Test if isolate is resistant to penicillin 0.5u, cefotaxime 0.5µg or ceftriaxone 0.5µg.

Table 2b. Surrogate disc testing, 2001. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc potency (µg)
<i>Campylobacter</i> spp.		
Tetracyclines	Tetracycline	30
<i>Enterobacteriaceae/ Vibriionaceae/ Acinetobacter</i> spp.		
Amoxycillin	Ampicillin	25
Cephalothin @	Ampicillin	25
Ceftriaxone	Cefotaxime	5
Cefotaxime	Ceftriaxone	5
Co-trimoxazole +	Sulphafurazole	300
Co-trimoxazole +	Trimethoprim	5
Piperacillin	Ampicillin	25
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Ticarcillin	Ampicillin	25
Enterococci		
Amoxycillin	Ampicillin	5
Benzympenicillin	Ampicillin	5
<i>Haemophilus influenzae/Haemophilus</i> spp.		
Amoxycillin	Ampicillin	5
Cefepime	Cefotaxime/Ceftriaxone	0.5
Cefotaxime	Ceftriaxone	0.5
Cefpirome	Cefotaxime/Ceftriaxone	0.5
Ceftazidime	Cefotaxime/Ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
Cephalexin	Cefuroxime/Cefaclor	30
Tetracyclines	Tetracycline	30
<i>Listeria</i> spp.		
Amoxycillin	Ampicillin	5
Benzympenicillin	Ampicillin	5
<i>Moraxella catarrhalis</i>		
Azithromycin	Erythromycin	5
Amoxycillin	Benzympenicillin	0.5 u
Ampicillin	Benzympenicillin	0.5 u
Augmentin	Cefuroxime/Cefaclor	30
Cephalosporins	Cefuroxime/Cefaclor	30
Clarithromycin	Erythromycin	5
Penicillin V	Benzympenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
<i>Neisseria meningitidis</i>		
Ampicillin	Benzympenicillin	0.5 u
Amoxycillin	Benzympenicillin	0.5 u
Cefotaxime	Ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
<i>Pasteurella multocida</i>		
Amoxycillin	Ampicillin	5
Benzympenicillin	Ampicillin	5
Tetracyclines	Tetracycline	30
<i>Pseudomonas</i> spp & <i>Burkholderia</i> spp.		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole +	Trimethoprim	5
Co-trimoxazole	Sulphafurazole	300

@ Not for *Acinetobacter* spp.

+ Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

Table 3a. Reference strains, 2001: Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
<i>Enterococcus faecalis</i> POW 1994		
(Blood Sensitest, air 35°C)		
Ampicillin	5	5.9 - 9.2
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Teicoplanin	15	3.1 - 5.3
Vancomycin	5	2.0 - 3.7
<i>Staphylococcus aureus</i> NCTC 6571		
(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	8.6 - 11.2
Fusidic acid	2.5	8.6 - 12.6
Gatifloxacin	2.5	10.1 - 14.9
Gentamicin	10	6.6 - 9.4
Kanamycin	50	7.8 - 9.6
Methicillin	5	8.8 - 12.0
Moxifloxacin	2.5	10.9 - 14.5
Nitrofurantoin	200	6.7 - 10.3
Rifampicin	1	9.3 - 12.5
Sulphafurazole	300	9.3 - 13.7
Teicoplanin	15	3.4 - 6.1
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	8.5 - 11.3 &
Vancomycin	5	2.8 - 4.9
<i>Streptococcus pneumoniae</i> ARL 10582		
(Blood Sensitest, 5% CO₂, 35°C)		
Ampicillin	5	10.8 - 15.2 &
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
Gatifloxacin	2.5	5.6 - 8.4
Moxifloxacin	2.5	5.6 - 8.6
Rifampicin	1	7.5 - 10.8
Teicoplanin	15	5.1 - 8.0
Tetracycline	30	9.2 - 14.5
Vancomycin	5	5.1 - 8.6

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

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. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.
- & Adjusted acceptable range.

Table 3b. Reference strains, 2001: Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
<i>Escherichia coli</i> NCTC 10418 #		
(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
Cefepime	10	11.9 - 15.3
Cefotaxime	5	9.7 - 13.7
Cefotetan	30	11.9 - 14.8
Cefoxitin	30	9.8 - 13.0
Cefpirome	10	11.9 - 14.6
Cefpodoxime	10	10.3 - 12.7
Ceftazidime	10	9.3 - 14.1 ^{&}
Ceftriaxone	5	10.5 - 14.3
Cefuroxime	30	7.5 - 10.1
Cephalexin	100	6.9 - 10.9
Chloramphenicol	30	8.7 - 11.9
Ciprofloxacin	2.5	12.4 - 15.8
Gatifloxacin	2.5	11.2 - 14.8
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
Moxifloxacin	2.5	10.0 - 13.4
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	8.8 - 13.6 ^{&}
<i>Escherichia coli</i> NCTC 11560		
(Sensitest, air, 35°C)		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
Tazocin	55	7.4 - 9.2

*The acceptable range (95% confidence limits) is the mean ± 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.

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. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

Table 3c. Reference strains, 2001: Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS CONTINUED

Antibiotic	Disc content (µg)	Acceptable range* (mm)
<i>Campylobacter jejuni</i> NCTC 11168 (Blood Sensitest, microaerophilic, 42°C)		
Ciprofloxacin	2.5	9.2 - 16.9
Erythromycin	5	6.4 - 12.4
Gentamicin	10	7.0 - 11.0
Tetracycline	30	10.3 - 16.0
<i>Haemophilus influenzae</i> NCTC 4560 (HTM® agar, 5%CO ₂ , 35°C)		
Ampicillin	5	7.0 - 11.1
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
Cefuroxime	30	8.3 - 12.8
Chloramphenicol	10	11.1 - 14.3 ^{&}
Ciprofloxacin	2.5	11.1 - 15.9
Co-trimoxazole	25	9.0 - 12.5
Gatifloxacin	2.5	13.5 - 17.1
Moxifloxacin	2.5	10.6 - 15.2
Tetracycline	30	9.9 - 13.3
<i>Haemophilus influenzae</i> NCTC 11315 (HTM® agar, 5%CO ₂ , 35°C)		
Augmentin	15	7.7 - 10.1
<i>Pseudomonas aeruginosa</i> NCTC 10662[#] (Sensitest, air 35°C)		
Amikacin	30	7.4 - 10.6
Aztreonam	30	8.3 - 13.1
Cefepime	10	8.1 - 11.3
Cefpirome	10	8.1 - 10.6
Ceftazidime	10	7.5 - 11.9
Ciprofloxacin	2.5	8.9 - 14.5
Gatifloxacin	2.5	7.8 - 11.4
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
Meropenem	5	9.7 - 14.8
Moxifloxacin	2.5	^s
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 ± 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. A new batch of medium is used.
- b. A new batch of discs is used.
- c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

^s Moxifloxacin MIC recorded with *Ps.aeruginosa* NCTC 10662 is 2mg/L and is above the susceptible MIC. Test *E. coli* NCTC 10418 instead.

Table 4, 2001: A guide to the testing/reporting of β -lactam antibiotics for *Enterobacteriaceae/Aeromonas spp.*, *Pseudomonas/Burkholderia spp.* and *Stenotrophomonas maltophilia*.

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

T = can be tested.

Organism/ Species	Antibiotic								
	AMP	AMC	ATM	CAZ	CXM/CL	CPD	CPO	CRO	CTT
Inducible β-lactamases present									
<i>Ent. cloacae</i> / <i>Ent. aerogenes</i>	R	R	R	R	R	R	T	R	R
<i>Cit. freundii</i>	R	R	R	R	R	R	T	R	R
<i>Ser. marcescens</i>	R	R	R	R	R	R	T	R	R
<i>Prov. stuartii</i> / <i>Prov. rettgeri</i>	R	R	T	R	R	R	T	R	R
<i>Morg. morganii</i>	R	R	T	R	R	R	T	R	R
<i>Prot. vulgaris</i> / <i>Prot. penneri</i> ¹	R	T	R	T	R	R	T	R	T
<i>Aeromonas</i> /A2 (most <i>A. sobria</i>)	R	R	T	T	T	T	T	T	T
<i>Aeromonas</i> /A1 & A2	R	R	T	R	R	R	T	R	R
<i>Enterobacteriaceae</i> with ESBL	R	T ²	R	R	R	R	R	R	T
<i>Enterobacteriaceae</i> with inducible β -ses and ESBL	R	R	R	R	R	R	R	R	R
<i>Pseudomonas</i> / <i>Burkholderia</i> spp.	R	R	T	T	R	R	T	R	R
<i>Steno. maltophilia</i>	R	R	R	R	R	R	R	R	R

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

1. Isolates with high β -lactamase activity may give no zone around CTX 5 μ g but show a "key-hole" effect which may be mistaken as an indication of the presence of an ESBL. However, they are susceptible to ceftazidime which can be tested.

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

Table 4, 2001(continued): A guide to the testing/reporting of β -lactam antibiotics for *Enterobacteriaceae*/ *Aeromonas* spp., *Pseudomonas/Burkholderia* spp. and *Stenotrophomonas maltophilia*.

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

T = can be tested.

Organism/ Species	Antibiotic								
	CTX	FEP	FOX	IPM	KZ	MEM	PRL	TIM	TZP
Inducible β-lactamases present									
<i>Ent. cloacae</i> / <i>Ent. aerogenes</i>	R	T	R	T	R	T	R	R	R
<i>Cit. freundii</i>	R	T	R	T	R	T	R	R	R
<i>Ser. marcescens</i>	R	T	R	T	R	T	R	R	R
<i>Prov. stuartii</i> / <i>Prov. rettgeri</i>	R	T	R	T	R	T	R	R	T
<i>Morg. morganii</i>	R	T	R	T	R	T	R	R	T
<i>Prot. vulgaris</i> / <i>Prot. penneri</i> ¹	R	T	T	T	R	T	R	T	T
<i>Aeromonas</i> /A2 (most <i>A. sobria</i>)	T	T	T	R	T	R	R	R	R
<i>Aeromonas</i> /A1 & A2	R	T	R	R	R	R	R	R	R
<i>Enterobacteriaceae</i> with ESBL	R	R	R	T	R	T	R	T ²	T ²
<i>Enterobacteriaceae</i> with inducible β -ses and ESBL	R	R	R	T	R	T	R	R	R
<i>Pseudomonas</i> / <i>Burkholderia</i> spp.	R	T	R	T	R	T	T	T	T
<i>Steno. maltophilia</i>	R	R	R	R	R	R	R	R	R

CTX=cefotaxime, FEP=cefepime, FOX=cefoxitin, IPM=imipenem, KZ=cefazolin, MEM=meropenem, TIM=Timentin, TZP=Tazocin.

1. Isolates with high β -lactamase activity may give no zone around CTX 5 but show a "key-hole" effect which may be mistaken producing an ESBL, but are susceptible to ceftazidime which can be tested.

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

Section B: Veterinary Session

The topic “CDS testing of Veterinary Isolates”, was presented by Dr. Jeanette Pham at Symposium 56 on “Antimicrobial Testing Standardisation for Veterinary Laboratories”. The talk was divided into two parts A and B

Part I: The performance of the CDS test (see CDS Concise Manual 1999, p. 6-9 and 24).

Part II: Special application for Veterinary Isolates

Veterinary Tables 1a and 1b of Calibrations, 2001

These tables are prepared for veterinary laboratories and contain some antibiotics that are used only in veterinary medicine.

Table 1a presents antibiotics calibrated, listed in alphabetical order, for the main groups of Gram-positive organisms ie. staphylococci, streptococci, enterococci, *Listeria* species. For each group of organisms, the media and the incubation conditions are shown in brackets under the group name. For example “Blood Sensitest, air, 35°C @” for streptococci means the media and the incubation conditions recommended for streptococci are Sensitest Agar supplemented with horse blood and incubation is in air at 35°C. The flag @ indicates that *Strep. pneumoniae* & *Strep. anginosus (milleri)* are incubated in 5% CO₂. For each antibiotic, the disc potency and the MIC breakpoint for susceptible strains are indicated clearly. If requested, the MIC ≤ ... mg/L may also be reported for susceptible strains. When there is an exception to the standard interpretation, it is clearly marked. For example, when testing enterococci against vancomycin, CDS User needs to see the foot note # that says: Irrespective of the annular radius measurement, a zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*VanB* type).

Table 1b lists antibiotics calibrated for Gram-negative organisms namely members of the *Enterobacteriaceae*, *Vibrionaceae* & *Acinetobacter* spp., *Pseudomonas* spp. & *Burkholderia* spp., *Pasteurella multocida* and *Campylobacter* spp.

For organisms not included in the Table of Calibrations, extrapolate the testing from that established for similar organisms.

Examples:

Corynebacterium spp. from streptococci.

Erysipelothrix spp. from streptococci in CO₂.

Pasteurella spp. from *Pasteurella multocida*.

Notes on Ceftiofur

The susceptibility to ceftiofur is obtained from surrogate testing (See Veterinary Tables 2a and 2b Surrogate disc testing 2001). The surrogate discs are methicillin 5 µg for staphylococci, benzylpenicillin 0.5 u for streptococci and cefazolin 30 µg for isolates of *Enterobacteriaceae*/ *Vibrionaceae*/ *Acinetobacter* spp.

Table 2 Surrogate disc testing, 2001

Surrogate disc testing is unique to the CDS method. It allows the reporting of other antibiotics of the same class based on the susceptibility results of an antibiotic disc known as the surrogate disc.

For example, the susceptibility of staphylococci to amoxycillin/ampicillin is inferred from the results recorded with benzylpenicillin 0.5 u. If the annular radius of the zone of inhibition is < 6 mm, the organism is **Resistant** to benzylpenicillin, amoxycillin and ampicillin. Do not use an ampicillin disc since this is not calibrated for testing staphylococci. Similarly, when testing staphylococci, if the annular radius of the zone of inhibition around a methicillin 5 µg disc is > 6mm, the organism is **susceptible** to methicillin, cloxacillin, flucloxacillin, Augmentin, ceftiofur and other cephalosporins such as cephalothin and cefazolin.

Quality Control

Tables 3a and 3b show the antibiotic disc content and the acceptable range of the annular radii of the zones of inhibition with the reference strains used for quality control in the CDS method. Tables 3a show the QC recommendations for *Staphylococcus aureus* NCTC 6571 and *Enterococcus faecalis* POW 1994. Tables 3b show the QC recommendations for *Escherichia coli* NCTC 10418, *Escherichia coli* NCTC 11560 and *Pseudomonas aeruginosa* NCTC 10662.

1. Testing of **reference strains**
 - Ideally, the reference strains are tested with each run of CDS testing or *at least once a week*
 - With each new batch of antibiotic discs
 - With each new batch of agar plates
2. Fresh cultures plates used for QC are **kept at 4°C** and subcultured again the week after for subsequent testing (CDS Manual 1999, p. 13-14). Unlike storage at room temperature, storage at 4°C results in a decrease in bacterial metabolism and lessens the probability of mutations occurring.
3. The subculture may be **repeated up to 30 times** and the reference strains then need to be subcultured from the stock kept at – 70°C.
4. Keeping the record of Quality Control: CDS Users are advised to record the measurement of the annular radius of the zone of inhibition each time a reference strain is tested. The following table is an example of how to keep the QC record.

<i>Staphylococcus aureus</i> NCTC 6571						
Annular radius (mm)						
Date	P 0.5 8.7-13.5	MET 5 8.8-12.0	E 5 7.1-10.7	TE 5 10.6-16.2	C 30 7.8-11.4	CIP 2.5 9.2 -12.4
18.9.01	12.5	9.5	10	11.5	10.5	11.5
15.9.01	11	9	9.5	12	9	11

5. **A strict compliance** of the described method is still the most important quality assurance.

Veterinary Table 1a. Calibrations, 2001: Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Enterococci (Blood Sensitest, air, 35°C)			
Ampicillin	5 ^Φ	4 mm ^Φ	≤ 4.0
Chloramphenicol	30	4 mm	≤ 8.0
Gentamicin	200	4 mm	≤ 512
Nitrofurantoin ⁺	200	4 mm	≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Vancomycin	5	(See foot note) [#]	≤ 4.0
Listeria spp. (Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Gentamicin	10		≤ 1.0
Staphylococci (Sensitest, air, 35°C)			
Ampicillin	5		≤ 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
* Novobiocin	5		≤ 1.0
Rifampicin	1		≤ 0.5
Sulphafurazole	300		≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Trimethoprim	5		≤ 2.0
Vancomycin	5	2 mm	≤ 4.0
Streptococci (Blood Sensitest, air, 35°C) [@]			
Ampicillin [↓]	5		≤ 2.0
Benzylpenicillin	0.5 u		≤ 0.25
Chloramphenicol	30		≤ 8.0
Co-trimoxazole	25		≤ 0.5/9.5
Erythromycin	5		≤ 0.5
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0

* Antibiotic calibrated specifically for veterinary medicine

^Φ Perform a nitrocefin based test to detect βlactamase activity if the zone of inhibition has a sharp edge zone and an annular radius > 4 mm. βlactamase-positive isolates are reported as resistant.

[#] A zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*VanB* type), irrespective of the annular radius measurement.

[@] *Strep. pneumoniae* & *Strep. anginosus (milleri)* are incubated in 5% CO₂.

[↓] To test strains with a benzylpenicillin 0.5 u zone < 6 mm.

Veterinary Table 1b. Calibrations, 2001: Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
<i>Campylobacter spp.</i>			
(Blood Sensitest, microaerophilic, 42°C)			
Ciprofloxacin	2.5		1.0
Erythromycin	5	4 mm	0.5
Gentamicin	10		1.0
Tetracycline	30		4.0
<i>Enterobacteriaceae, Vibrionaceae, & Acinetobacter spp.</i>			
(Sensitest, air, 35°C) #			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Apramycin*	25		≤ 8.0
Augmentin	60		≤ 16.0/8.0
Cefazolin	30		≤ 16.0
Cefotetan	30		≤ 8.0
Cefoxitin	30		≤ 8.0
Cephalexin	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Enoxacin	10		≤ 4.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Neomycin*	30		≤ 4.0
Spectinomycin*	25		≤ 32.0
Streptomycin*	25		≤ 16.0
Sulphafurazole	300		≤ 64.0
Tetracycline	30		≤ 4.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
<i>Pseudomonas spp. & Burkholderia spp.</i>			
(Sensitest, air, 35°C)			
Amikacin	30	4 mm	≤ 16.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10	4 mm	≤ 4.0
Imipenem	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm	≤ 1.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm	≤ 4.0
Trimethoprim	5		≤ 2.0
<i>Pasteurella multocida</i>			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Ciprofloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0

* Antibiotic calibrated specifically for veterinary medicine.

Yersinia enterocolitica is incubated in air at 30°C.

Veterinary Table 2. Surrogate disc testing, 2001. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc potency (µg)
<i>Enterobacteriaceae/ Vibrionaceae/ Acinetobacter spp.</i>		
Amoxicillin/ Cephalothin	Ampicillin	25
Ceftiofur	Cefazolin	30
Co-trimoxazole ⁺	Sulphafurazole	300
Co-trimoxazole ⁺	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Ticarcillin	Ampicillin	25
Enterococci		
Amoxicillin/ Benzylpenicillin	Ampicillin	5
Listeria spp.		
Amoxicillin/ Benzylpenicillin	Ampicillin	5
Pasteurella multocida		
Amoxicillin/ Benzylpenicillin	Ampicillin	5
Tetracyclines	Tetracycline	30
Pseudomonas spp & Burkholderia spp.		
Colistin	Polymyxin	300 u
Co-trimoxazole ⁺	Trimethoprim	5
Co-trimoxazole	Sulphafurazole	300
Staphylococci		
Amoxicillin/ Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Ceftiofur/ other cephalosporins ^{@*}	Methicillin	5
Clindamycin/Lincomycin	Erythromycin	5
Cloxacillin/Dicloxacillin/Flucloxacillin	Methicillin	5
Co-trimoxazole ⁺	Sulphafurazole	300
Co-trimoxazole ⁺	Trimethoprim	5
Enrofloxacin*	Ciprofloxacin	2.5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Streptococci		
Amoxicillin/ Ampicillin	Benzylpenicillin	0.5 u
Amoxicillin/ Benzylpenicillin	Ampicillin [↓]	5
Ceftiofur/ other cephalosporins*	Benzylpenicillin	0.5 u
Clindamycin/Lincomycin	Erythromycin	5
Tetracyclines	Tetracycline	30

⁺ Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

* Antibiotic used only in veterinary medicine.

[↓] Strains with reduced susceptibility to benzylpenicillin.

[@] Except ceftazidime

Veterinary Table 3a. Reference strains, 2001: Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
<i>Enterococcus faecalis</i> POW 1994		
(Blood Sensitest, air 35°C)		
Ampicillin	5	5.9 - 9.2
Chloramphenicol	30	6.6 - 9.9
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Teicoplanin	15	3.1 - 5.3
Vancomycin	5	2.0 - 3.7
<i>Staphylococcus aureus</i> NCTC 6571		
(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	8.6 - 11.2
Fusidic acid	2.5	8.6 - 12.6
Gentamicin	10	6.6 - 9.4
Kanamycin	50	7.8 - 9.6
Methicillin	5	8.8 - 12.0
Novobiocin	5	6.1 - 12.5
Rifampicin	1	9.3 - 12.5
Sulphafurazole	300	9.3 - 13.7
Teicoplanin	15	3.4 - 6.1
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	8.5 - 11.3
Vancomycin	5	2.8 - 4.9
<i>Streptococcus pneumoniae</i> ARL 10582		
(Blood Sensitest, 5% CO₂, 35°C)		
Benzylpenicillin	0.5u	8.3 - 14.8
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
Teicoplanin	15	5.1 - 8.0
Tetracycline	30	9.2 - 14.5
Vancomycin	5	5.1 - 8.6

* 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.

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.
- d. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

Veterinary Table 3b. Reference strains, 2001: Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
<i>Escherichia coli</i> NCTC 10418 #		
(Sensitest, air, 35°C)		
Amikacin	30	6.7 - 10.3
Ampicillin	25	7.5 - 10.7
Apramicin	15	5.3 - 7.9
Cefazolin	30	6.7 - 12.7
Cefotetan	30	11.9 - 14.8
Cefoxitin	30	9.8 - 13.0
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
Neomycin	30	6.0 - 8.6
Spectinomycin	25	5.0 - 7.8
Streptomycin	25	6.2 - 7.8
Sulphafurazole	300	5.0 - 9.4
Tetracycline	30	5.8 - 11.0
Tobramycin	10	6.4 - 8.4
Trimethoprim	5	8.8 - 13.6
<i>Escherichia coli</i> NCTC 11560		
(Sensitest, air, 35°C)		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
<i>Pseudomonas aeruginosa</i> NCTC 10662 #		
(Sensitest, air 35°C)		
Amikacin	30	7.4 - 10.6
Ciprofloxacin	2.5	8.9 - 14.5
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
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 ± 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.

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

- a. A new batch of medium is used.
- d. A new batch of discs is used.
- e. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.