Newsletter 12

REPORT
ON
CDS USERS GROUP WORKSHOP

ANNUAL SCIENTIFIC MEETING

ASM & NZMS

AUCKLAND – NEW ZEALAND 2003

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Introduction

The CDS Workshop was held on Wednesday October 1 2003 at the ASM Scientific Meeting in Auckland. It was attended by about 60 participants. We presented information on a variety of topics related to the CDS method of susceptibility testing and there was full audience participation on the various aspects of developments in the method. The feedback from members was of considerable value to us in planning the way ahead for the CDS.
The meeting concluded with a get together sponsored by Oxoid.
This report is a summary of the proceedings of the workshop. Also, in view of reports of *Salmonella* spp. with decreased susceptibility to ciprofloxacin, we have added a section on the testing of these isolates. (page 5).

The CDS Web Site

The meeting was reminded of the URL of the web site at http://www.med.unsw.edu.au/pathology-cds/ and also those who were not members of the CDS Users group were invited to join by going to http://www.med.unsw.edu.au/pathology-cds/how_to_join_the_cds_users_group.htm.
A demonstration was given of Version Control of the CDS Manual. Changes are best seen in the pdf view that has the version number and the date issued contained on the index (first) page of the manual. The locations of changes are indicated by a "post-it" note in the index and by blue text in the relevant section of the manual.

New Calibrations for the CDS

Ertapenem

Ertapenem is a recently introduced carbapenem and a slide showing the comparative activities of the three carbapenem is shown here (Fig. 1). A satisfactory calibration was achieved using a 10mg disc. The results will be included in the next version of the manual.

Helicobacter pylori

Final calibrations of the calibration of antibiotics used in the eradication of *Helicobacter pylori* are shown in the following slide (Fig. 2). These results also will be included in the next version of the manual.

Replacement for the Methicillin Disc

The meeting was informed that the supply of methicillin discs will cease within 6 months of the date of the meeting. A presentation was given of various alternatives to the methicillin disc and the meeting agreed that at present there is no single disc that could be used to accurately test methicillin susceptibility in both coagulase negative and coagulase positive staphylococci. The oxacillin 1µg disc was demonstrated to be the most satisfactory disc for testing coagulase negative staphylococci and was shown to be superior to the methicillin disc in demonstrating resistance in these species. However oxacillin was not a satisfactory disc in testing Staph. aureus (see below BORSA) but a cefpodoxime 10µg disc was a satisfactory alternative to the methicillin disc (see Fig. 3).
Postscript – subsequent to the meeting we have been informed that cefpodoxime probably will be withdrawn from sale and the prospects of obtaining discs containing this agent in the future are poor. Work is proceeding in the CDS Laboratory on a suitable alternative disc and the results will be reported as soon as they are available.

MRSA WITH LOW RESISTANCE TO METHICILLIN and BORSA

1. *Methicillin resistant Staph. aureus (MRSA) with low resistance to methicillin.*

Occasionally, we come across strains of non-multiple resistant MRSA (NMRMRSA) with a very low level of resistance to methicillin. The annular radius of the methicillin zone is around 5 mm. Some CDS Users also isolated MRSA with a heterogeneous resistance. These strains have a methicillin zone around 7 mm with only a few resistant colonies inside the zone (plate 1c, p58 CDS Manual 2002). These phenotypes were frequent 25 years ago when MRSA first emerged in hospitals. It was for this reason we used to use Mannitol Salt Agar (MSA) to test suspected strains of MRSA. The high level of salt in MSA keeps the cells affected by methicillin intact, giving PBP 2' the time to be induced. Tested on MSA at 35°C, MRSA strains with low or heterogeneous resistance to methicillin have no zone around methicillin disc or a zone clearly < 6mm in annular radius (Figs. 4 & 6).

2. *Borderline oxacillin - resistant Staphylococcus aureus (BORSA).*

The oxacillin MIC of some strains of *Staph. aureus* with a high β-lactamase activity lands just above the susceptible breakpoint of oxacillin recommended by the NCCLS which is 2mg/L, therefore the name of **Borderline oxacillin-resistant *Staph. aureus*** or **BORSA.** BORSA are *mecA* gene negative. Methicillin being more resistant to the hydrolysis by penicillinase than oxacillin, BORSA are susceptible to methicillin. In the CDS test, BORSA have a zone 7-8 mm in annular radius with methicillin 5 µg disc (Fig.5). Rare strains may have small colonies at the edge of the zone. If unsure, repeat the test on Mannitol Salt Agar (MSA). The methicillin zone on MSA is > 6mm in annular radius (Fig.7). Contrast these results with those seen with a strain of NMRMRSA with low-level resistance to methicillin (Figs. 4 & 6).
Note that infections with these strains respond to treatment with the anti-staphylococcal drugs flucloxacillin, dicloxacillin and cephalothin.

Summary:
If unsure, retest on MSA at 35°C.
MRSA: no zone or a zone clearly < 6mm
BORSA: zone > 6mm

UPDATE ON THE β-LACTAMASES OF THE GRAM-NEGATIVES

1. Carbapenemases and ESBLs in *Enterobacteriaceae*.

Carbapenemases are the zinc-dependent metallo-enzymes that hydrolyse the penicillins and the carbapenems, ie. imipenem, meropenem and ertapenem, a new carbapenem.

These zinc-dependent metallo-enzymes have been described in *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and members of the *Enterobacteriaceae* isolated in several countries in Asia, Japan, Korea, China, Malaysia.

Carbapenemase activity is inhibited by chelating agents such as EDTA. This property is used for the detection of carbapenemases just like the inhibition of extended spectrum beta-lactamases by clavulanic acid is used for the detection of ESBL.
Fig.8 shows a strain of *Klebsiella pneumoniae* isolated from the urine of an in-patient of a hospital in Melbourne. The strain was saved and kindly sent to us by a CDS User. The resistance to imipenem (IPM) alone and the large zone observed with an imipenem disc loaded with 750 µg EDTA suggests the presence of a metallo-β-lactamase, a carbapenemase.
The synergy observed between aztreonam and Augmentin^{R} reveals the presence of an additional β-lactamase, an ESBL that hydrolyses aztreonam.

This strain is also resistant to cefotetan (CTT). There is a slight synergy between EDTA diffused from IPM+EDTA and the CTT disc suggesting that the resistance to CTT is due to the metallo-enzyme carbapenemase.
The isolate was sent to a laboratory in France specialising in carbapenemases. The molecular investigation showed the presence of a 160-kb plasmid that carried bla_{SHV-12} gene, a gene coding for an ESBL quite widespread in Australia and a 150-kb plasmid that carried bla_{IMP-4} and bla_{TEM-1} genes.
The agar dilution MIC of imipenem of the strain is 4 mg/L, ie. right on the susceptible breakpoint. When a high inoculum of 10^6 is used, the imipenem MIC is 64 mg/L. So using the agar or broth dilution method, one would not be able to recognise the isolate as resistant to imipenem. The relatively high inoculum of 10^7 cfu/ml used in the CDS test helps with the recognition of carbapenemase. IMP zone of inhibition is reduced to < 6 mm with numerous resistant colonies at the edge of the zone. The same CDS User also sent us an *E. coli* which also had a 150-kb plasmid, carrying bla_{IMP-4} and bla_{TEM-1} genes.
As far we know, only one other isolate of carbepenemase producing *Klebsiella pneumoniae* has been recently isolated in Australia.
We need to be aware and keep an eye on isolates of the *Enterobacteriaceae* that show a reduced imipenem or meropenem zone. You can send them to us or you can perform the detection of carbapenemase yourself by adding 750 µg EDTA to an IPM disc.
To prepare EDTA solution containing 750µg per 25 µl drop, prepare a 1/6 dilution in water of a disodium 0.5 M solution (Sigma). One drop of 25 µl contains approximately 750 µg EDTA.

2. The Chromosomal β-lactamases of *Aeromonas* .

Aeromonas species are known to produce an inducible cephalosporinase of Bush functional group 1 similar to that produced by *Enterobacter cloacae* and a carbapenemase.
For a long time, we have suspected that *Aeromonas caviae* do not produce carbapenemase. The minimum inhibitory concentration (MIC) of imipenem performed on *A. caviae* strains using a high inoculum of 10^6 cfu was 0.25 to 0.5 mg/L. On the other hand, with *Aeromonas hydrophila* and *Aeromonas sobria* strains, the MIC of imipenem using a high inoculum of 10^6 cfu were 32 to 64 mg/L although the conventional MIC of some strains of *A. hydrophila* was under the susceptible breakpoint. These strains also had an imipenem zone > 6mm in annular radius with only a few resistant colonies.
For these reasons, in the past, we recommended that all *Aeromonas* be reported as resistant to carbapenems because there is no simple test to recognise the presence of carbapenemase.

In a recent study, Walsh and colleagues (Walsh et al. 1997 JAC vol. 40, 171-78) found that all *A. hydrophila* produce an inducible cephalosporinase and a carbapenemase (Fig. 9), most *A. veronii* biovar *sobria* produce only the carbapenemase (Fig. 10) and *A. caviae* produce an inducible cephalosporinase but lack the carbapenemase (Fig. 11). They also found that some *Aeromonas* strains produce β-lactamase similar to TEM-1 in *E. coli.*

Given the findings on the β-lactamases of *Aeromonas* sp., see the updated Table 4 on Testing and Reporting of β-lactam antibiotics.

3. Salmonella spp. with decreased susceptibility to ciprofloxacin.

Treatment failure has been reported with invasive *Salmonella* infections where the MIC of ciprofloxacin was ≥ 0.125 mg/L (Tariq Butt *et al*. 2003. Ciprofloxacin treatment failure in typhoid fever case, Pakistan. *Emerg. Inf. Dis.* 9:). These authors recommended that *Salmonella* spp. should be tested against both nalidixic acid 30 µg (NA 30) and ciprofloxacin 2.5 µg (CIP 2.5). A single point mutation in the quinolone resistance-determining region (QRDR) of the topoisomerase gene *gyrA* in Salmonella spp. confers resistance to nalidixic acid with an associated decrease in the susceptibility to ciprofloxacin (Antti Hakanen et al 1999. Detection of Decreased Fluoroquinolone Susceptibility in Salmonellas and Validation of Nalidixic Acid Screening Test. 2003. *J. Clin. Microbiol.* **37**: 3572-3577).

Although we have had limited experience with this phenomenon and to date have tested only two such strains we suggest CDS users, at this stage, should follow the recommendation and test all strains of Salmonella causing systemic disease to both nalidixic acid and ciprofloxacin. We also would be appreciate it if you find any strains with this type of resistance would you please send them to us. The susceptibility of the isolate to ciprofloxacin is reported as follows:

Annular radius of the zone of inhibition ≥ 6 mm around nalidixic acid 30 µg and ≥ 6 mm around ciprofloxacin 2.5 µg = **SUSCEPTIBLE** to ciprofloxacin. The MIC of ciprofloxacin is < 0.125 mg/L.

Annular radius of the zone of inhibition < 6 mm (usually 0 mm) around nalidixic acid 30 µg and ≥ 6 mm around ciprofloxacin 2.5 µg = **REDUCED SUSCEPTIBLITY** to ciprofloxacin. The MIC of ciprofloxacin is ≥ 0.125 mg/L.

If the isolate is invasive, a report should be issued "There is reduced susceptibility to ciprofloxacin with the MIC between 0.125 mg/L and 1 mg/L. Treatment failure with ciprofloxacin has been reported with these strains." The MIC of ciprofloxacin should be determined and the isolate sent to a Reference Centre.

3. Annular radius of the zone of inhibition < 6 mm (usually 0 mm) around nalidixic acid 30 µg and < 6 mm around ciprofloxacin 2.5 µg = **RESISTANT** to ciprofloxacin. The MIC of ciprofloxacin is > 1 mg/L. The MIC of ciprofloxacin should be determined and the isolate sent to a Reference Centre.