

## FIELD TRIAL OF SUSCEPTIBILITY TESTING OF ENTEROCOCCI TO AMPICILLIN AND VANCOMYCIN BY THE CDS METHOD

### *Introduction*

With the emergence of  $\beta$ -lactamase producing *Enterococcus faecalis* and the development of low level resistance of enterococci to vancomycin, susceptibility testing to ampicillin and vancomycin has become increasingly difficult in practice. The CDS method of disc testing performs well in this regard in the Reference Laboratory and yields reproducible results. However, to achieve these results the test demands a degree of care and precision considerably higher than that generally required with other species. Interpretation of the results is also complex and often involves more than a simple measurement of zone sizes.

This report contains the result of a field trial carried out by members of the CDS Users Group to ascertain if the CDS method would yield reproducible results when enterococci were tested against ampicillin and vancomycin by a broad spectrum of diagnostic laboratories.

### *Method*

Eighty three members of the CDS Users Group responded to a call for volunteers and participated in the trial. There were 55 specialist microbiology laboratories, 21 general pathology laboratories and 7 non-specified laboratories in the group. Each participant was sent 8 isolates of enterococci that were to be tested according to the method of the CDS test as described in Newsletter No. 10. Each was requested to report on the size of the inhibitory zone with ampicillin and vancomycin, the nature of the zone edge, the results of nitrocefin testing (if performed) and to give an interpretation of the results of the test.

### *Results*

*Ampicillin:* Overall there were 38 errors in the 664 tests (6%). Thirty five of the errors appeared to be technical, ie., the participant reported zone sizes outside either the susceptible or resistant reference ranges; three errors were in interpretation where the zone size was within the correct reference range but was incorrectly interpreted and two errors were obviously clerical. The details of ampicillin testing of each of the eight strains are shown in Table 1.

*Vancomycin:* Overall there were 63 errors in the 664 tests (10%). However, with the seven strains of the pathogenic species, *E. faecalis* and *E. faecium*, there were only five errors in 664 tests (<1%). Of the four technical errors, two were due to the participant's failure to notice the hazy edge of the zone of inhibition of *E. faecium* with low vancomycin resistance (strain No 1). The cause of the other two technical errors was unclear (hazy zone reported with strain 4 and 6). There was also one clerical error recorded with strain 7.

Participants had considerable difficulty recognising the *VanC* type resistance (natural, low level resistance) in strain 3 which was an *E. gallinarum*. Only 22 of the 83

participants recognise this type of resistance. The details with each strain are shown in Table 2.

**Table 1: Results of CDS Testing of Enterococci to Ampicillin.**

Strain No/ Identity Observations	Reference Lab. Report	Participants Results
1. <i>E. faecium</i> Annular radius Zone edge Nitrocefin Interpretation	0 N/A Negative Resistant	0 (83/83) N/A (79/83) ND (69/83), Neg (14/83) Resistant (83/83)
2. <i>E. faecalis</i> Annular radius Zone edge Nitrocefin Interpretation	6.5-8 Hazy Negative Susceptible	5 – 9.5 mm (79/83) Hazy (79/83) ND (58/83), Neg (23/83) Susceptible (83/83)
3. <i>E. gallinarum</i> Annular radius Zone edge Nitrocefin Interpretation	6-7 Hazy Negative Susceptible	4– 8 mm (79/83) Hazy (79/83) ND (58/83), Neg (23/83) S (67/83), RS (15/83), R (1/83)
4. <i>E. faecalis</i> Annular radius Zone edge Nitrocefin Interpretation	7.5-8.5 Hazy Negative Susceptible	6-8 (78/83), 5-5.5 (5/83) Hazy (79/83) ND (58/83), Neg (25/83) S (78/83), RS (5/83)
5. <i>E. faecalis</i> Annular radius Zone edge Nitrocefin Interpretation	3.5-5 Sharp Positive Resistant	4-5 (83/83) Sharp (79/83) Pos (75/83), Neg (1/83), ND (7/83) R (82/83), S (1/83)
6. <i>E. faecium</i> Annular radius Zone edge Nitrocefin Interpretation	0 N/A Negative Resistant	0 (83/83) N/A (78/83) ND (69/83), Neg (14/83) Resistant (83/83)
7. <i>E. faecium</i> Annular radius Zone edge Nitrocefin Interpretation	0 N/A Negative Resistant	0 (82/83), 3 (1/83) N/A (78/83), Sharp (1/83) ND (66/83), Neg (16/83), Pos (1/83) Resistant (83/83)
8. <i>E. faecalis</i> Annular radius Zone edge Nitrocefin Interpretation	4-5.5 Hazy Negative Susceptible	2-4 (6/83), 4-5.5 (68/83), 6-8 (9/83) Hazy (79/83) ND (8/83), Neg (75/83) R (7/83), RS (67/83), S (9/83)

N/A = not applicable; ND = not detected; R = resistant; RS = reduced susceptibility  
S = susceptible

**Note:** 4/83 participants did not record the appearance of the edge of the zone, only zone sizes, S or R.

**Table 2: Results of CDS Testing of Enterococci to Vancomycin**

Strain No/ Identity	Reference Lab. Report	Participants Results
Characteristics		
1. E. faecium Annular radius Zone edge Interpretation	0-2 (light growth) Hazy Resistant	0-4 Hazy (79/83) R (81/83), S (2/83)
2. E. faecalis Annular radius Zone edge Interpretation	0-2 (light growth) Hazy Resistant	0-2 Hazy (79/83) R (83/83)
3. E. gallinarum Annular radius Zone edge Interpretation	1.5 Sharp VanC type	2-3 (77/83), 1-1.5 (6/83) Sharp (79/83), Hazy (1/83) S (58/83), E. gal. or Van C (20/83) R (2/83), No comments (3/83)
4. E. faecalis Annular radius Zone edge Interpretation	2.5 Sharp Susceptible	2-4 (79/83) Sharp (78/83), Hazy (1/83) S (82/83), R (1/83)
5. E. faecalis Annular radius Zone edge Interpretation	2.5 Sharp Susceptible	2-4 Sharp (79/83) S (83/83)
6. E. faecium Annular radius Zone edge Interpretation	2.5 Sharp Susceptible	4-7 Sharp (78/83), Hazy (1/83) S (82/83), R (1/83)
7. E. faecium Annular radius Zone edge Interpretation	0-2 (light growth) Hazy Resistant	0 (82/83), 2 (1/83) N/A (82/83), Sharp (1/83) R (82/83), S (1/83)
8. E. faecalis Annular radius Zone edge Interpretation	2.5 Sharp Susceptible	2-4 Sharp (79/83) S (83/83)

R = resistant; S = susceptible

**Note:** 4/83 participants did not record the appearance of the edge of the zone, only zone sizes, S or R.

## ***Discussion***

The results with ampicillin were in many respects encouraging. Excluding the clerical error, which appeared to be due to a transposition of the results with strains 5 and 8 in one laboratory, all participants detected resistance in the pathogenic species of *E. faecalis* and *E. faecium* and further defined susceptibility of those strains which were clearly susceptible when tested by the Reference Laboratory.

Although the MIC of ampicillin in the test strain was only one twofold dilution above the cut-off MIC (2 mg/L), the majority of participants (81%) were able to detect this reduction in susceptibility. Nevertheless, the survey did draw attention to the fact that 19% of participants recorded measurements outside what is, admittedly, a very tight range. It indicated that, at least with ampicillin and enterococci, there is considerable room for improvement in reducing the distribution of zone sizes recorded by the users of the CDS. This will be the subject of a future tutorial in "What's New".

In regard to the difficulty that some laboratories experienced with detecting the reduced susceptibility of enterococci to ampicillin, this problem may be solved, at least temporarily, by our decision to respond to pressure and accept the higher cut-off MIC of 4 mg/L which is used by other methods. As a result strains with a zone size of 4mm annular radius or above will be interpreted as susceptible.

Vancomycin testing also yielded satisfactory results with the pathogenic species of enterococci. Errors in technique and interpretation were at a rate of <1% with strains of *E. faecium* and *E. faecalis*. However, the results in the trial indicated that, in practice, users of the CDS method are unable to clearly identify *E. gallinarum*, an Enterococcus with the natural *VanC* type resistance. This deficiency is of little consequence as *E. gallinarum* is seldom associated with infection and is commonly found in the screening of stool samples for vancomycin-resistant enterococci. This organism is susceptible to ampicillin and vancomycin is therefore not used clinically for this organism. A positive aspect of the results is that CDS Users did not falsely recognise this organism as a VRE, this term being reserved for Vancomycin-Resistant *E. faecalis* and *E. faecium* in the screening of VRE.

We would like to thank all those who participated in the study and, in particular, the CDS State Representatives who subcultured and distributed the test strains for us.