

P0826

Paper Poster Session

Phenotypic susceptibility testing of Gram-positive organisms

**EUCAST MIC broth microdilution of *Streptococcus* spp. and *Haemophilus influenzae*. Evaluation of broth with and without beta-NAD and with different concentrations of lysed horse blood**

Erika Matuschek\*<sup>1</sup>, Jenny Åhman<sup>1</sup>, Cecilia Alexandersson<sup>1</sup>, Gunnar Kahlmeter<sup>2</sup>

<sup>1</sup>*Eucast Development Laboratory, Växjö, Sweden*

<sup>2</sup>*Central Hospital, Clinical Microbiology, Växjö, Sweden*

**Background:** For broth microdilution (BMD) of fastidious organisms, EUCAST recommends Mueller-Hinton (MH) broth with 5% lysed horse blood (LHB) and 20 mg/L  $\beta$ -NAD (MH-F). The ISO standard for BMD, 20776-1, recommends MH broth with 2.5-5% LHB for streptococci, but there is no recommendation for *H. influenzae*. The aim of this study was to investigate the effect of i) adding  $\beta$ -NAD to the broth (streptococci) and ii) varying the LHB concentration (streptococci and *H. influenzae*).

**Material/methods:** Three different broths, MH-F5.0 (5% LHB and 20 mg/L  $\beta$ -NAD), MH-F2.5 (2.5% LHB and 20 mg/L  $\beta$ -NAD) and MH-LHB (2.5% LHB only) were prepared according to the manufacturer's instructions. The horse blood was lysed by repeated freezing (-20°C) and thawing (4 repetitions) and clarified by centrifugation. The three broths were produced on the same day from the same lot of broth powder, horse blood and  $\beta$ -NAD. BMD was performed according to the ISO standard 20776-1 with a final inoculum of  $5 \times 10^5$  CFU/mL on custom Sensititre panels (TREK/Thermo Fisher Scientific). Sealed panels were incubated in ambient air at 35°C for 16-20 h. MIC values were read manually using a mirror. The panels included agents from several antimicrobial classes: 24 agents for streptococci and 13 agents for *H. influenzae*. Tests were performed for *H. influenzae* (n=25), *S. pneumoniae* (n=25),  $\beta$ -haemolytic streptococci (n=18) and viridans group streptococci (n=7), selected to represent a wide range of MIC values for important agents. *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 were used as quality control.

**Results:** For streptococci, MICs with MH-F5.0 and MH-F2.5 were randomly distributed within  $\pm 1$  dilution of MH-LHB, with 2159/2400 (90%) being equal. The highest proportion of MICs equal to MH-LHB was observed for MH-F2.5 (**Table 1**), which implies that adding  $\beta$ -NAD to the broth had a very small or no effect, but that there might be some effect of varying concentration of LHB.

**Conclusions:** The results from this study imply that adding  $\beta$ -NAD to the broth for BMD had a very small or no effect on MICs for streptococci. The possible effect of varying the concentration of lysed horse blood on streptococci and *H. influenzae* should be investigated further.

Table 1. Differences in dilutions (number of MICs) for *Streptococcus* spp.<sup>1</sup> comparing MH-F2.5<sup>2</sup> and MH-F5.0<sup>3</sup> with MH-LHB<sup>4</sup>.

| Broth<br>Antimicrobial agent<br>/Differences in dilutions (No of MICs) | MH-F2.5 <sup>2</sup> |             |            | MH-F5.0 <sup>3</sup> |             |            |
|--|----------------------|-------------|------------|----------------------|-------------|------------|
|  | -1                   | 0           | +1         | -1                   | 0           | +1         |
| Benzympenicillin   | 3                    | 45          | 2          | 2                    | 43          | 5          |
| Ampicillin   | 0                    | 49          | 1          | 0                    | 45          | 5          |
| Amoxicillin  | 0                    | 46          | 4          | 3                    | 41          | 6          |
| Amoxicillin-clavulanic acid  | 0                    | 49          | 1          | 1                    | 46          | 3          |
| Piperacillin-tazobactam  | 2                    | 43          | 5          | 4                    | 41          | 5          |
| Cefepime   | 1                    | 49          | 0          | 3                    | 47          | 0          |
| Cefotaxime   | 1                    | 45          | 4          | 1                    | 42          | 7          |
| Ceftriaxone  | 1                    | 46          | 3          | 1                    | 46          | 3          |
| Cefuroxime   | 3                    | 46          | 1          | 5                    | 42          | 3          |
| Imipenem   | 4                    | 44          | 2          | 3                    | 43          | 4          |
| Meropenem  | 3                    | 45          | 2          | 2                    | 41          | 7          |
| Ciprofloxacin  | 3                    | 46          | 1          | 9                    | 39          | 2          |
| Levofloxacin   | 3                    | 45          | 2          | 6                    | 44          | 0          |
| Moxifloxacin   | 1                    | 45          | 4          | 4                    | 45          | 1          |
| Vancomycin   | 0                    | 48          | 2          | 0                    | 48          | 2          |
| Azithromycin   | 1                    | 44          | 5          | 5                    | 44          | 1          |
| Clarithromycin   | 0                    | 49          | 1          | 2                    | 48          | 0          |
| Erythromycin   | 0                    | 47          | 3          | 2                    | 46          | 2          |
| Clindamycin  | 1                    | 49          | 0          | 3                    | 46          | 1          |
| Tetracycline   | 2                    | 45          | 3          | 4                    | 45          | 1          |
| Doxycycline  | 3                    | 46          | 1          | 4                    | 45          | 1          |
| Linezolid  | 2                    | 47          | 1          | 9                    | 40          | 1          |
| Rifampicin   | 5                    | 40          | 5          | 7                    | 38          | 5          |
| Trimethoprim-sulfamethoxazole  | 1                    | 49          | 0          | 2                    | 47          | 1          |
| <b>TOTAL (No)</b>  | <b>40</b>            | <b>1107</b> | <b>53</b>  | <b>82</b>            | <b>1052</b> | <b>66</b>  |
| <b>TOTAL (%)</b>   | <b>3.3</b>           | <b>92.3</b> | <b>4.4</b> | <b>6.8</b>           | <b>87.7</b> | <b>5.5</b> |

<sup>1</sup> *S. pneumoniae* (n=25),  $\beta$ -haemolytic n=18) and viridans group (n=7) streptococci.

<sup>2</sup> Mueller-Hinton broth with 2.5% lysed horse blood and 20 mg/L  $\beta$ -NAD.

<sup>3</sup> Mueller-Hinton broth with 5.0% lysed horse blood and 20 mg/L  $\beta$ -NAD.

<sup>4</sup> Mueller-Hinton broth with 2.5% lysed horse blood only.