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16 August 2000

Dear Colleague,

CDS USERS GROUP

Newsletters No. 10

This Newsletter contains the proceedings of the CDS Users Group Workshop conducted at the Australian Society for Microbiology Annual Meeting in Cairns in July 2000. It also includes reference to the establishment of the CDS website and a proposed field test of the CDS method of testing of enterococci.. There are updated Tables 2000, an updated list of CDS Representatives and other matters of importance.

The field test of enterococci susceptibility testing to Ampicilcillin and Vancomycin is being undertaken to determine if all CDS users can reproduce the results achieved in the Antibiotics Laboratory. Participation in the field test is voluntary and anonymous and we strongly urge you to join in. If you wish to do so please contact your CDS representatives in your area will provide you with a set of enterococci labelled 1 to 8 The instructions and a form to record your results are contained in this Newsletter. Please post or fax your results to the CDS Reference Laboratory. We strongly encourage CDS Users not only to help us validate the methodology in practice but to take this opportunity to become familiar with the testing of enterococci. We will feed back the results to CDS users as soon as they become available.

The CDS web site referred to in the Newsletter is under construction and will contain details of the method, updates and other information relevant to the CDS.

Also we hope to distribute future information via the Web site or via email. To do this we need to update our members data base could you please let us have your email address and indicate whether you have access to the WWW.

Yours sincerely

S M Bell

CDS USERS GROUP NEWSLETTER No. 10

PROCEEDINGS OF THE CDS USERS GROUP WORKSHOP

ASM SCIENTIFIC MEETING

CAIRNS JULY 2000

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Section

1. Business meeting

In Cairns this year, for the first time, we held a business meeting prior to the scientific part of the workshop. The purpose of the meeting was to explore ways to strengthen the base of the organisational structure supporting the CDS and ensure that the method continues to be maintained and developed in response to changing clinical circumstances. There were several helpful suggestions and as a result, a number of members volunteered to form a steering committee to help us at the Antibiotics Reference Laboratory (Randwick) to develop some firm proposals for the CDS Users Group.

The members who volunteered for the steering committee were:

Rebecca Guy	Cairns Base Hospital
Raymond Lin	KK Womens and Childrens Hospital Singapore
Sandra Lutwyche	Capital Pathology Canberra
Keith Wise	Illawarra Area Pathology Wollongong
Trevor Warren	Oxoid Sydney

Since the members of the steering committee are scattered across the country and beyond, our discussions will be largely by e-mail and telephone. We are seeking suggestions that you think are important for the future of the method. So please don't hesitate to contact us by any means of communication shown on the front of this Newsletter.

One very popular suggestion raised in Cairns was that we should have a CDS Users Web Site that includes descriptions of the method and updates etc. We have followed up this suggestion and have secured a site which is being constructed by the School of Pathology at the University of New South Wales. The construction of the site is in progress.

2. A Comparison of the CDS and the NCCLS methods

This comparison of the CDS and NCCLS methods of susceptibility testing was prepared in response to requests from several members of the CDS Users Group. There appeared to be a lack of awareness of the differences between the two methods and in some circumstances, this has led to the adoption of a hybrid method, combining elements of the CDS and NCCLS methods. This practice has the potential to introduce serious errors into susceptibility testing and laboratories are advised that the first rule of Quality Assurance for any method is strict adherence to each procedural step. In addition, the results can only be reported according to the interpretation prescribed in the method otherwise they have no validity.

General Points

Both methods of susceptibility testing use high potency discs where the inhibitory zone sizes have been calibrated to the Minimum Inhibitory Concentration (MIC) for each antibiotic. In both methods, the different categories of susceptibility are related to the Cut-off MICs (Breakpoints for the NCCLS) which are derived using one or more parameters. The values may be self-selected as a result of a natural process (eg. a bimodal distribution of susceptibility within a species), by correlation with a clinical response, by extrapolation from the results obtained with similar antibiotics, on the basis of expected blood, tissue or urinary concentrations and finally is some cases, on an arbitrary basis.

The similarity of the two methods ends here with the major difference between the methods being a philosophical approach to susceptibility testing. With the CDS method, the emphasis is on defining "susceptibility" ie. in identifying susceptible isolates. The CDS method is less concerned with attempting to grade more finely the non-susceptible category into "resistant" and "intermediate". As far as the CDS method is concerned, the purpose is to indicate which antibiotics are likely to be effective against the organism under test. As a result, we attempt to enhance the specificity of the test (ie. increase the likelihood of reporting only truly susceptible strains as susceptible). In doing so, we are prepared to sacrifice the sensitivity of the test (in the specificity/sensitivity sense of the word) and as a results, some strains which may appear susceptible by the NCCLS method would not be defined as such by the CDS method. This is often reflected by the Cut-off MICs being different with the two methods. Although these differences are generally minor, the use of lower Cut-off MICs in the CDS method may lead to a vast improvement in the positive predictive value of the test.

Specific Differences

The Test Medium : The base test medium used with the CDS method is Sensitest Agar (except for testing *Haemophilus* spp.) whilst Mueller Hinton Agar is the base used with the NCCLS method. There is not a great deal of difference in the performance of the two media but when the CDS method was developed, Mueller Hinton was unsatisfactory for testing sulphonamides and later the combination of sulphonamide/trimethoprim.

The inoculum: With the CDS method, the inoculum is slightly heavier than that of NCCLS and was chosen because it was easier to prepare, more consistent in size and gave a much more uniform lawn of growth. As the CDS method evolved, this latter characteristic was advantageous in facilitating the measurement of zone sizes and assessing the state of the zone edge, a valuable tool for either defining or confirming susceptibility.

Disc Potency: The disc potencies for use with the CDS method were chosen so that with the vast majority of antibiotics and bacterial species, a uniform zone size separated susceptible from resistant categories. The convenience of a

uniform cut-off zone size which obviates the need for interpretive tables is the most obvious advantage. Moreover, technically, for the designer of the test, it endows the test with a much greater degree of in built precision. With some exceptions, the diffusion constant is similar with the common groups of antibiotics and a cut-off point at 6mm annular radius (18mm diameter) falls on the optimum part of the diffusion curve facilitating better discrimination between different MICs. An added advantage of the CDS method is that because it was developed independently, the choice of disc potencies was not subject to pressure from pharmaceutical firms. This has given the CDS method far greater superiority in testing several antibiotics, for example, the third generation cephalosporins.

Measurement of the Annular Radius: Although the measurement of the annular radius in preference to the diameter is no longer as important as it was when Multodiscs were used with the CDS method when the determination of a diameter was difficult, we have been reluctant to change. Many CDS Users report that having the edge of a disc as a fixed point improves the accuracy of measurement and enables them to choose the optimum site of the edge of the inhibitory zone for measurement if required.

Reporting the Result: The CDS method reports only "susceptible" and "resistant" (see above) for several reasons. First we don't believe that any disc test can further divide resistant zones into two with any degree of accuracy. Secondly we don't believe it matters in all but the rare case of a serious infection caused by what appears to be an organism resistant to all antibiotics where the preferred option is to use MIC determination methods.

Features Unique to the CDS method

Testing pneumococci: The CDS but not the NCCLS method is able to test pneumococci against penicillin directly and does not need to use an additional oxacillin disc. It recognises the need and is able to test and report different interpretations of susceptibility of isolates from CSF and sputum.

Testing streptococci: Unlike the NCCLS method, different *Streptococcus* species can be tested against penicillin. using the CDS method. The use of the lower potency penicillin disc not only improves the accuracy and extends the range of the test but enables the results to be used to indicate susceptibility to other β–lactams (surrogate testing).

Testing enterococci: Only the CDS method can directly detect resistance mediated by β–lactamase and recognise certain types of resistance to vancomycin.

Testing Enterobacteriaceae : The CDS method will readily detect and report the susceptibilities of those strain possessing an inducible β–lactamase not just hyperproducers of the enzyme which is the only type detected by the NCCLS method. In addition, as the result of using disc approximation tests with the CDS method and a cefotaxime 5μg disc as opposed to 30 μg as used by NCCLS, the method has no difficulty in identifying those strains which produce an Extended Spectrum β–lactamase (ESBL).

Surrogate Disc Testing : The most appropriate representative from a closely related family of antibiotics is tested and interpreting the results obtained from the representative is used to indicate the susceptibility of other members of the family. Surrogate testing increases the accuracy of the result and reduces the need for unnecessary discs.

Recognising specific mechanisms of resistance : The CDS method details a variety of observations which enable the user to recognise by disc testing, specific mechanisms of resistance. For example, enzyme mediated resistance, high rates of mutation and heterogeneous resistance any one of which may confer resistance will be recognised by the CDS User.

Advantages of Using the CDS method in Australasia

Relevance : Antibiotics used in Australasia are tested by the CDS method and calibrations are performed when new antibiotics enter the market. In addition, feedback to the CDS Reference Laboratory results in the enhancement of the method to cater for specific mechanisms of resistance that become apparent in the local environment.

Updating the CDS : The CDS test is regularly updated by way of Newsletters and the Workshops held at the ASM Annual Scientific Meeting.

"Help Desk" : The CDS Reference Laboratory maintains an advisory service for Users and responds to queries raised in person, by telephone, e-mail, fax and by post.

3. Calibration of moxifloxacin for the CDS method

Moxifloxacin, another new quinolone will be available shortly in Australia and 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., *Stenotrophomonas maltophilia*, *Branhamella catarrhalis*, *Haemophilus* spp, *Neisseria meningitidis*, *Campylobacter* spp. and *Pasteurella multocida*. (See Table 1a, 1b, 1c Calibrations 2000, p. 7-9).
Note that moxifloxacin MICs recorded with *Pseudomonas aeruginosa* including the reference strain NCTC 10662 are ≥ 1 mg/L whilst the MICs observed with other species of *Pseudomonas* may be as low as 0.008 mg/L. As a result, the reference strain *Ps. aeruginosa* NCTC 10662 IS NOT included for the Quality Control testing of the moxifloxacin 2.5 μg disc. Instead, *Escherichia coli* NCTC 10418 is used for Quality Control testing of moxifloxacin.

4. Campylobacter jejuni NCTC 11168 as a reference strain for Quality Control

In response to requests by a number of CDS Users, *Campylobacter jejuni* NCTC 11168 is now available for Quality Control. The acceptable range of the inhibitory zone sizes, when tested on Blood Sensitest agar, in micro-aerophilic conditions, at 42° C is shown in Table 3b. Reference Strains 2000, p. 12.

5. Reporting Strep. pneumoniae sent out by the RCPA Quality Assurance Program

According to the report issued by RCPA Quality Assurance Program on item 2000:1:2B, it appears that CDS Users need to be more aware of how to test and report correctly the susceptibility of *S. pneumoniae*. The CDS Manual 1999, section 2.3.1, page 9 states clearly how to test and report *S. pneumoniae* from CSF and other sites.

Note: If CDS is the chosen method (circled, pages 14 & 15) the testing and reporting need to conform with the CDS recommendations.

CSF: Isolates are tested using a benzylpenicillin 0.5 u disc and a cefotaxime or a ceftriaxone 0.5 µg disc (Table 1a). When the inhibitory zone is < 6 mm, report as Resistant (MIC ≥ 0.5 mg/L). If an E-test is performed, report eg. "MIC = … mg/L by E-test" (See example p. 13).

Sites other than CSF: As well as testing the lower potency CSF discs, isolates are tested with an ampicillin 5 µg disc and a higher potency cefotaxime or ceftriaxone 5 µg disc (Table 1a). If the annular radius of the inhibitory zone is < 6 mm with the CSF low potency discs (P 0.5 u, CTX/CRO 0.5) but ≥ 6 mm with the higher potency discs, report as "Susceptible" but add the following note: "Reduced susceptibility to benzylpenicillin (or cefotaxime/ceftriaxone) with the MIC between 0.5 and 2.0 mg/L". In such cases, oral administration may not be suitable and the physician may need to use a parental dose of benzylpenicillin, cefotaxime or ceftriaxone.

Laboratories performing an E-test SHOULD also report as above, "Susceptible" but add the following note: "Reduced susceptibility to benzylpenicillin (or cefotaxime/ceftriaxone), MIC = … mg/L by E-test" (See example p. 14).

6. Enterococci and the CDS method

Ampicillin

In recent years, the appearance of enterococci that are resistant to ampicillin due to the production of β–Lactamase has necessitated a modification of the CDS method and drawn attention to follow carefully the instructions when performing the test. If this is done using an ampicillin 5 µg disc and an inoculum of 10^7 cfu/ml, the CDS method is the only routinely used antibiotic disc diffusion test that is able to detect β–lactamase producing *Enterococcus faecalis*. Some strains of *E. faecalis* may express only low levels of β–lactamase under the conditions of the test, and as a result, the annular radius of the zone of inhibition for susceptible strains has been modified provisionally from ≥ 4 mm to ≥ 6 mm. When the annular radius of the inhibitory zone is between 4mm to 6 mm the isolate should be examined for β–lactamase activity using a nitrocefin-based test. β–Lactamase-positive isolates are reported as resistant. There are very rare strains of β–lactamase negative *E. faecalis* where the inhibitory zone sizes may be between 4 mm and 6 mm because of reduced susceptibility to ampicillin due to changes in penicillin-binding proteins. The interpretation of the test with these strains is still under review, but at this stage, they should be reported as susceptible and a note made indicating "Reduced susceptibility to ampicillin."

Vancomycin

The interpretation of vancomycin inhibitory zone has been modified from **2 mm** to **2 mm** [#] (refer to the Table of calibrations 1a) in order to detect low level resistance to vancomycin (Van B type) in *Enterococcus faecium*. **A hazy edge** ie. fine growth seen at the edge of the zone of inhibition, is typical of *E. faecium* with the Van B type of resistance. Note that the annular radius measured from the edge of the confluent growth may be > 2mm. A zone with a **sharp edge** but **smaller** than that of the reference strain *E. faecalis* indicates Van C type resistance ie. low natural resistance seen in *E. gallinarum*, *E. casseliflavus* and *E. flavescens.*. Rare strains of *E. faecalis* may have a zone slightly smaller than that of the reference strain around the 5 µg disc. The vancomycin MIC of such strains is 2 mg/L using a 10^4 cfu inoculum and 4 mg/L with 10^6 cfu inoculum.

Enterococci (Table of calibrations 1a, 2000) (Blood Sensitest, air 35°C)	Disc potency (µg)		MIC for susceptible strains (mg/L)
Ampicillin	5	6 mm [Φ]	2.0
Chloramphenicol	30	4 mm	8.0
Gentamicin	200	4 mm	512
Nitrofurantoin	200	4 mm	64
Teichoplanin	15	2mm	8.0
Vancomycin	5	2 mm [#]	4.0

[Φ] Perform a nitrocefin-based test to detect β–lactamase activity when the annular radius is < 6 mm. β–Lactamase-positive isolates are reported as "Resistant". For β–lactamase negative isolates with an annular radius between 4 mm and 6 mm, report as "Susceptible" but add a note: "Reduced susceptibility to ampicillin".

[#] Hazy edge indicates low level of resistance to vancomycin (Van B type) ie. Resistant. The annular radius measured from the edge of the confluent growth may be > 2 mm. Always COMPARE with the reference strain *E. faecalis* POW 1994.

Important points to remember when testing enterococci:

1. Use Blood Sensitest Agar at 35°C in air.
2. Use the correct CDS inoculum of 10^7 cfu/ml ie. cellular material must be visible at the tip of the straight wire. A correct inoculum yields a lawn of confluent growth after incubation.
3. Incubate 18 to 24 hours.
4. Always COMPARE with the reference strain *E. faecalis* POW 1994.
5. Observe the edge of the inhibitory zones around vancomycin and nitrofurantoin.
6. Reduced zone of inhibition around ampicillin ie. < 6 mm and a sharp edge indicate the presence of β–lactamase.
7. Confirmation of β–lactamase activity using a nitrocefin-based test.

7. Assessment of the Reproducibility of Testing enterococci

The testing of enterococci against ampicillin and vancomycin requires a degree of precision and care not usually demanded of other tests with the CDS method. The question that needs to be answered is, can all CDS Users reproduce this degree of precision when testing enterococci in practice? When we polled the CDS Users at Cairns, the vast majority volunteered to participate in a field trial to test these antibiotics using the CDS method. The study will consist of sending out a number of enterococcal isolates to each participant and requesting them to determine the susceptibility to ampicillin and vancomycin. We wish the trial to be regarded as a training exercise also and we hope that as many members of the CDS Users Group as possible will participate. A set of 8 strains of enterococci was sent to all CDS Representatives. Please contact CDS Representatives in your area if you would like to participate in the exercise. The results will be reported back to the CDS Users as soon as they are collated.

8. Testing the Reference Strains for Quality Control

CDS Users expressed the need of having a separate statement about the testing of reference strains for Quality Control. It was clearly outlined at the bottom of Tables 3a and 3b, p. 22-23 of the CDS Manual 1999, that the testing of 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 a week.

9. Detection of Extended Spectrum β–Lactamases (ESBLs) in Enterobacteriaceae producing Class I inducible cephalosporinases

Strains of *Enterobacter cloacae* and *Citrobacter freundii* producing an ESBL have been isolated from clinical specimens. These organisms may be completely resistant to cefotaxime or cephalexin due to the co-existence of a derepressed cephalosporinase and therefore synergy between cefotaxime 5 µg and an Augmentin^R 60 µg or a Timentin^R 85 µg disc cannot be demonstrated. As a result, it is necessary to use a cefepime 10 µg disc instead of a cefotaxime 5 µg for the detection of an ESBL if the organism expresses a derepressed Class I cephalosporinase.

10. Trimethoprim

A number of CDS Users asked about the presence of small colonies at the edge of the zone of inhibition (> 6 m) around a trimethoprim 5 µg disc sometimes observed with *Enterobacteriaceae* isolated from urine specimens. The colonies are not resistant mutants. Trimethoprim is a bacteriostatic antibiotic and a small percentage of cells exposed to the low level of trimethoprim at the edge of the inhibitory zone might have started to grow again. One can verify that these colonies are not mutants by retesting with a trimethoprim 5 µg disc. The culture will give the same zone size as that of the original test. On the contrary, resistant mutants are, large healthy colonies which when retested with a trimethoprim 5 µg disc give no zone of inhibition.

11. E-mail address

It has been suggested that CDS Newsletters be transmitted to CDS Users Group members by e-mail. We think it is an excellent idea. Please send e-mails to our addresses shown on page 1 of this Newsletter so that we can compile an "e-mailing list".

12. Calibration of antibiotics used in Veterinary Medicine

The calibration of antibiotics used in veterinary medicine has been on the agenda of the CDS Reference Laboratory since 1996. A Table of Additional Calibrations 2000 for veterinary medicine together with the acceptable range of the annular radii of the zones of inhibition for the reference strains used for Quality Assurance and a Table for surrogate testing of enrofloxacin and ceftiofur is included.

Additional Calibrations 2000 for Veterinary Medicine. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and the incubation conditions used.

Antibiotic strains	Disc potency (µg)		MIC for susceptible (mg/L)
Enterobacteriaceae **(Sensitest, air, 35° C)**			
Apramicin	15	4 mm *	≤ 8.0
Ceftiofur	5		≤ 1.0
Neomycin	30	4 mm *	≤ 4.0
Spectinomycin	25	4 mm *	≤ 32.0
Streptomycin	25	4 mm *	≤ 16.0
Staphylococci **(Sensitest, air, 35° C)**			
Novobiocin	5	4 mm *	≤ 1.0

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

Quality Control with Reference Strains 2000 for Veterinary Medicine. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zone of inhibition obtained with the reference strains

Antibiotic & disc content (µg)		Acceptable range (mm)
Escherichia coli **NCTC 10418** **(Sensitest, air, 35° C)**		
Apramicin	15	5.3 – 7.9
Ceftiofur	5	7.7 – 10.1
Neomycin	30	6.0 – 8.6
Spectinomycin	25	4.1 – 6.9
Streptomycin	25	6.2 – 7.8
Staphylococcus aureus NCTC 6571 **(Sensitest, air, 35° C)**		
Novobiocin	5	6.1 – 12.5

Surrogate Disc Testing for Veterinary Medicine. Antibiotic that can be reported based on the susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc used (µg))
Enterobacteriaceae		
Enrofloxacin	Ciprofloxacin	2.5
Staphylococci		
Ceftiofur	Methicillin	5
Enrofloxacin	Ciprofloxacin	2.5
Streptococci		
Ceftiofur	Benzylpenicillin	0.5 u

13. Illustrations

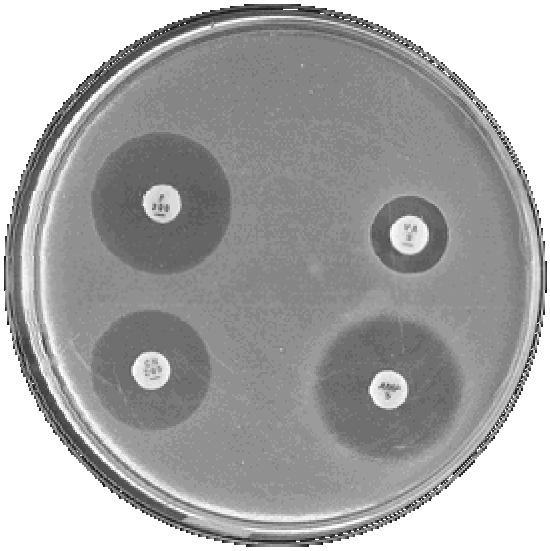

Fig. 1 *E. faecalis* POW 1994

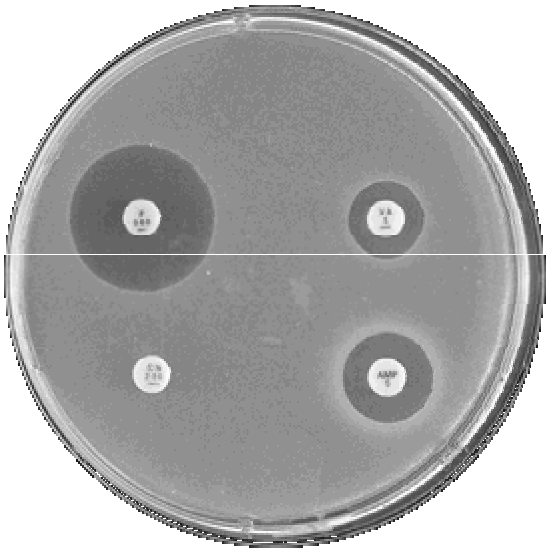

Fig. 2 *E. faecalis* resistant to ampicillin (ß- lactamase positive).

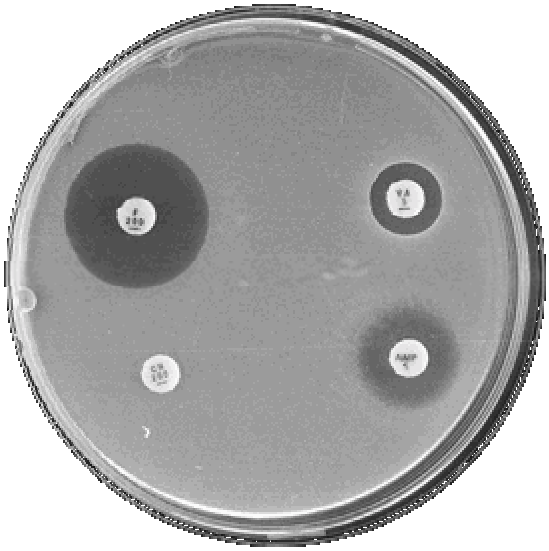

Fig. 3 *E. faecalis* susceptible with a reduced susceptibility to ampicillin (ß-lactamase negative).

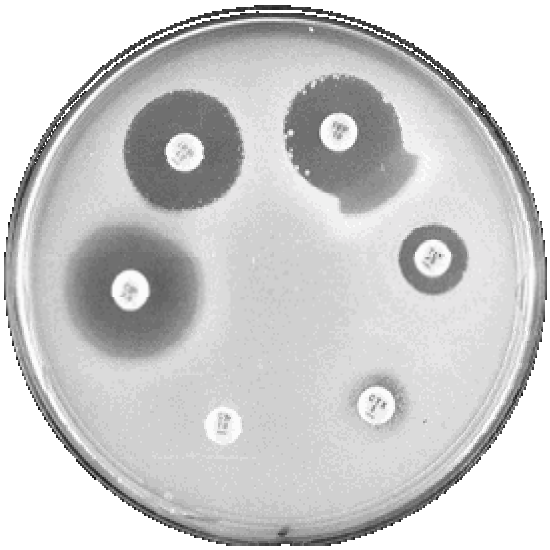

Fig. 4 Detection of ESBL in *E. cloacae* using a cefepime 10 µg and a Timentin^{R} 85 µg disc.

Table1a Calibrations 2000 Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and the incubation conditions used**.**

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)		MIC for susceptible strains (mg/L)
Enterococci			
(Blood Sensitest, air 35° C)			
Ampicillin	5 Φ	6 mm Φ	≤ 2.0
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	2 mm #	≤ 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	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	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

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

© The annular radius of the zone of inhibition for susceptible strains is ≥ 2 mm.

• ONLY for testing isolates of *Staph. saprophyticus***.**

& ONLY for testing *Strep. pneumoniae* and group B streptococci**.**

+ For testing urinary isolates only.

$ NOT for testing *Staph. saprophyticus*.

@ *Strep. pneumoniae* & *Strep. anginosus* (*milleri*) are incubated in 5 % CO_2.

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 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg then test ampicillin 5 µg, cefotaxime 5 µg and ceftriaxone 5 µg.

Φ Perform a nitrocefin-based test to detect β–lactamase activity on strains with an annular radius < 6 mm. β–Lactamase-positive isolates are reported as resistant. β–Lactamase-negative isolates are reported as "isolate with reduced susceptibility to ampicillin.

Hazy edge indicates low level of resistance to vancomycin (Van B type) ie Resistant. The annular radius measured to the edge of confluent growth may be > 2 mm. Always COMPARE with the reference strain *E. faecalis* POW 1994.

Table 1b. Calibrations 2000. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and the incubation conditions used.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (μg)		MIC for susceptible strains (mg/L)
***Enterobacteriaceae, Vibrionaceae* & *Acinetobacter* spp. (Sensitest, air, 35º 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	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
***Pseudomonas* spp. & *Burkholderia* spp. (Sensitest, air, 35º 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	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
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm *	≤ 4.0
Trimethoprim	5		≤ 2.0

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

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

[#] *Yersinia enterocolitica* is incubated in air at 30^0 C.

[+] For testing urinary isolates only.

Table 1c. Calibrations 2000. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and the incubation conditions used.

MISCELLANEOUS GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (μg)		MIC for susceptible strains (mg/L)
Moraxella catarrhalis **(Blood Sensitest, 5 % CO_2, 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	2.5		≤ 1.0
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
Moxifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
***Haemophilus influenzae*/*Haemophilus* spp.** **(HTM [@] agar, 5 % CO_2, 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	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Neisseria meningitidis **(Blood Sensitest, 5 % CO_2, 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
Moxifloxacin	2.5		≤ 1.0
Rifampicin	1		≤ 0.5
Pasteurella multocida **(Blood Sensitest, air, 35° C)**			
Ampicillin	5		≤ 1.0
Ciprofloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Moxifloxacin	2.5		≤ 1.0
Stenotrophomonas maltophilia **(Sensitest, air, 35°C)**			
Sulphafurazole	300		≤ 64.0

* The annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm.
[@] Haemophilus Test Medium containing 15 mg/L of freshly prepared haematin and NAD.

Table 2. Surrogate Disc Testing 2000. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Moraxella catarrhalis		
Azithromycin	Erythromycin	5
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefuroxime/cefaclor	30
Cephalosporins	Cefuroxime/cefaclor	30
Clarithromycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
***Campylobacter* spp.**		
Tetracyclines	Tetracycline	30
***Enterobacteriaceae/Vibrionaceae/Acinetobacter* 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
Benzylpenicillin	Ampicillin	5
***Haemophilus influenzae/Haemophilus* 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
***Listeria* spp.**		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
Neisseria meningitidis		
Ampicillin	Benzylpenicillin	0.5 u
Amoxycillin	Benzylpenicillin	0.5 u
Cefotaxime	Ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
Pasteurella multocida		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
Tetracyclines	Tetracycline	30
***Pseudomonas* spp. & *Burkholderia* spp.**		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole [+]	Trimethoprim	5
Co-trimoxazole [+]	Sulphafurazole	300

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Staphylococci (except S. saprophyticus 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 [$]	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5
Roxithromycin	Erythromycin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
***Staphylococcus saprophyticus* from urine**		
Amoxycillin	Ampicillin	5
Augmentin	Cephalexin	100
Benzylpenicillin	Ampicillin	5
Cephalosporins [&]	Cephalexin	100
Cloxacillin	Cephalexin	100
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Dicloxacillin	Cephalexin	100
Flucloxacillin	Cephalexin	100
Norfloxacin [$]	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
Stenotrophomonas maltophilia		
Co-trimoxazole	Sulphafurazole	300

[&] Ceftazidime is considered inactive against Gram-positive organisms.

orting of norfloxacin is for urine isolates only.

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

Γ for testing *Strep. pneumoniae* from CSF. Test if isolate is resistant to penicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg.

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

[@] Not for *Acinetobacter* spp.

Table 3a. Reference Strains 2000. 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.

Antibiotic & disc content (µg)		Acceptable range* (mm)
***Staphylococcus aureus* NCTC 6571**		
(Sensitest, air, 35⁰ 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	5.9 - 8.7
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	7.3 - 10.1
Vancomycin	5	2.8 - 4.9
***Streptococcus pneumoniae* ARL 10582**		
(Blood Sensitest, 5 % CO_2, 35⁰ C)		
Benzylpenicillin	0.5 u	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
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

Antibiotic & disc content (µg)		Acceptable range* (mm)
***Escherichia coli* NCTC 10418 #**		
(Sensitest, air 35⁰ 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	8.7 - 11.9
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
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	8.7 - 11.1

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

Table 3b. Reference Strains 2000. 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.

Antibiotic & disc content (µg)	Acceptable range* (mm)	
***Enterococcus faecalis* POW 1994**		
(Blood Sensitest, air, 35⁰ 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
***Pseudomonas aeruginosa* NCTC 10662 #**		
(Sensitest, air, 35⁰ 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
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
Meropenem	5	9.7 - 14.8
Netilmicin	30	6.4 - 10.4
Piperacillin	50	8.1 - 12.9
Polymyxin	300 u	5.2 - 7.2
Ticarcillin	75	7.3 - 12.1
Tobramycin	10	7.0 - 10.6

Antibiotic & disc content (µg)		Acceptable range* (mm)
***Escherichia coli* NCTC 11560**		
(Sensitest, air, 35⁰ C)		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
Tazocin	55	7.4 - 9.2
***Haemophilus influenzae* NCTC 4560**		
(HTM@ agar, 5 % CO_2, 35⁰ 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	9.8 - 12.6
Ciprofloxacin	2.5	11.1 - 15.9
Co-trimoxazole	25	9.0 - 12.5
Moxifloxacin	2.5	10.6 - 15.2
Tetracycline	30	9.9 - 13.3
***Haemophilus influenzae* NCTC 11315**		
(HTM@ agar, 5 % CO_2, 35⁰ C)		
Augmentin	15	7.7 - 10.1
***Campylobacter jejuni* NCTC 11168**		
(Blood Sensitest, microaerophilic, 42⁰ 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
Moxifloxacin	2.5	9.0 - 13.0

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

17. Examples of RCPA Quality Assurance of *s.pneumoniae*

S20

RCPA QUALITY ASSURANCE PROGRAMS PTY LIMITED
A.C.N 003 520 172

ANTIBIOTIC SUSCEPTIBILITIES LAB. NO. 21375

ITEM : :
ORGANISM S. pneumoniae (CSF)

Method: CDS | | NCCLS | | Microscan | | Vitek | | Other(state)

Antibiotic	Zone Diameter	Annular Radius	M.I.C.	Test Result	Result reported
Ampicillin/ Amoxycillin		✓			
Augmentin		✓			
Cefotaxime/ Ceftriaxone 0.5 µg		1		R	R ①
Ceftazidime		✓			
Cephalexin		✓			
Cephalothin		✓			
Ciprofloxacin		✓			
Erythromycin		✓			
Fusidic Acid		✓			
Gentamicin		✓			
Imipenem		✓			
Methicillin		✓			
Norfloxacin		✓			
Nitrofurantoin		✓			
Oxacillin		✓			
Penicillin 0.5 Unit		1		R	R ①
Piperacillin					
Rifampicin					
Tetracycline					
Timentin					
Trimethoprim					
Vancomycin 5 µg		6		S	S
Other:					

ESBL producer? YES NO

① MIC ≥ 0.5 mg/L , MIC by E-Test .... mg/L

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S20

RCPA QUALITY ASSURANCE PROGRAMS PTY LIMITED
A.C.N 003 520 972

ANTIBIOTIC SUSCEPTIBILITIES

LAB. NO. 21375

ITEM 00:1 : 2B

ORGANISM S. pneumoniae (sputum)

Method					
CDS		NCCLS		Microscan	
Vitek		Other(state)			

Antibiotic	Zone Diameter	Annular Radius	M.I.C.	Test Result	Result reported
Ampicillin/ Amoxycillin 5 µg		8		S (1)	S (1)
Augmentin		—			
Cefotaxime/ Ceftriaxone 0.5 µg 5 µg		1 8		S (1)	S (1)
Ceftazidime		—			
Cephalexin		—		R	Not reported
Cephalothin		—		R	Not reported
Ciprofloxacin		—			
Erythromycin		12		S	S
Fusidic Acid		—			
Gentamicin		—			
Imipenem		—			
Methicillin		—			
Norfloxacin		—			
Nitrofurantoin		—			
Oxacillin		—			
Penicillin 0.5 Unit		1		S (1)	S (1)
Piperacillin		—			
Rifampicin		—			
Tetracycline		12		S	Not reported
Timentin		—			
Trimethoprim		—			
Vancomycin 5		6		S	Not reported
Other:					

ESBL producer?	YES	NO

(1) SUSCEPTIBLE with "Reduced Susceptibility" (MIC = 0.5 – 2 mg/L

18. CDS Representatives (2000)

As the liaison between the CDS Reference Laboratory and members of the CDS Users Group, the role of CDS Representatives is increasingly important. With restricted resources, the CDS Reference Laboratory cannot function adequately without the assistance of CDS Representatives. Besides distributing references strains for Quality Control, some CDS Representatives also organise "mini" workshops to exchange and share information with their colleagues. We would like to take this opportunity to thank all CDS Representatives. There have been some changes in facsimile and telephone numbers since CDS Representatives 1999 list. Also two CDS Representatives, Christopher Pearce and Ted Anderson are no longer with us. If you would like to be a CDS Representative, please let us know.

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