THE PRINCE OF WALES HOSPITAL

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

5th January , 1996.

Dear Colleague,

CDS USERS GROUP

Newsletter No. 7

This newsletter largely reports the proceedings of the Group Workshop at the Australian Society for Microbiology Annual meeting in Canberra in September, 1995. The workshop was attended by over 150 participants and it was characterised by a free and, at times, lively discussion. Considerable feedback on laboratories' needs and problems associated with susceptibility testing was received from the audience.

The attention of users of the CDS method is drawn in particular to important updated information in this newsletter. Please replace existing tables headed "calibration", "surrogate disc testing" and "reference strains" with the three updated tables numbered 1, 2 and 3 contained in this newsletter.

I would like to thank all who participated in the 1995 Workshop, the Australian Society for Microbiology for its support and add a special thanks to my colleagues in the Antibiotics Laboratory for the effort they put into preparing material for the meeting.

Please make a note of changes in our communication numbers.

Telephone: **(02) 3829053**
Facsimile: **(02) 3829098**
e-mail: smbell@unsw.edu.au

We intend to conduct our next workshop this year at the ASM Annual Meeting in Christchurch, New Zealand. I look forward to seeing you there.

Yours faithfully,

S. M. BELL,
Antibiotics Laboratory,
Department of Microbiology.

CDS USERS GROUP NEWSLETTER No. 7

Report on the

CDS USERS GROUP WORKSHOP

ANNUAL SCIENTIFIC MEETING

ASM

CANBERRA 1995

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The Antibiotics Laboratory

S M Bell, B J Gatus, J N Pham & J S Jupp.

1. Additions and Modifications to Antibiotic Calibrations.

Calibrations have been performed with three recently available antibiotics and a number of recalibrations have been undertaken with either new disc potencies or changed growth conditions. In others, the MIC of susceptible strains has been re-evaluated. The information is now included in Table 1 and is shown by the symbol c.

1.i Tazocin 55 µg.

Tazocin 55 µg (piperacillin 50 µg and tazobactam 5 µg) was calibrated for the testing of Gram-negative bacilli. For other species, use a surrogate disc test (see below).

NOTE: *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, any organism which possesses an extended spectrum ß-lactamase (ESB), *Aeromonas* species and *Stenotrophomonas maltophilia* are considered RESISTANT to Tazocin (Table 6).

SURROGATE DISC TESTING (Table 2a)

Staphylococci (except *Staph. saprophyticus*)	Methicillin 5 µg
Streptococci	Penicillin 0.5 u
Enterococci	Ampicillin 25 µg
Haemophilus influenzae (non-encapsulated)	Cefaclor 30 µg
Branhamella catarrhalis	Cefaclor 30 µg

1.ii Cefpodoxime 10 µg.

Cefpodoxime 10 µg, an oral 3rd generation cephalosporin, was calibrated for the testing of members of the *Enterobacteriaceae*, *Haemophilus influenzae* and *Branhamella catarrhalis*. Use a surrogate disc test for Gram-positive cocci (see below).

NOTE: *E. cloacae*, *E. aerogenes*, *C. freundii*, *S. marcescens*, *M. morganii*, *P. vulgaris*, *P. penneri*, *Prov. rettgeri*, *Prov. stuartii,* organsims which possess an extended spectrum ß-lactamase (ESB), enterococcci, *Acinetobacter* species, *Pseudomonas* species, *Burkholderia* species and *Stenotrophomonas maltophilia* are considered RESISTANT to cefpodoxime (Table 6).

SURROGATE DISC TESTING for Gram-positive cocci (Tables 2a and 2b)

Staphylococci (except *Staph. saprophyticus*)	Methicillin 5 µg
Streptococci	Penicillin 0.5 u

1.iii Cefpirome 10 µg.

Cefpirome 10 µg was calibrated for the testing of **Gram-negative bacilli.**

However, cefpirome 10 µg discs are not yet available and until they are surrogate disc testing can be performed although this is not the ideal situation**.** Information concerning the calibration of cefpirome is included in Table 1 to prevent duplication of a subsequent table.

NOTE: *E. cloacae*, *E. aerogenes*, *C. freundii*, *S. marcescens*, *M. morganii*, *P. vulgaris*, *P. penneri*, *Prov. rettgeri*, *Prov. stuartii*, organisms which possess an extended spectrum ß-lactamase (ESB), enterococci and *Stenotrophomonas maltophilia* are considered RESISTANT to cefpirome (Table 6).

Surrogate Disc Testing (Table 2a)

Staphylococci (except *Staph. saprophyticus*)	Methicillin 5 µg
Streptococci	Penicillin 0.5 u
Aeromonas species	Cefotaxime 5 µg or ceftriaxone 5 µg
Enterobacteriaceae	Cefotaxime 5 µg or ceftriaxone 5 µg
Pseudomonas aeruginosa	Ceftazidime 10 µg
Branhamella catarrhalis	Cefpodoxime 10 µg
Haemophilus influenzae (non-encapsulated)	Cefpodoxime 10 µg

1.iv Cefotaxime 0.5 µg & ceftriaxone 0.5 µg for *Streptococcus pneumoniae* and *Neisseria meningitidis.*

Cefotaxime and ceftriaxone were recalibrated with 0.5 µg discs for testing *Streptococcus pneumoniae* and *Neisseria meningitidis* on blood Sensitest Agar at 35^{o}C in 5% CO_2.

The disc potencies, the annular radius of the zone of inhibition and the MIC for susceptible strains are:

Cefotaxime 0.5 µg ≥ 6 mm	MIC ≤ 0.25 mg/L
Ceftriaxone 0.5 µg ≥ 6 mm	MIC ≤ 0.25 mg/L

1.v ***Burkholderia cepacia*** **and** ***Burkholderia*** **species.**

Antibiotics used to test *Burkholderia* species are the same as those used for *Pseudomonas aeruginosa* with the addition of trimethoprim.

NOTE. Several types of ß-lactamase were described in *B. cepacia* including a carbapenem (imipenem) hydrolysing metallo-enzyme and strains resistant to all antibiotics including imipenem were isolated from patients with cystic fibrosis.

1.vi **Aztreonam 30 µg potency discs.**

Aztreonam 10 µg discs are no longer available, aztreonam 30 µg discs are used instead. The new range of the annular radii of inhibition with the reference strain *Escherichia coli* NCTC 10418 is shown in Table 3.

1.vii **Modification of MIC breakpoints and cut-off zone sizes.**

The MIC breakpoint of susceptible strains was modified with several antibiotics as information became available of the MIC of resistant strains previously unencountered. Calibration graphs for *Aeromonas* species and aminoglycosides were reviewed and the cut-off zone sizes for aminoglycoside were increased to 6mm. A changed value in either MIC breakpoint or cut-off zone size is indicated by the symbol c in Table 1.

1.viii ***Vibrionaceae*** **(except** ***Aeromonas*** **species).**

The calibration of the *Enterobacteriaceae* (Tables 1) has been extended to include members of the *Vibrionaceae* (with the exception of *Aeromonas* species).

2. Modification of the CDS Test for *Streptococcus pneumoniae* and *Streptococcus anginosus (milleri)*.

A number of isolates of these species do not grow well in air. Consequently, it was necessary to modify the method of preparing the inoculum and change the conditions of growth. These important changes are:

2.i The inoculum.

It is critical to obtain an inoculum which results in confluent growth. With a straight wire held at an angle of approximately 45^{o}, move it in one direction along the edge of confluent growth until cellular material is visible on the tip of the wire. Note that there is a risk that the inoculum prepared this way may not be pure.

2.ii Atmosphere of incubation.

All antibiotics were recalibrated on blood Sensitest Agar incubated at 35^{o}C in 5% CO_2 (Gas generating kit carbon dioxide system Oxoid BR039A is suitable).

3. Testing reference strains.

Two new reference strains *Streptococcus pneumoniae* ARL 10582 and *Enterococcus faecalis* POW 1994 have been added since the last newsletter. Also, acceptable ranges with the reference strains for new antibiotics or changed disc potencies and recommendations with regard to the frequency and indications for testing the reference strains are now included with the above data in Table 3.

3.i ***Streptococcus pneumoniae*** **ARL 10582** was selected as the reference strain for testing antibiotics on blood Sensitest Agar. Note that incubation is at 35^{O}C in an atmosphere of 5% CO_2. The acceptable ranges (mm) of the annular radii of the zones of inhibition (95 % confidence limits) of benzylpenicillin, chloramphenicol, erythromycin, rifampicin, tetracycline, vancomycin, cefotaxime and ceftriaxone for the reference strain *Streptococcus pneumoniae* ARL 10582 are shown in Table 3.

3.ii ***Enterococcus faecalis*** **POW 1994** was selected as the reference strain for testing enterococci. The acceptable ranges (mm) of the annular radii of the zones of inhibition (95 % confidence limits) of ampicillin, gentamicin (200 µg), nitrofurantoin and vancomycin for the reference strain tested on blood Sensitest Agar at 35^{O}C in air are shown in Table 3.

3.iii **Disc potencies and appropriate reference strains for new calibrations.**

The acceptable ranges (mm) of the annular radii of the zones of inhibition with the reference strains and recently calibrated antibiotics are shown in Table 3. The antibiotic disc potencies and the appropriate reference strains are:

Cefotaxime 0.5 µg	*Streptococcus pneumoniae* ARL 10582 *Haemophilus influenzae* NCTC 4560
	Streptococcus pneumoniae ARL 10582 *Haemophilus influenzae* NCTC 4560
Tazocin 55 µg	*Escherichia coli* NCTC 11560
Cefpodoxime10 µg	*Escherichia coli* NCTC 10418 *Haemophilus influenzae* NCTC 4560
Cefpirome 10 µg	*Escherichia coli* NCTC 10418 *Pseudomonas aeruginosa* NCTC 10662
Gentamicin 200 µg	*Enterococcus faecalis* POW 1994

4 CDS Representatives.

In order to keep The CDS Users Group running efficiently we have formed an accredited CDS Representatives Group whose role is to help CDS Users by supplying them with reference strains required for Quality Control of the CDS method. The names and addresses of people from different centres (state based) who kindly offered to be CDS Representatives are:

Australian Capital Territory.

Leon Tetlow,
Doctors Barratt & Smith & Moran,
63 Denison Street,
Deakin, ACT. 2600.
Tel: (06) 2859846.

New South Wales.

Rodney Jones,
Microbiology Department,
Wollongong Hospital,
Wollongong, NSW 2500.
Tel: (042) 201359.

Greg Farrow,
Pathology Department,
Queanbeyan District Hospital,
Queanbeyan, NSW 2620.
Tel: (06) 2989258.

Mike Burgess,
North Coast Pathology Service,
Manning Base Hospital,
P.O. Box 35,
Taree, NSW 2430.
Tel: (065) 511511.

Queensland.

Peter Lowe,
Central Queensland Pathology,
40 Carlyle Street,
Mackay, Queensland 4740.
Tel: (079) 513577.

Russell Lowry,
Pathology Department,
Nambour General Hospital,
Hospital Road,
Nambour, Queensland 4560.
Tel: (074) 459741.

Sabina Kopinski,
Pathology Department,
1st Military Hospital,
Kadumba Street,
Yeronga, Queensland 4104.
Tel: (07) 38920286.

David Winwood,
Micro Diagnostics,
1-3 Dixon Street,
P.O. Box 220,
New Farm, Queensland 4005.
Tel: (07) 2541644.

Tasmania.

Barbara Henderson,
Diagnostic Services,
Launceston Pathology,
159 St. John Street,
Launceston, Tasmania 7250.
Tel: (003) 343636.

Victoria.

Christopher Pearce,
St. John of God Pathology,
PO Box 20,
Ballarat, Victoria 3353.
Tel: (053) 316701.

Cathy Carolan,
Trezise Pathology,
582 Heidelberg Road,
Fairfield, Victoria 3078.
Tel: (03) 94862000.

Victoria cont.

Robert Clark,
Geelong Pathology,
68 Myers Street,
Geelong, Victoria 3220.
Tel: (052) 222488.

Angela Irving,
University of Melbourne,
Veterinary Clinical Centre,
Princes Highway,
Werribee, Victoria 3030.
Tel: (03) 7428273.

Mike Norriss,
Dorevitch Pathology Pty. Ltd.,
693 Burke Road,
Camberwell, Victoria 3124.
Tel: (03) 2440348.

Western Australia.

Jim Wells,
Western Diagnostic Pathology,
74 Mc Coy Street,
Myaree, W.A. 6154.
Tel: (09) 3171999.

5. Answers to questions from members of the CDS Users Group.

5.i *Stenotrophomonas maltophilia* and combination testing.

We have received numerous enquiries regarding what to test and how to treat serious infections caused by *Stenotrophomonas maltophilia* if the organism is resistant to sulphafurazole.

Stenotrophomonas maltophilia is considered resistant to all ß-lactams, aminoglycoside and quinolone antibiotics. However, it is possible that a combination of antibiotics may be useful for therapy if an isolate is resistant to sulphafurazole.

If an isolate of *S. maltophilia* is found to be resistant to sulphafurazole and the infection is serious then it may be justifiable to perform CDS testing using TIM 85 µg, CIP 2.5 µg and ATM 30 µg and telephone the CDS Reference Laboratory to discuss the zones of inhibition obtained with the 3 antibiotics.

5.ii *Haemophilus influenzae* (non-encapsulated strains) and the susceptibility to cefaclor and ampicillin.

When testing *H. influenzae* (non-encapsulated strains only) against cefaclor and ampicillin the following are possible.

Susceptible to cefaclor and susceptible to ampicillin = (S) to both antibiotics.

Susceptible to cefaclor and resistant to ampicillin	= TEM ß-lactamase present.	(S) to Augmentin.
Resistant to cefaclor and resistant to ampicillin	= Intrinsic resistance to both antibiotics or	(R) to Augmentin.
	= Intrinsic resistance to both antibiotics & TEM ß-lactamase.	(R) to Augmentin.
Resistant to cefaclor and susceptible to ampicillin	= Intrinsic resistance to cefaclor only.	(S) to Augmentin.

5.iii *Aeromonas* species susceptibility to ß-lactam antibiotics.

Aeromonas species may possess 1 or 2 types of ß-lactamase:

Class 3 inducible penicillinase/carbapenemase A2 which hydrolyses penicillins and carbapenems is produced by most strains, therefore these should be considered resistant to all penicillins and imipenem.

Class 1 inducible cephalosporinase A1 is also present in some species. This enzyme is inhibited by aztreonam and if it is present then a flattened zone between CTX 5 and IPM 10 is visible. In this case report the isolate resistant to all cephalosporins, but test aztreonam (T in table 6) (also see Figure 10a).

If the inducible cephalosporinase A1 is absent ie. there is no flattened zone between CTX 5 and IPM 10: test for cephalosporins susceptibility (T in table 6) (also see Figure 10b).

5.iv Testing co-trimoxazole in a single disc.

Co-trimoxazole is not used in the CDS method for testing any organism. Instead, trimethoprim and sulphafurazole are tested separately. However, note that these agents cannot be tested on either "chocolate agar" or blood Sensitest Agar.

5.v Calibrations for testing *Neisseria gonorrhoeae*.

Neisseria gonorrhoeae can be tested in accordance with the recommendations of the National Gonococcal Surveillance Programme.

5.vi Testing enterococci resistant to ampicillin?

Thirteen different species of enterococci have been described. In routine medical microbiology laboratories, two species namely *Enterococcus faecalis* and *Enterococcus faecium* are commonly encountered and are resistant to a wide variety of antibiotics including: all cephalosporins, cephamycins (cefoxitin and cefotetan), carbapenems (imipenem), quinolones, tetracyclines, macrolides (erythromycin, clindamycin & lincomycin), rifampicin, fusidate and sulphonamides.

Antibiotics which have been calibrated are:

Ampicillin 25 µg, nitrofurantoin 200 µg (for urinary isolates only), vancomycin 60 µg and gentamicin 200 µg (if endocarditis is suspected).

NOTE: At present, no enterococci which produce ß-lactamase have been described in Australia although with the CDS method, an ampicillin 25 µg disc can detect strains producing the enzyme. Enterococci resistant to ampicillin (MIC $\geq$ 16 mg/L) encountered so far have been identified as *Enterococcus faecium.*

5.vii Calibration of erythromycin for testing *Haemophilus influenzae*.

Haemophilus influenzae is considered resistant to erythromycin and roxithromycin and therefore these antibiotics are not tested by the CDS method.

5.viii Detection of extended-spectrum ß-lactamase (ESB) producing organisms.

More than 50 different types of extended-spectrum ß-lactamases have been described but their prevalence in Australia remains unknown. To detect an ESB, use disc approximation tests where either Timentin 85 µg or Augmentin 60 µg is placed adjacent to either cefotaxime 5 µg or cephalexin 100 µg in a disc dispenser along with other anbiotics. The patterns produced were described in Newsletter No. 6, 1993. (Also see Figures 6a, 6b & 7b in this Newsletter).

5.ix Which antibiotics to test against Haemophilus influenzae.

a. Systemic isolates (type b strains).

For *H. influenzae* type b isolated from CSF or blood culture, the detection of ß-lactamase using nitrocefin is performed before formal results of susceptibility testing are obtained. Appropriate antibiotics to test are ampicillin 2 µg, cefotaxime 0.5 µg, ceftriaxone 0.5 µg and chloramphenicol 10 µg.

b. Respiratory isolates (non-encapsulated strains).

Isolates from the respiratory tract are commonly non-encapsulated and it would be appropriate to test ampicillin 2 µg, cefaclor 30 µg, cefpodoxime 10 µg and oxytetracycline 30 µg.

5.x Erythromycin 5 µg as the surrogate disc for reporting the susceptibility of lincomycin and clindamycin against *Staphylococcus aureus* and streptococci.

Both lincomycin and clindamycin should not be tested against *Staphylococcus aureus* and streptococci. The susceptibility to lincomycin and clindamycin is inferred from the susceptibility to erythromycin.

Staphylococci exposed to erythromycin may develop resistance not only to erythromycin but to other related macrolide antibiotics. With agar disc diffusion tests, some strains of staphylococci and streptococci are resistant to erythromycin but appear to be susceptible to lincomycin and clindamycin. For this reason, test erythromycin only and report the susceptibility to lincomycin and clindamycin based on erythromycin as the surrogate antibiotic.

5.xi The testing of vancomycin and enterococci.

Vancomycin 60 µg was calibrated for the detection of high level resistance to this antibiotic in enterococci.

5.xii The shelf life of Sensitest and blood Sensistest Agar: storage and methods of packaging.

Sensitest Agar plates can be stored at 4^{o}C for up 2 weeks without plastic bags and for up to 6 weeks in plastic bags at 4^{o}C.

Blood Sensistest Agar can be stored at 4^{o}C for up 2 weeks without plastic bags and for up to 4 weeks in plastic bags at 4^{o}C.

5.xiii Testing of *Enterobacteriaceae* isolated from patients with systemic infections against Augmentin. Can an extrapolation be made from the susceptibility to Timentin?

Augmentin 60 µg should be used for the testing of members of the *Enterobacteriaceae* from the URINE ONLY. It should not be tested for isolates obtained from systemic infections.

Timentin is calibrated for testing members of the *Enterobacteriaceae* isolated from systemic sites and in this context an extrapolation from Augmentin cannot be made.

Test cefpodoxime 10µg if an oral ß-lactam antibiotic is required.

5.xiv Direct Sensitivity Testing: Is it valid?

There have been numerous questions concerning direct susceptibility testing of bacteria from urine.

The 'so-called' standardisation of the inoculum was based on the estimation of the number of organisms seen on microscopy which is a subjective test and is not standardised. The culture may be mixed and substances may be present in the urine which interfere with testing.

Although direct susceptibility testing may give some laboratories what they consider to be useful information, it does not comply with the rigid rules of standardisation used in the CDS method and therefore cannot be endorsed.

5.xv Heterogeneous resistance to methicillin in Staphylococcus aureus: Tips on detection.

The issue of testing staphylococci and the detection of heterogeneous resistance to methicillin was addressed in Newsletter No. 3, 1991.

IN SUMMARY: Resistance to methicillin is due to the presence of PBP 2a which has poor affinity for methicillin and all other ß-lactams.

Test all *Staphylococcus* species (except *Staph. saprophyticus*) on Sensitest Agar at 35^{o}C in air, conditions which allow optimum growth and expression of resistance to methicillin.

There is no need for testing at 30^{O}C or on Mannitol Salt Agar.
Resistant = a zone < 6mm in annular radius or presence of colonies within the zone of inhibition around a methicillin 5 µg disc (See Figure 1).

5.xvi The use of the CDS method in veterinary medicine.

The following antibiotics; apramycin, ceftiofur, neomycin, novobiocin, spectinomycin and streptomycin have been calibrated and the definitive results should be available later in a separate Veterinary Supplement.

5.xvii Mucoid *Pseudomonas aeruginosa*.

Stab the colony with the straight wire and pick up bacterial material as usual. If a satisfactory inoculum is not achieved, then tease the colony apart with the straight wire before picking up bacterial material.

5.xviii Who is right? NCCLS or CDS when testing *Stenotrophomonas maltophilia* ?

The following were observed when testing *Steno. maltophilia:*

Stable resistant mutants (MIC 32 to 64 fold greater than the wild strains) arose at a frequency of 10^{-4} to 10^{-6} when exposed to an aminoglycoside, a quinolone and any ß-lactam antibiotic (See Figure 11a, 11b). According to the CDS method, *Steno. maltophilia* is considered RESISTANT to all these antibiotics.

5.xix The testing of group B streptococci and cephalosporins.

The treatment of choice for serious group B streptococcal infections is penicillin, ampicillin or amoxycillin with or without an aminoglycoside. However, cephalosporins may be indicated for the treatment of less serious infections. See Surrogate Table 2a in this newsletter as the guide for testing and reporting cephalosporins.

6. PAPERS PRESENTED AT THE WORKSHOP.

6.i Susceptibility testing of *Streptococcus pneumoniae* from the CSF with cefotaxime and ceftriaxone.

Dr. B. J. Gatus

Mechanism of resistance to penicillin, cefotaxime & ceftriaxone.
There are 5 high molecular weight penicillin binding proteins (PBPS) 1A, 1B, 2A, 2B & 2X which are enzymes in the cell wall of *Strep. pneumoniae* and are the target sites for ß-lactam antibiotics. Changes in one or more PBP's which arise because of genetic tranformation result in an increase in the MIC of penicillin, cefotaxime and ceftriaxone.

Resistance to penicillin.
Changes in 4 PBP's are required to give high level resistance to penicillin (MIC > 2 mg/L).
In general the following changes in specific PBP's result in an increase in the penicillin MIC:

Changes in PBP's:	Penicillin MIC
1A & 2	≤ 0.1 mg/L
2 B	≥ 0.1 mg/L
1A, 2X & 2B	0.25 - 2.0 mg/L
1A, 2X, 2B & 2A	> 2.0 mg/L

There is a lack of correlation between resistance to OXACILLIN and PENICILLIN since the PBP changes which result in resistance to these agents are different.

Resistance to cefotaxime & ceftriaxone.
Changes in 2 PBP's are required to give high level resistance to cefotaxime and ceftriaxone.

Changes in PBP's	Cefotaxime & ceftriaxone MIC
1A & 2X	4.0 mg/L

Resistance to cefotaxime and ceftriaxone may not be associated with resistance to penicillin and as a consequence resistance to penicillin cannot be extrapolated from resistance to cefotaxime and ceftriaxone and *vice versa*. Therefore penicillin, cefotaxime and ceftriaxone must be tested separately.

***Streptococcus pneumoniae* meningitis.**
Failure of the therapy of pneumococcal meningitis with penicillin, cefotaxime and ceftriaxone was observed when the MIC of these antibiotics was > 0.5 mg/L.
We propose that the MIC of penicillin, cefotaxime and ceftriaxone for susceptible strains of *Streptococcus pneumoniae* is ≤ 0.25 mg/L.

Agar disc diffusion screening tests used by other workers.
Previously agar disc diffusion screening tests were devised by others workers.
Ceftizoxime 30 µg distinguished strains with MIC of cefotaxime & ceftriaxone ≥ 4 mg/L.
Cefuroxime 30 µg distinguished strains with MIC of cefotaxime & ceftriaxone ≥ 2 mg/L.

Calibration of cefotaxime & ceftriaxone for the CDS method.

Conditions of the test: Blood Sensitest Agar, 5 % CO_2 at 35^{o} C.
Cefotaxime 0.5 µg & ceftriaxone 0.5 µg.

MIC of both antibiotics for susceptible strains ≤ 0.25 mg/L.
Annular radius of inhibition ≥ 6 mm.
DEFINITELY: If MIC is 1 mg/L then annular radius of inhibition < 6 mm.

Conclusion.
MIC of penicillin, cefotaxime & ceftriaxone for susceptible strains ≤ 0.25 mg/L.
Disc potencies: Benzylpenicillin 0.5 u
Cefotaxime 0.5 µg
Ceftriaxone 0.5 µg

Must test all three antibiotics. Figures 2, 3, 4 & 5 on page 11 demonstrate different patterns of susceptibility using penicillin, cefotaxime and ceftriaxone.

6.ii The ß-lactamases of Gram-negative bacilli.

Dr. J. N. Pham

The issue of resistance to ß-lactam antibiotics of members of the *Enterobacteriaceae* which produce an inducible ß-lactamase requires clarification. The phenomenon of induction of ß-lactamase indicates that the bacterial species possesses a gene on the chromosome which codes for the production of a ß-lactamase whose activity is greatly increased by an inducer. This may be demonstrated by using disc approximation tests where the presence of a flattened zone between an imipenem 10 µg disc (IPM10) and another ß-lactam antibiotic disc eg. cefotaxime 5 µg (CTX 5) or cefotetan 10 µg (CTT 10) is visible.

However, resistance to ß-lactam antibiotics is due to the selection of stably derepressed mutants which produce a high level of ß-lactamase in the absence of an inducer. Therefore, if it is well established that for a species (eg. *Enterobacter cloacae*), stably derepressed resistant mutants arise at a high frequency then isolates belonging to that species are considered resistant to ß-lactam antibiotics with some exceptions eg. imipenem which can be tested. On the other hand, if stably derepressed resistant mutants arise at a low frequency, these are less likely to be selected during treatment. Isolates belonging to these species (eg. *Pseudomonas aeruginosa*) can be tested against antibiotics which have been calibrated (See Table 6).

There are 3 main classes of ß-lactamase:

I. **Classes 1 and 1'** ß-lactamases are inducible cephalosporinases not inhibited by clavulanic acid.
Figures 7a and 10a illustrate the patterns of recognition of an *Enterobacter cloacae* (Figure 7a) and an *Aeromonas* species, most likely to be *A. caviae* or *A. hydrophila* (Figure 10a) producing Class 1 ß-lactamase.
Clues: Flattened zone between CTX 5 and IPM 10 (ß-lactamase induction) and also resistance to AMC 60 (Figure 7a). Resistant colonies are seen sometimes within the zones of inhibition eg CTX (cefotaxime), CRO (ceftriaxone)...

Figures 8a and 8b illustrate the patterns of recognition of *Providencia* spp. or *Morganella* spp. producing Class 1' ß-lactamase.
Clues: Flattened zone between CTX 5 and IPM 10 and resistance to AMC 60 indicating the presence of an inducible cephalosporinase not inhibited by clavulanic acid (Figure 8a). In Figure 8b, the Class 1' ß-lactamase is inhibited by ticarcillin contained in Timentin but not by clavulanic acid.

II. **Class 2** is very complex and comprises Class 2a, 2b, 2c, 2d and 2e all of which are inhibited by clavulanic acid and some of them are inhibited also by tazobactam.

*** Class 2a** ß-lactamases comprise the penicillinases of *Staphylococcus* species.

*** Class 2b** comprises the common broad spectrum ß-lactamases produced by *E. coli*, *Haemophilus* species and *Klebsiella* species eg. TEM -1, TEM-2, SHV-1...etc which confer resistance to ampicillin.

*** Class 2b'** comprises the extended spectrum ß-lactamases (ESB) derived by mutation from the original TEM and SHV ß-lactamases of class 2b.
Figures 6a and 6b illustrate a *Klebsiella pneumoniae* producing an ESB, a non-inducible cephalosporinase inhibited by clavulanic acid which shows as an elliptical clearing area (Figure 6a) or a "key hole" clearing area (Figure 6b).

ESB's have been observed in other members of the *Enterobacteriaceae*. Figure 7b illustrates an *Enterobacter cloacae* producing a Class 1 inducible ß-lactamase and an ESB. Clues: "Key hole" between AMC 60 and CTX 5 indicates the presence of an ESB whilst the resistance to AMC 60 and the flattened zone of CTT 10 near IPM 10 indicates the presence of Class 1 inducible cephalosporinase.

*** Classes 2c** and **2d** ß-lactamases comprise the plasmid mediated ß-lactamases that may be present in *Ps. aeruginosa.* These ß-lactamases confer resistance to carbenicillin and ticarcillin.

*** Class 2e** includes chromosomally mediated inducible cephalosporinases of *Proteus vulgaris* or *Proteus penneri* and enzyme L2 of *Steno. maltpphilia.* These ß-lactamases confer resistance to cephalosporins.
Figures 9a and 9b illustrate the patterns of recognition of *Proteus vulgaris* or *Proteus penneri:* Flattened zone between CTX 5 and IPM 10 and a zone ≥ 6mm to AMC 60 that is the presence of an inducible cephalosporinase inhibited by clavulanic acid. Resistant colonies may or may not be present in CTX 5 zone (Figure 9a). Strains with high ß-lactamase activity may give no zone around CTX 5 disc but show a "key hole" effect (Figure 9b) resembling an ESB which is also a

cephalosporinase inhibited by clavulanic acid. NB: *P. penneri* (indole negative, maltose positive) may be mistaken as *P. mirabilis* (indole negative, maltose negative) producing an ESB.

III. Class 3 ß-lactamases comprise the carbapenem hydrolysing metallo-enzymes which hydrolyse penicillins and carbapenems very effectively and confer resistance to penicillins and imipenem. Amongst those are enzyme A2 produced by *Aeromonas* species (Figure 10b) and enzyme L1 produced by *Steno. maltophilia* (Figure 11b).

Table 4 is a simplified and modified version of the classification of bacterial ß-lactamases adapted from Bush 1988, 1989. This classification is based pragmatically on inhibitor profiles. The inhibitors are clavulanic acid, aztreonam, tazobactam and ticarcillin. An understanding of Table 4 helps with the use of Table 6.

Table 5 summarises data on the ß-lactamases of Gram-negative bacilli and the reasons why certain organisms are considered as resistant (R) to some ß-lactam antibiotics whilst others can be tested (T).

Table 6 is a guide to the testing and reporting of ß-lactam antibiotics for Gram-negative bacilli based on Tables 4 & 5.

ILLUSTRATIONS OF PATTERNS OF ANTIBIOTIC SUSCEPTIBILITY.

Staphylococcus aureus

Figure 1

Methicillin resistant *Staph. aureus* (MRSA) with **heterogeneous resistance** to methicillin shows resistant colonies in MET 5 zone when tested at **35^{O}C** on **Sensitest Agar** in air.

NB: Some isolates would give a larger zone with no obvious resistant colonies if incubated at 30^{O}C on Sensitest Agar or Mannitol Salt Agar (MSA) due to the poor growth at 30^{O}C especially on MSA.

Streptococcus pneumoniae.

Figure 2
Streptococcus pneumoniae.
The isolate is fully susceptible to benzylpenicillin, cefotaxime & ceftriaxone (MIC = 0.015 mg/L).

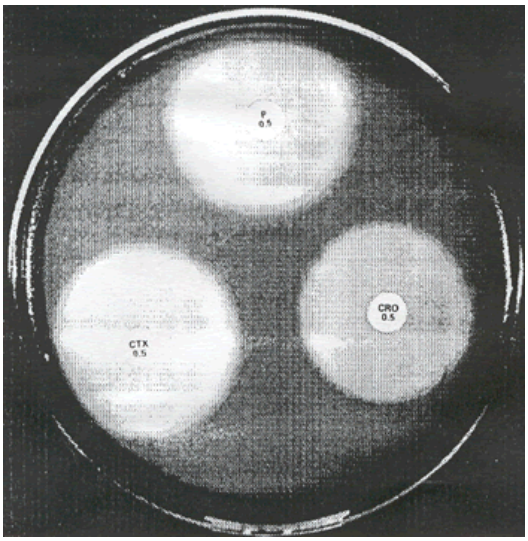


Figure 3
Streptococcus pneumoniae.
The isolate is less susceptible to benzylpenicillin, (MIC = 0.5 mg/L) than cefotaxime & ceftriaxone (MIC = 0.25 mg/L).

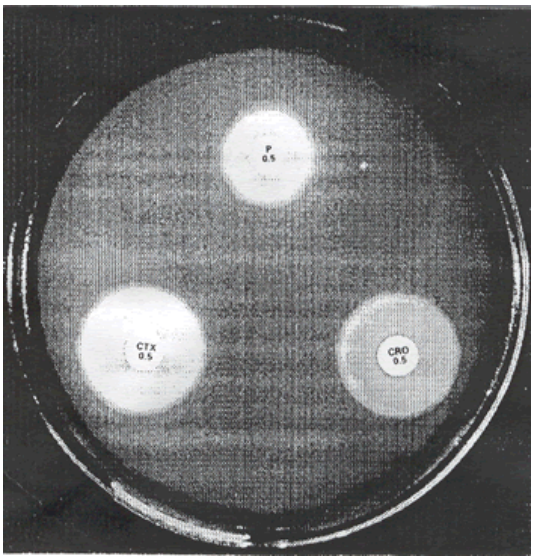


Figure 4
Streptococcus pneumoniae.
The isolate is resistant to benzylpenicillin (MIC = 2.0 mg/L) cefotaxime & ceftriaxone (MIC = 1.0 mg/L).

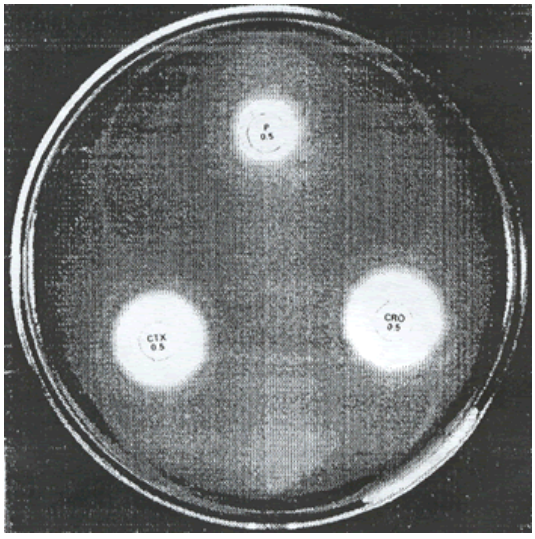
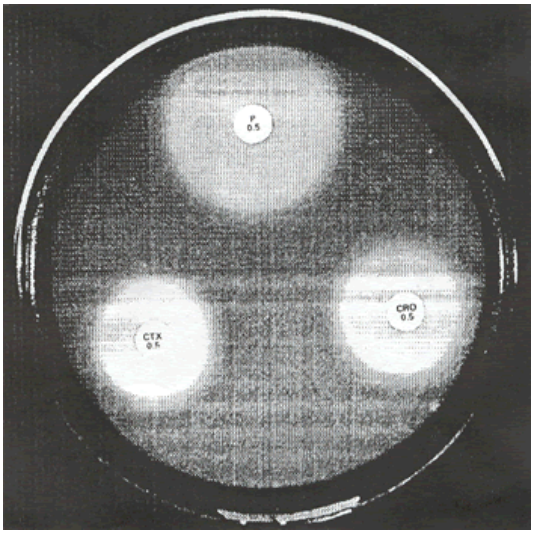


Figure 5
Streptococcus pneumoniae.
The isolate is unusual because the MIC of benzylpenicillin (MIC = 0.03 mg/L) is less than that of cefotaxime & ceftriaxone (MIC = 0.25 mg/L).



Gram-negative bacilli

Figures 6a, 6b

Klebsiella pneumoniae producing ESB, a non-inducible cephalosporinase inhibited by clavulanic acid, shows either an elliptical clearing area (Figure 6a) or the "key hole" effect (Figure 6b).

NOTE: 1. For strains producing high levels of ß-lactamase, the two discs need to be closer to show these effects.

2. *K. oxytoca* and *K. ozaenae* may produce ß-lactamases (MJ-1, K1; Bush 1989) similar to ESB found in *K. pneumoniae*. All are inhibited by clavulanic acid.

Figure 6a

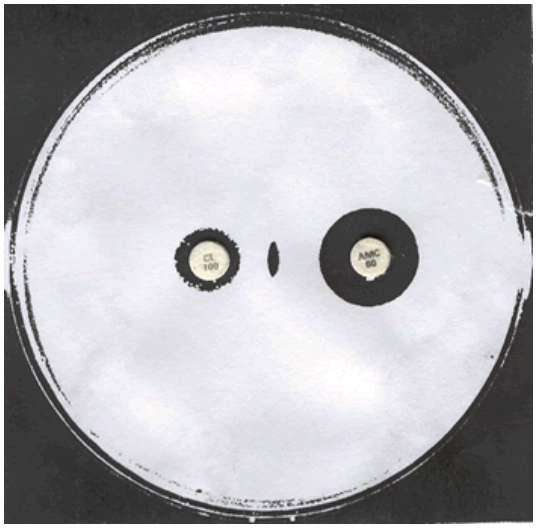
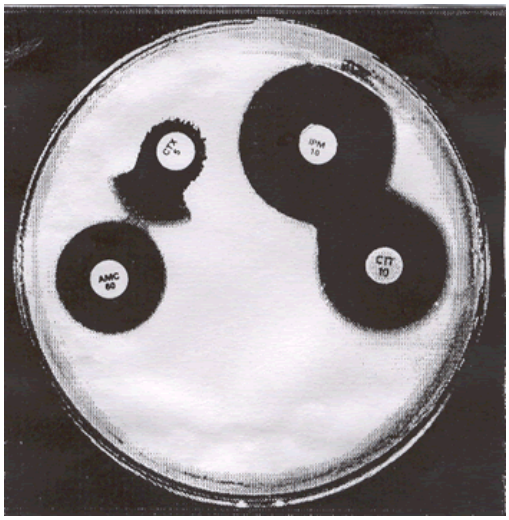


Figure 6b



Figures 7a, 7b

Enterobacter cloacae (Figure 7a): Class 1 ß-lactamase, inducible cephalosporinase not inhibited by clavulanic acid. Clues: Flattened zone between CTX 5 and IPM 10 (ß-lactamase induction) and resistance to AMC 60.

Enterobacter cloacae and ESB (Figure 7b): Other members of the *Enterobacteriaceae* may also produce an ESB. Clues: "Key hole" between AMC 60 and CTX 5 indicates the presence of an ESB whilst the resistance to AMC 60 and the flattened zone of CTT 10 near IPM 10 indicates the presence of Class 1 inducible cephalosporinase.

Figure 7a

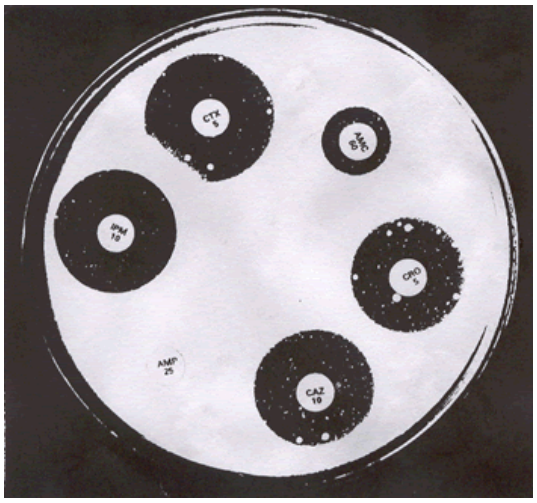
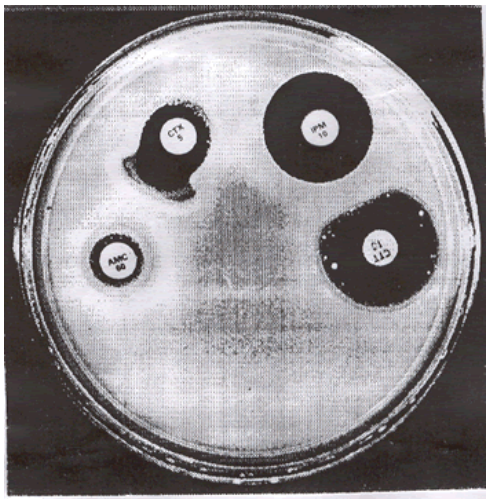


Figure 7b



Figures 8a, 8b

Providencia spp. or *Morganella* spp. of Class 1' ß-lactamase.

Clues: Flattened zone between CTX 5 and IPM 10 and resistance to AMC 60 indicating the presence of an inducible cephalosporinase not inhibited by clavulanic acid (Figure 8a). In Figure 8b, the Class 1' ß-lactamase is inhibited by ticarcillin contained in Timentin but not by clavulanic acid.

Figure 8a

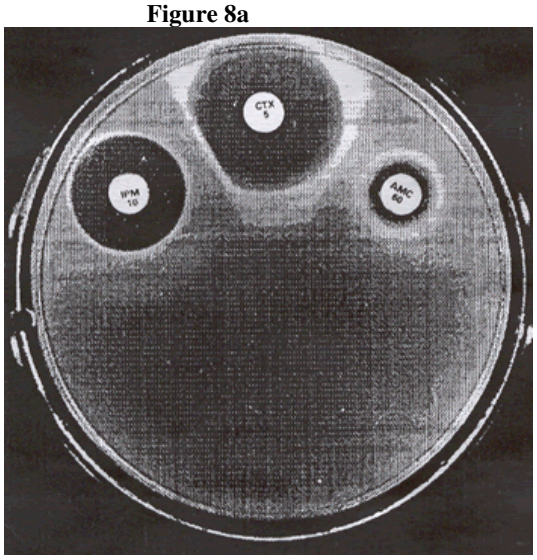
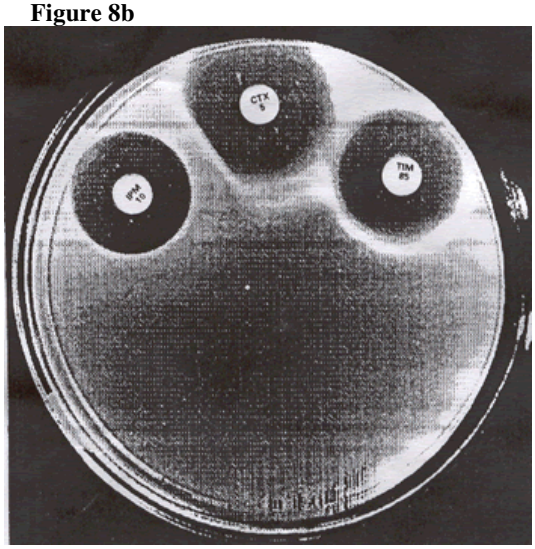


Figure 8b



Figures 9a, 9b

P. vulgaris or *P. penneri.*

1. Flattened zone between CTX 5 and IPM 10 and a zone ≥ 6mm to AMC 60 ie. presence of an inducible cephalosporinase inhibited by clavulanic acid. Resistant colonies may or may not be present in CTX 5 zone (Figure 9a).
2. Strains with high ß-lactamase activity may give no zone around CTX 5 disc but show a "key hole" effect **resembling an ESB** which is also a cephalosporinase inhibited by clavulanic acid (Figure 9b). NB: *P. penneri* (indole negative, maltose positive) may be mistaken as *P. mirabilis* (indole negative, maltose negative) producing an ESB.

Figure 9a

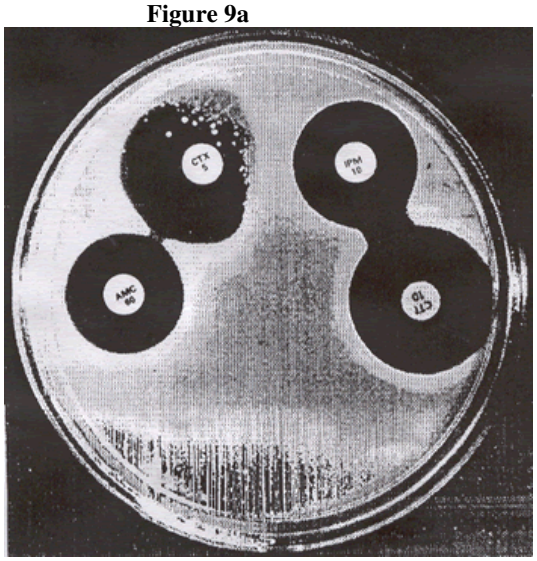
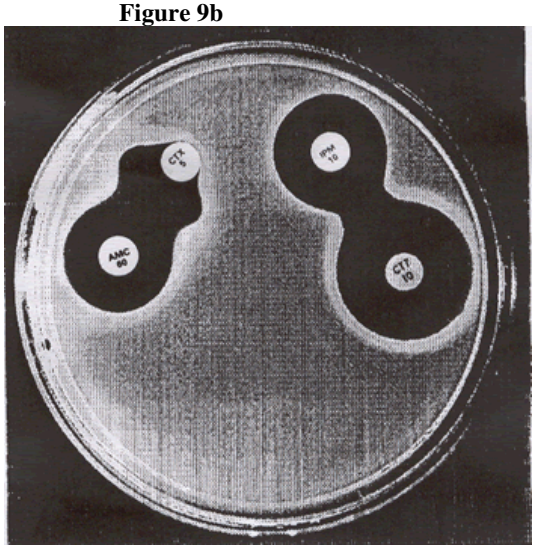


Figure 9b



Figures 10a, 10b

Aeromonas species (predominantly *A. caviae* & *A. hydrophila*) producing both the inducible cephalosporinase A1 (flattened zone between CTX 5 and IPM 10) and the carbapenemase A2 (Figure 10a).
Aeromonas species (predominantly *A. sobria*) lacking the inducible cephalosporinase A1 (no flattened zone between CTX 5 and IPM 10) and produce the penicillinase/carbapenemase (imipenemase) A2 only (Figure 10b).

Figure 10a

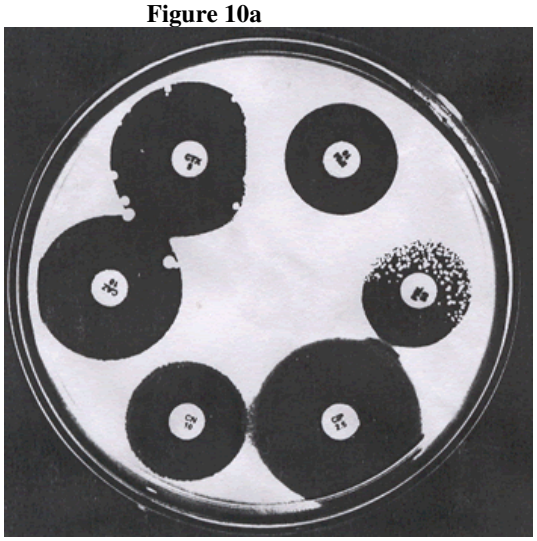
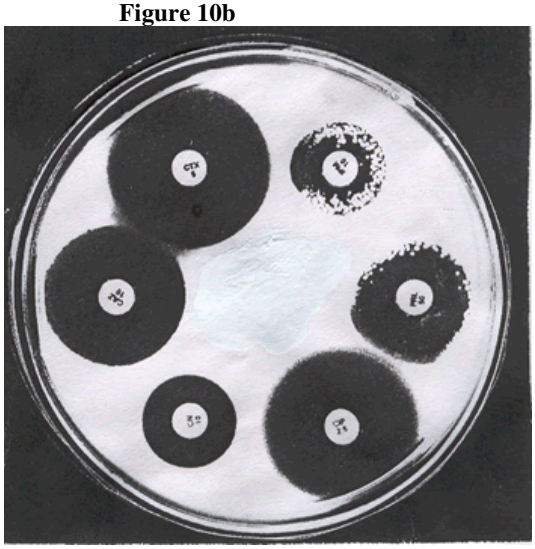


Figure 10b



Figures 11a, 11b

Stenotrophomonas maltophilia shows resistant mutants which arise at a very high frequency (Figure 11a).
A susceptibility pattern typical of *S. maltophilia* (Figure 11b) with no zone to IPM 10 (imipenem) and the synergy between W 5 (trimethoprim) and S 300 (sulphafurazole). These two features are often used as an aid to identification of S. maltophilia.

Figure 11a

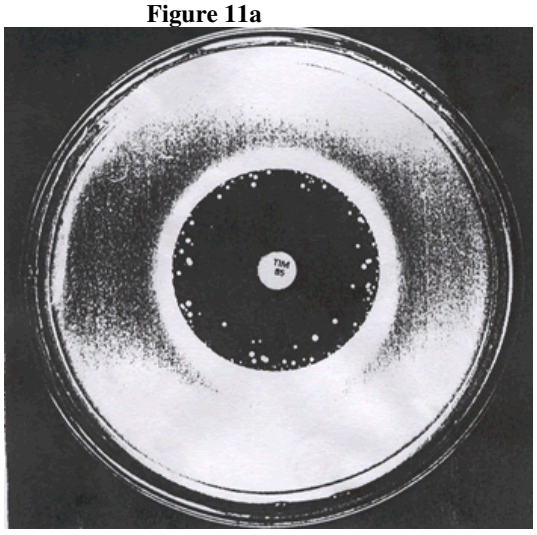


Figure 11b

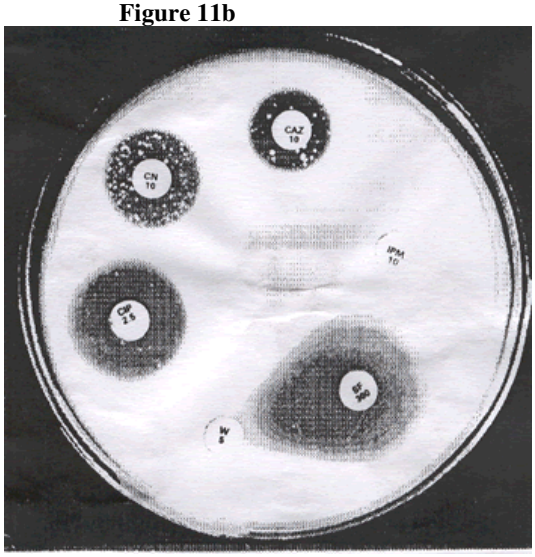


Table 1a. Calibrations: Antibiotics, disc potencies, the MIC for susceptible strains, media used and incubation conditions used. (c indicates changed disc potencies, MIC breakpoints or zone sizes).

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
Acinetobacter spp. (Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin *	60		≤ 8.0/4.0
Cefpirome	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Netilmicin	30		≤ 2.0
Norfloxacin *	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Aeromonas spp. (Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0 c
Aztreonam	30		≤ 8.0
Cefotaxime	5		≤ 1.0
Cefotetan	10		≤ 4.0
Cefoxitin	30		≤ 8.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cephalexin *	100		≤ 16.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0 c
Netilmicin	30		≤ 2.0 c
Tetracycline	30		≤ 4.0
Tobramycin	10		≤ 1.0 c
Branhamella catarrhalis (Blood Sensitest, CO_2, 35°C)			
Benzylpenicillin	0.5 u		≤ 0.25 c
Cefaclor	30		≤ 4.0
Cefpodoxime	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Tetracycline	30		≤ 4.0
Campylobacter spp. (Blood Sensitest, microaerophilic, 42°C)			
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5	4 mm†	≤ 0.5
Gentamicin	10		≤ 1.0
Tetracycline	30		≤ 4.0

* ONLY for isolates from urine. † The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm.

Table 1b. Calibrations (continued): Antibiotics, disc potencies, the MIC for susceptible strains, media used and incubation conditions used. (c indicates changed disc potencies, MIC breakpoints or zone sizes).

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
***Enterobacteriaceae* & *Vibrionaceae* (except *Aeromonas* spp.)**			
(Sensitest, air, 35°C)			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin *	60		≤ 8.0/4.0
Aztreonam	30		≤ 8.0
Cefotaxime	5		≤ 1.0
Cefotetan	10		≤ 4.0
Cefoxitin	30		≤ 8.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cephalexin*	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Nalidixic acid *	30		≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin *	200		≤ 32.0
Norfloxacin *	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Tetracycline	30		≤ 4.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
Enterococci			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 4.0 c
Gentamicin	200	4 mm†	≤ 512
Nitrofurantoin *	200		≤ 32.0
Vancomycin	60	4 mm†	≤ 16.0 c
Haemophilus influenzae			
(Chocolate agar, air, 35°C)			
Ampicillin	2		≤ 1.0
Cefaclor +	30		≤ 4.0
Cefotaxime	0.5		≤ 0.25 c
Cefpodoxime+	10		≤ 2.0
Ceftriaxone	0.5		≤ 0.25 c
Chloramphenicol	10		≤ 2.0
Ciprofloxacin +	2.5		≤ 1.0
Oxytetracycline +	30		≤ 4.0
Listeria monocytogenes			
(Blood Sensitest, air, 35°C)			
Ampicillin	25		≤ 2.0 c
Gentamicin	10		≤ 1.0
Neisseria meningitidis			
(Blood Sensitest, CO_2, 35°C)			
Benzylpenicillin	0.5 u	4 mm†	≤ 0.25 c
Cefotaxime	0.5		≤ 0.25 c
Ceftriaxone	0.5		≤ 0.25 c
Chloramphenicol	10		≤ 2.0

* ONLY for isolates from urine. † The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm.
+ ONLY for non-encapsulated strains.

Table 1c. Calibrations (continued): Antibiotics, disc potencies, the MIC for susceptible strains, media used and incubation conditions (c indicates changed disc potencies, MIC breakpoints or zone sizes).

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
Pasteurella multocida			
(Blood Sensitest, air, 35°C)			
Amoxycillin	10		≤ 0.5
Ciprofloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Pseudomonas aeruginosa, Burkholderia cepacia & other pseudomonads			
(Sensitest, air, 35°C)			
Amikacin	30	4 mm†	≤ 16.0
Aztreonam	30		≤ 8.0
Cefpirome	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin	2.5		≤ 2.0
Gentamicin	10	4 mm†	≤ 4.0
Imipenem	10		≤ 4.0
Netilmicin	30	4 mm†	≤ 8.0
Norfloxacin *	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm†	≤ 1.0
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
Staphylococci			
(Sensitest, air, 35°C)			
Amoxycillin §	10		≤ 0.5
Benzylpenicillin #	0.5 u		≤ 0.06
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Gentamicin	10		≤ 1.0
Kanamycin	50		≤ 8.0
Methicillin #	5		≤ 4.0
Nitrofurantoin *	200		≤ 32.0
Rifampicin	1		≤ 0.5
Sulphafurazole *	300		≤ 64.0
Tetracycline	30		≤ 4.0
Trimethoprim *	5		≤ 2.0
Vancomycin	60	4 mm†	≤ 16.0 c

* ONLY for isolates from urine. † The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm.
§ ONLY for testing isolates of *S. saprophyticus*. # Not for testing *S. saprophyticus.*
$ For testing *Burkholderia cepacia.*

Table 1d. Calibrations (continued): Antibiotics, disc potencies, the MIC for susceptible strains, media used and incubation conditions used. (c indicates changed disc potencies, MIC breakpoints or zone sizes).

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
Streptococci (except *Strep. pneumoniae* & *Strep. anginosus*) (Blood Sensitest, air, 35°C)			
Benzylpenicillin	0.5 u		≤ 0.25 c
Chloramphenicol	30		≤ 8.0
Erythromycin	5		≤ 0.5
Tetracycline	30		≤ 4.0
Vancomycin	60	4 mm†	≤ 16.0 c
Streptococcus pneumoniae* & *Strep. anginosus (Blood Sensitest, CO_2, 35°C)			
Benzylpenicillin	0.5 u		≤ 0.25 c
Cefotaxime •	0.5		≤ 0.25
Ceftriaxone •	0.5		≤ 0.25
Chloramphenicol	30		≤ 8.0
Erythromycin	5		≤ 0.5
Rifampicin	1		≤ 0.5
Tetracycline	30		≤ 2.0
Vancomycin	60	4 mm†	≤ 16.0 c
Stenotrophomonas maltophilia (Sensitest, air, 35°C)			
Sulphafurazole	300		≤ 64.0
Yersinia enterocolitica (Sensitest, air, 30°C)			
Amikacin	30		≤ 4.0
Augmentin	3		≤ 2.0/1.0
Aztreonam	30		≤ 8.0
Cefotaxime	5		≤ 1.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Netilmicin	30		≤ 2.0
Sulphafurazole	300		≤ 64.0
Tetracycline	30		≤ 4.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0

† The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm.
• For *Streptococcus pneumoniae* only.

Table 2a. . **Surrogate disc testing. Antibiotics of therapeutic relevance.** Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic	Disc potency (µg)	MIC for susceptible strains (mg/L)
***Acinetonacter* spp.**		
Amoxycillin	Ampicillin	25
Co-trimoxazole	Sulphafurazole	300
Sulphonamides	Sulphafurazole	300
***Aeromonas* spp.**		
Cefaclor*	Cephalexin	100
Tetracyclines	Tetracycline	30
Branhamella catarrhalis		
Azithromycin	Erythromycin	5
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefaclor	30
Cephalexin	Cefaclor	30
Cefotaxime	Cefpodoxime	10
Cefpirome	Cefpodoxime	10
Ceftriaxone	Cefpodoxime	10
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
***Campylobacter* spp.**		
Azithromycin	Erythromycin	5
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
***Enterobacteriaceae & Vibrionaceae (except Aeromonas* sp.)**		
Amoxycillin	Ampicillin	25
Cefaclor *	Cephalexin	100
Cefotaxime	Ceftriaxone	5
Ceftriaxone	Cefotaxime	5
Cephalothin	Ampicillin	25
Cephazolin	Ampicillin	25
Co-trimoxazole$^{\phi}$	Sulphafurazole	300
Co-trimoxazole $^{\phi}$	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Enterococci		
Amoxycillin	Ampicillin	25
Benzylpenicillin	Ampicillin	25
Haemophilus influenzae		
Amoxycillin	Ampicillin	2
Augmentin^{+}	Cefaclor	30
Cefpirome^{+}	Cefotaxime	0.5
Ceftazidime^{+}	Cefotaxime	0.5
Ceftriaxone	Cefotaxime	0.5
Cephalexin^{+}	Cefaclor	30
Tetracyclines^{+}	Oxytetracycline	30
Listeria monocytogenes		
Amoxycillin	Ampicillin	25
Benzylpenicillin	Ampicillin	25
Staphylococci (except *S. saprophyticus*)		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Azithromycin	Erythromycin	5
Cefaclor	Methicillin	5
Cefpodoxime	Methicillin	5
Cephalexin	Methicillin	5
Cephalothin	Methicillin	5
Cephazolin	Methicillin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole *$^{\phi}$	Sulphafurazole	300
Co-trimoxazole *$^{\phi}$	Trimethoprim	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
***Staphylococcus saprophyticus* from urine**		
Ampicillin	Amoxycillin	10
Augmentin	Amoxycillin	10
Benzylpenicillin	Amoxycillin	10
Cefaclor	Amoxycillin	10
Cephalexin	Amoxycillin	10
Cephalothin	Amoxycillin	10
Cephazolin	Amoxycillin	10
Cloxacillin	Amoxycillin	10
Co-trimoxazole *$^{\phi}$	Sulphafurazole	300
Co-trimoxazole *$^{\phi}$	Trimethoprim	5
Flucloxacillin	Amoxycillin	10
Norfloxacin *	Ciprofloxacin	2.5
Penicillin V	Amoxycillin	10
Sulphonamides *	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Streptococci #		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Cefaclor	Benzylpenicillin	0.5 u
Cefpodoxime	Benzylpenicillin	0.5.u
Cephalexin	Benzylpenicillin	0.5 u
Cephalothin	Benzylpenicillin	0.5 u
Cephazolin	Benzylpenicillin	0.5 u
Clindamycin	Erythromycin	5
Lincomycin	Erythromycin	5

Neisseria meningitidis		
Ampicillin	Benzylpenicillin	0.5u
Pasteurella multocida		
Ampicillin	Amoxycillin	10
Benzylpenicillin	Amoxycillin	10
Tetracyclines	Tetracycline	30
***Pseudomonas* & *Burkholderia* spp.**		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole $	Trimethoprim	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
Xanthomonas maltophilia		
Co-trimoxazole	Sulphafurazole	300
Yersinia enterocolitica		
Ceftazidime	Cefotaxime	5
Ceftriaxone	Cefotaxime	5
Co-trimoxazole ϕ	Sulphafurazole	300
Co-trimoxazole ϕ	Trimethoprim	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30

* ONLY for isolates from urine.

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

+ ONLY for non-encapsulated strains.

$ Only for Burlholderia species.

\# Not for *Strep pneumoniae* from CSF.

Table 2b. Surrogate disc testing. Antibiotics of questionable therapeutic relevance. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic	Disc potency (µg)	MIC for susceptible strains (mg/L)
***Acinetonacter* spp.**		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
Branhamella catarrhalis		
Azlocillin	Benzylpenicillin	0.5 u
Piperacillin	Benzylpenicillin	0.5 u
***Enterobacteriaceae & Vibrionaceae (except Aeromonas* sp.)**		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
Ticarcillin	Ampicillin	25
Enterococci		
Azlocillin	Ampicillin	25
Piperacillin	Ampicillin	25
Tazocin	Ampicillin	25
Haemophilus influenzae		
Azlocillin[+]	Ampicillin	2
Piperacillin[+]	Ampicillin	2
Tazocin[+]	Cefaclor	30
Ticarcillin	Ampicillin	2
Timentin[+]	Cefaclor	30
Staphylococci (except *S. saprophyticus*)		
Azlocillin	Benzylpenicillin	0.5 u
Cefotaxime	Methicillin	5
Cefpirome	Methicillin	5
Ceftriaxone	Methicillin	5
Imipenem	Methicillin	5
Piperacillin	Benzylpenicillin	0.5 u
Tazocin	Methicillin	5
Ticarcillin	Benzylpenicillin	0.5 u
Timentin	Methicillin	5
Streptococci		
Azlocillin	Benzylpenicillin	0.5 u
Cloxacillin	Benzylpenicillin	0.5 u
Flucloxacillin	Benzylpenicillin	0.5 u
Imipenem	Benzylpenicillin	0.5 u
Piperacillin	Benzylpenicillin	0.5 u
Ticarcillin	Benzylpenicillin	0.5 u

[+] Only for non-encapsulated strains

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

Antibiotic and disc content (µg)	Disc content	Acceptable range* (mm)
Staphylococcus aureus **NCTC 6571**		
Amoxycillin	10	11.5 - 5.9
Benzylpenicillin	0.5 u	8.7 - 13.5
Chloramphenicol	30	7.8 - 11.4
Ciprofloxacin	2.5	9.2 - 12.4
Erythromycin	5	7.1 - 10.7
Fusidic acid	2.5	8.6 - 12.6
Gentamicin	10	6.6 - 9.4
Kanamycin	50	5.9 - 8.7
Methicillin	5	8.8 - 12.0
Nitrofurantoin	200	6.7 - 10.3
Rifampicin	1	9.3 - 12.5
Sulphafurazole	300	9.3 - 13.7
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	7.3 - 10.1
Vancomycin	60	5.4 - 7.8
Haemophilus influenzae **NCTC 4560**		
Ampicillin	2	6.0 - 9.2
Chloramphenicol	10	7.7 - 10.9
Cefaclor	30	7.3 - 10.9
Cefotaxime	5	8.9 - 14.1
Ciprofloxacin	2.5	9.7 - 14.9
Oxytetracycline	30	6.6 - 9.0
Yersinia enterocolitica **IP 22273**		
Amikacin	30	6.0 - 8.4
Aztreonam	10	10.1 - 13.7
Augmentin	3	6.4 - 8.4
Chloramphenicol	30	6.7 - 11.9
Ciprofloxacin	2.5	12.1 - 16.1
Gentamicin	10	6.0 - 8.0
Imipenem	10	11.1 - 15.1
Netilmicin	30	8.1 - 10.5
Sulphafurazole	300	8.9 - 13.1
Tetracycline	30	9.9 - 13.9
Timentin	85	10.6 - 15.4
Tobramycin	10	6.1 - 8.1
Trimethoprim	5	9.6 - 13.2
Enterococcus faecalis **POW 1994**		
Ampicillin	25	8.0 - 12.4
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Vancomycin	60	5.4 - 6.3
Escherichia coli **NCTC 11560**		
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 or *Yersinia enterocolitica* IP 22273 as well and vice versa.

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

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

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

Simplified classification of bacterial ß-lactamases

Adapted from K. Bush (1988, 1989)

	Class 1	Class 1'	Class 2a	Class 2b/2b'
Inhibited by				
Clav. acid	-	-	+	+
Aztreonam	+	+	-	-
Tazobactam	-	+	+	v
Ticarcillin	v	+	-	-
Type	**Inducible Ceph-se**[1]	**Inducible Ceph-se**[1]	**Pen-se**[2]	**2b. Broad spectrum ß-se**[3]
Representatives	*E. cloacae*	*Providencia*	*Staph.* spp	TEM-1, 2
	E. aerogenes	*Morganella*		SHV-1
	C. freundii			ROB-1,...
	S. marcescens			
	Ps. aeruginosa			**2b'. ESB**[6]
	**Aeromonas* A1			TEM-3,...
				SHV-2,...

v: variable
* Depressed mutants not selected by aztreonam ie. can test aztreonam (T).
** *S. malto*. L1 and *Aeromonas* A2 carbapenem-hydrolysing metallo-enzymes are inhibited by EDTA.
[1]Ceph-se=cephalosporinase, [2]Pen-se=penicillinase, [3]ß-se=ß-lactamase, [4]Carb-se=carbenicillinase, [5]Clox-se=cloxacillinase, [6]ESB=extended spectrum ß-lactamase.

Table 4 (continued).

Simplified classification of bacterial ß-lactamases

Adapted from K. Bush (1988, 1989)

	Class 2c	Class 2d	Class 2e	Class 3
Inhibited by				
Clav. acid	+	+	+	v
Aztreonam	-	-	-	v
Tazobactam	+	+	v	-
Ticarcillin	-	-	-	-
Type	**Carb-se**[4]	**Clox-se**[5]	**Inducible Ceph-se**[1]	**Metallo-enzyme**
Representatives	PSE CARB	OXA	*P. vulgaris* *P. penneri* *S. malto*. L2	***S. malto*. L1 ***Aero.* A2

v: variable
* Depressed mutants not selected by aztreonam ie. can test aztreonam (T).
** *S. malto*. L1 and *Aeromonas* A2 carbapenem-hydrolysing metallo-enzymes are inhibited by EDTA.
[1]Ceph-se=cephalosporinase, [2]Pen-se=penicillinase, [3]ß-se=ß-lactamase, [4]Carb-se=carbenicillinase,
[5]Clox-se=cloxacillinase, [6]ESB=extended spectrum ß-lactamase.

Table 5a.

The ß-lactamases of the *Enterobacteriaceae*

Class 1 ß-lactamase

Type : Inducible cephalosporinase
Substrate: Cephalosporins, penicillins
Derepressed resistant mutants arise at a HIGH frequency.

Enterobacter cloacae
Enterobacter aerogenes
Citrobacter freundii
Serratia marcescens

RESISTANT to all ß-lactam antibiotics except (T): Imipenem.

Class 1' ß-lactamase

Inhibited by: Aztreonam, ticarcillin, tazobactam

Providencia stuartii
Providencia rettgeri
Morganella morganii

RESISTANT to all ß-lactam antibiotics except (T): Tazocin, ticarcillin,Timentin, imipenem, aztreonam.

Class 2b/2b' ß-lactamases.

Type: Non-inducible ß-lactamases
Inhibited by: Clavulanic acid, tazobactam

2b. Broad spectrum (common ß-lactamase, eg. TEM-1,-2, SHV-1, ...)
RESISTANT to ampicillin, piperacillin, ticarcillin, cephalothin, cefazolin. (T): All other ß-lactams.

2b'. Extended spectrum ie ESB (eg. TEM-3, ..., SHV-2, ...)
RESISTANT to all ß-lactams except (T) Augmentin, cefotetan, cefoxitin, imipenem.

Class 2e ß-lactamases

Type : Inducible cephalosporinase
Substrate: Penicillins, cephalosporins
Inhibited by: Clavulanic acid, tazobactam
Derepressed mutants arise at a HIGH frequency.

Proteus vulgaris
Proteus penneri

RESISTANT to all ß-lactams except (T): Augmentin, cefotetan, cefoxitin, ceftazidime, Tazocin, Timentin, imipenem.

Note: When a CTX 5 disc is placed between an IMP 10 disc and an AMC 60 (or TIM 85) disc in the "combined disc approximation test", *P. vulgaris* and *P. penneri* show either induction or "key hole". Strains with a large zone of inhibition around CTX 5 show a "flattened" zone between IPM 10 and CTX 5 demonstrating the presence of an inducible cephalosporinase. Strains with a small zone or no zone of inhibition show an elliptical clearing area or 'key hole" between CTX 5 and AMC 60 or TIM 85.
T = The antibiotic can be tested.

Table 5b.

The ß-lactamases of Other Gram-negative bacilli

Aeromonas species

Type: Class 1 inducible cephalosporinase A1
Class 3 inducible penicillinase/carbapenemase (metallo-enzyme) A2

Derepressed resistant mutants of A1 and A2 arise at a HIGH frequency.
Most strains produce A2 and therefore are considered RESISTANT to penicillins/**imipenem**.

If A1 present: RESISTANT to all cephalosporins, TEST (T) aztreonam
If A1 absent: TEST (T) cephalosporins

Pseudomonas aeruginosa and *Pseudomonas* species

Pseudomonas aeruginosa

Type: Class 1 inducible cephalosporinase

Derepressed resistant mutants arise at a LOW frequency.
Test (T): ceftazidime, cefpirome, piperacilin, Tazocin, ticarcillin, Timentin, imipenem, aztreonam

***Pseudomonas* species**

The calibration has included other ***Pseudomonas* species.**

Burkholderia cepacia and *Burkholderia* species

The testing of ***Burkholderia*** species is extended from the testing of *Pseudomonas aeruginosa* with the exception that *Burkholderia* species can be tested against trimethoprim.

Notes: Several types of ß-lactamase were described in ***B. cepacia*** including a cephalosporinase and a carbapenem (imipenem) hydrolysing metallo-enzyme.

Strains of ***B. cepacia*** resistant to all ß-lactam antibiotics were isolated from patients with cystic fibrosis.

Stenotrophomonas maltophilia

Type: Class 3 penicillinase/carbapenemase metallo-enzyme L1
Class 2e cephalosporinase L2

Derepressed resistant mutants of L1 and L2 arise at a HIGH frequency of 10^{-4} to 10^{-6}
RESISTANT to all ß-lactam antibiotics.

Note: When a CTX 5 disc is placed between an IMP 10 disc and an AMC 60 (or TIM 85) disc in the "combined disc approximation test", *P. vulgaris* and *P. penneri* show either induction or "key hole". Strains with a large zone of inhibition around CTX 5 show a "flattened" zone between IPM 10 and CTX 5 demonstrating the presence of an inducible cephalosporinase. Strains with a small zone or no zone of inhibition show an elliptical clearing area or 'key hole" between CTX 5 and AMC 60 or TIM 85.
T = The antibiotic can be tested.

Table 6. A guide to the testing and reporting of the susceptibility of the named species or genera of bacteria to ß-lactam antibiotics in accord with the CDS method. **R**=report as resistant irrespective of the test result. **T**=the organism can be tested.

Organism	Antibiotic									
	AMP	LOT	FAZ	CEC	CL	CPD	AMC	FOX	CTT	CTX
Ent. cloacae	R	R	R	R	R	R	R	R	R	R
Ent. aerogenes	R	R	R	R	R	R	R	R	R	R
Cit. freundii	R	R	R	R	R	R	R	R	R	R
Ser. marcescens	R	R	R	R	R	R	R	R	R	R
Prov. stuartii	R	R	R	R	R	R	R	R	R	R
Prov. rettgeri	R	R	R	R	R	R	R	R	R	R
Morg. morganii	R	R	R	R	R	R	R	R	R	R
TEM-1/2, SHV-1	R	R	R	**T+**	**T**	**T**	**T**	**T**	**T**	**T**
ESB	R	R	R	R	R	R	**T**	**T**	**T**	R
Prot. vulgaris	R	R	R	R	R	R	**T**	**T**	**T**	R
Prot. penneri	R	R	R	R	R	R	**T**	**T**	**T**	R
Aeromonas (A1 & A2)	R	R	R	R	R	R	R	R	R	R
(A2)	R	R	R	**T+**	**T**	**T**	R	**T**	**T**	**T**
Ps. aeruginosa	R	R	R	R	R	R	R	R	R	R
Burk. cepacia	R	R	R	R	R	R	R	R	R	R
Sten. maltophilia	R	R	R	R	R	R	R	R	R	R
Acinetobacter spp.	**T**	R	R	R	R	R	**T**	R	R	R

AMP=ampicillin, LOT=cephalothin, FAZ=cefazolin, CEC=cefaclor, CL=cephalexin, CPD=cefpodoxime, AMC=Augmentin, FOX=cefoxitin, CTT=cefotetan, CTX=cefotaxime,

* Ampicillin 25 µg is used as the surrogate disc.

+ Cephalexin 100 µg is used as the surrogate disc for URINE only.

Table 6.Continued A guide to the testing and reporting of the susceptibility of the named species or genera of bacteria to ß-lactam antibiotics in accord with the CDS method. **R**=report as resistant irrespective of the test result. **T**=the organism can be tested.

Organism	Antibiotic								
	CRO	CAZ	CPO	PRL	TZP	TIC	TIM	IPM	ATM
Ent. cloacae	R	R	R	R	R	R	R	**T**	R
Ent. aerogenes	R	R	R	R	R	R	R	**T**	R
Cit. freundii	R	R	R	R	R	R	R	**T**	R
Ser. marcescens	R	R	R	R	R	R	R	**T**	R
Prov. stuartii	R	R	R	R	**T**	**T**	**T**	**T**	**T**
Prov. rettgeri	R	R	R	R	**T**	**T**	**T**	**T**	**T**
Morg. morganii	R	R	R	R	**T**	**T**	**T**	**T**	**T**
TEM-1/2, SHV-1	**T**	**T**	**T**	R	**T**	R	**T**	**T**	**T**
ESB	R	R	R	R	R	R	R	**T**	R
Prot. vulgaris	R	**T**	R	R	**T**	R	**T**	**T**	R
Prot. penneri	R	**T**	R	R	**T**	R	**T**	**T**	R
Aeromonas (A1 & A2)	R	R	R	R	R	R	R	R	**T**
(A2)	**T**	**T**	**T**	R	R	R	R	R	**T**
Ps. aeruginosa	R	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**
Burk. cepacia	R	**T**	**T**	**R**	**T**	**T**	**T**	**R**	**T**
Sten. maltophilia	R	R	R	R	R	R	R	R	R
Acinetobacter spp.	R	R	**T**	**T***	**T**	**T**	**T**	**T**	R

CRO=ceftriaxone, CAZ=ceftazidime, CPO=cefpirome, PRL=piperacillin, TZP=Tazocin, TIC=ticarcillin, TIM=Timentin, IPM=imipenem, ATM=aztreonam.

* Ampicillin 25 µg is used as the surrogate disc.

+ Cephalexin 100 µg is used as the surrogate disc for URINE only.