eP192

# Comparison of commercial methods with broth microdilution for determining beta-lactam susceptibilities of *Streptococcus pneumoniae* isolates

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## Background

*Streptococcus pneumoniae* is a common cause of pneumonia and meningitis with substantial mortality and morbidity worldwide. In January 2008, the Clinical and Laboratory Standards Institute (CLSI) revised clinical breakpoints for penicillin and ceftriaxone to improve correlation between susceptibility test results (MIC) and clinical outcomes.

*S. pneumoniae* isolates with elevated beta-lactam MICs are no longer a rare event and underscore the importance of accurate detection of resistance. However, we have observed discordance between Sensititre and E-test susceptibility test methods.

The goal of this study was to compare commercially available methods: Etest®, Vitek-2®, M.I.C.Evaluator™ (M.I.C.E) and Sensititre ™ to the reference broth microdilution (BMD) for beta-lactam susceptibility testing of *S. pneumoniae*.

## Methods

**Bacterial strains:** All *S. pneumoniae* sterile site isolates with an E-test penicillin MIC >0.06 collected from adult and pediatric Albertan cases from 2011 to 2013 were selected for this study. All isolates were characterized and serotyped at the Alberta Provincial Laboratory for Public Health, Edmonton, AB. *S. pneumoniae* ATCC 49619 (low MIC) and ATCC 51915 (high MIC) were were tested by each method over ten consecutive days to establish precision, reproducibility and quality control.

**Systems tested:** The method for the Sensititre (Trek Diagnostic Systems) and the Vitek2 AST-ST01 (BioMérieux) was followed according to the manufacturer's instructions. BMD served as the reference method and was performed as described by CLSI M07-A9. Etest (BioMérieux) and M.I.C.E strips (Thermo Scientific-Oxoid) were performed on Mueller-Hinton large plates supplemented with 5% sheep blood, inoculated with 0.5 McFarland standard. All isolates were tested for penicillin, amoxicillin, ceftriaxone and meropenem MICs. CLSI breakpoint interpretations were made according to M100-S21 (2011).

**Interpretation of results:** The performance of the four testing methods (E-test, M.I.C.E, Vitek and Sensititre) was determined using essential agreement (EA) and categorical agreement (CA). Very major errors, major errors, and minor errors were determined according to Cumitech 31A.

## Breakpoints

**Table I. CLSI Breakpoints for *S. pneumoniae* M-100 2013**

| | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| **Penicillin** | | | |
| Oral | ≤0.06 | 0.12-1 | ≥2 |
| Meningitis IV | ≤0.06 | | ≥0.12 |
| Non-meningitis IV | ≤2 | 4 | ≥8 |
| **Ceftriaxone** | | | |
| Meningitis | ≤0.5 | 1 | ≥2 |
| Non-meningitis IV | ≤1 | 2 | ≥4 |
| **Amoxicillin** | ≤2 | 4 | ≥8 |
| **Meropenem** | ≤0.25 | 0.5 | ≥1 |

## Results

**Table II. Evaluation of the Performance of E-test Compared to CLSI BMD**

| | Susceptible | | Intermediate | | Resistant | | EA | CA | VME | ME | MiE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Etest | BMD | Etest | BMD | Etest | BMD | | | | | |
| **Penicillin** | | | | | | | 95% | | | | |
| Oral | **14** | 16 | **77** | 66 | **0** | 9 | | 84% | 0% | 0% | 16% (15) |
| Meningitis (IV) | **14** | 16 | N/A | N/A | **77** | 75 | | 93% | 3% (2) | 25% (4) | 0% |
| Non-men (IV) | **91** | 82 | **0** | 9 | **0** | 0 | | 90% | 0% | 0% | 10% (9) |
| **Ceftriaxone** | | | | | | | 98% | | | | |
| Meningitis (IV) | **61** | 57 | **29** | 17 | **1** | 17 | | 77% | 6% (1) | 0% | 22% (21) |
| Non-men (IV) | **83** | 74 | **7** | 17 | **0** | 0 | | 88% | 0% | 0% | 11% (10) |
| **Amoxicillin** | **90** | 76 | **1** | 3 | **0** | 12 | 75% | 84% | 92% (11) | 0% | 4% (4) |
| **Meropenem** | **69** | 63 | **22** | 11 | **0** | 17 | 86% | 71% | 6% (1) | 0% | 27% (25) |

**Table III. Evaluation of the Performance of M.I.C.E Compared to CLSI BMD**

| | Susceptible | | Intermediate | | Resistant | | EA | CA | VME | ME | MiE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MICE | BMD | MICE | BMD | MICE | BMD | | | | | |
| **Penicillin** | | | | | | | 93% | | | | |
| Oral | **9** | 16 | **81** | 66 | **1** | 9 | | 84% | 0% | 0% | 16% (15) |
| Meningitis (IV) | **9** | 16 | N/A | N/A | **82** | 75 | | 92% | 0% | 44% (7) | 0% |
| Non-men (IV) | **90** | 82 | **1** | 9 | **0** | 0 | | 91% | 0% | 0% | 9% (8) |
| **Ceftriaxone** | | | | | | | 97% | | | | |
| Meningitis (IV) | **70** | 57 | **19** | 17 | **2** | 17 | | 68% | 6% (1) | 0% | 31% (28) |
| Non-men (IV) | **89** | 74 | **2** | 17 | **0** | 0 | | 84% | 0% | 0% | 16% (15) |
| **Amoxicillin** | **84** | 76 | **7** | 3 | **0** | 12 | 92% | 85% | 50% (6) | 0% | 9% (8) |
| **Meropenem** | **78** | 63 | **13** | 11 | **0** | 17 | 78% | 69% | 24% (4) | 0% | 26% (24) |

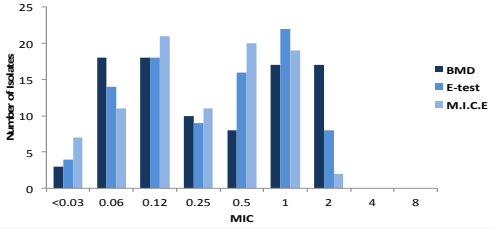


Figure I: MIC Distribution for Penicillin as Measured by Gradient Diffusion and BMD

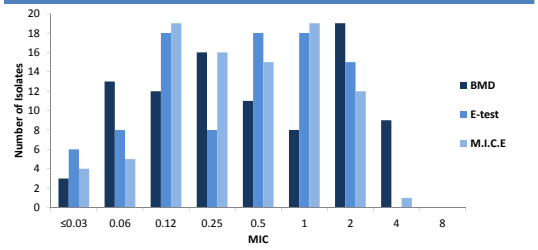


Figure II: MIC Distribution for Ceftriaxone as Measured by Gradient Diffusion and BMD

**Table IV. Evaluation of the Performance of Sensititre Compared to CLSI BMD**

| | Susceptible | | Intermediate | | Resistant | | EA | CA | VME | ME | MiE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sens | BMD | Sens | BMD | Sens | BMD | | | | | |
| **Penicillin** | | | | | | | 96% | | | | |
| Oral | **14** | 16 | **62** | 66 | **15** | 9 | | 89% | 0% | 0% | 11% (10) |
| Meningitis (IV) | **14** | 16 | N/A | N/A | **77** | 75 | | 96% | 1% (1) | 19% (3) | 0% |
| Non-men (IV) | **76** | 82 | **14** | 9 | **1** | 0 | | 92% | 0% | 0% | 8% (7) |
| **Ceftriaxone** | | | | | | | 95% | | | | |
| Meningitis (IV) | **54** | 57 | **16** | 17 | **21** | 17 | | 90% | 0% | 4% (2) | 8% (7) |
| Non-men (IV) | **70** | 74 | **19** | 17 | **2** | 0 | | 91% | 0% | 0% | 9% (8) |
| **Amoxicillin** | **73** | 76 | **5** | 3 | **13** | 12 | 98% | 95% | 0% | 1% (1) | 4% (3) |
| **Meropenem** | **73** | 63 | **5** | 11 | **13** | 17 | 67% | 91% | 0% | 0% | 9% (8) |

**Table IV. Evaluation of the Performance of Vitek Compared to CLSI BMD**

| | Susceptible | | Intermediate | | Resistant | | EA | CA | VME | ME | MiE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vitek | BMD | Vitek | BMD | Vitek | BMD | | | | | |
| **Penicillin** | | | | | | | 90% | | | | |
| Oral | **14** | 16 | 73 | 66 | 4 | 9 | | 86% | 0% | 0% | 14% (13) |
| Meningitis | **14** | 16 | N/A | N/A | 77 | 75 | | 96% | 1% (1) | 19% (3) | 0% |
| Parenteral | **87** | 82 | 4 | 9 | 0 | 0 | | 90% | 0% | 0% | 10% (9) |
| **Ceftriaxone** | | | | | | | 93% | | | | |
| Meningitis | **65** | 57 | 19 | 17 | 7 | 17 | | 76% | 12% (2) | 0% | 22% (20) |
| Parenteral | **84** | 74 | 4 | 17 | 3 | 0 | | 85% | 0% | 1% (1) | 14% (13) |
| **Amoxicillin** | **N/A** | 76 | **N/A** | 3 | **N/A** | 12 | | | | | |
| **Meropenem** | **N/A** | 63 | **N/A** | 11 | **N/A** | 17 | | | | | |

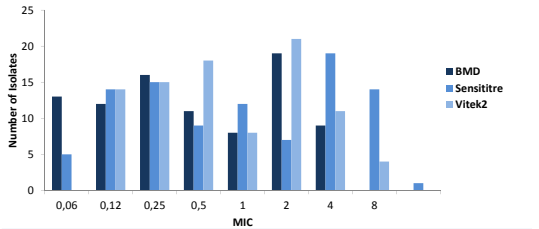


Figure III: MIC Distribution for Penicillin as Measured by Vitek2, Sensititre and BMD

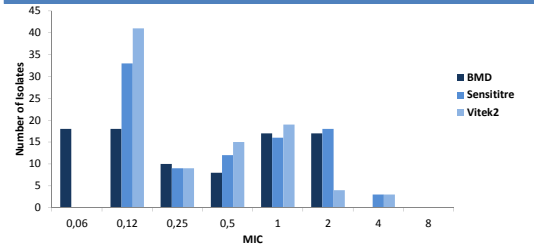


Figure IV: MIC Distribution for Ceftriaxone as Measured by Vitek2, Sensititre and BMD

## Results: Control Strains

Table VI. Replicate MICs for ATCC 51915 as Measured by All Test Methods

| | BMD | E-test | M.I.C.E | Sensititre* | Vitek2* |
|---|---|---|---|---|---|
| **Penicillin** | | | | | |
| MIC=8 | 9 | - | 2 | - | - |
| MIC=4 | 1 | 3 | 8 | 10 | 10 |
| MIC=3 | - | 7 | - | - | - |
| **Ceftriaxone** | | | | | |
| MIC=8 | 10 | - | 1 | - | - |
| MIC=6 | - | 1 | - | - | - |
| MIC=4 | - | 2 | 9 | 10 | 10 |
| MIC=3 | - | 7 | - | - | - |
| MIC=2 | - | - | - | - | - |
| **Amoxicillin** | | | | | |
| MIC=16 | - | - | - | 10 | N/A |
| MIC=8 | 10 | - | 2 | - | N/A |
| MIC=4 | - | - | 8 | - | N/A |
| MIC=3 | - | 10 | - | - | N/A |
| MIC=2 | | - | - | - | N/A |
| **Meropenem** | | | | | |
| MIC=2 | 8 | - | - | 5 | N/A |
| MIC=1 | 2 | 2 | 1 | 5 | N/A |
| MIC=0.75 | - | 8 | - | - | N/A |
| MIC=0.5 | - | - | 9 | - | N/A |

* Sensititre max MIC for penicillin is 4 and ceftriaxone is >2 (MIC =4 in this table); Vitek2 maximum MIC for penicillin and ceftriaxone is 4.

*S. pneumoniae* ATCC 49619 (low MIC) and ATCC 51915 (high MIC) were tested by each method over ten consecutive days to establish precision. The precision for all test methods was > 98% for both control strains. MICs for ATCC 49619 was within +/- 1 dilution of BMD for all test methods.

## Discussion

In our setting, pneumococcal susceptibilities are routinely determined by E-test, isolates that have a penicillin MIC > 0.06 are confirmed using Sensititre. However, discrepancies between the two test methods became apparent for more resistant clinical isolates.

In this study, we were able to confirm that in a controlled setting all methods display acceptable performance and are in essential agreement with BMD. Meanwhile, the categorical agreements were surprisingly low for all methods except for Sensititre. This is probably a reflection of the changes in CLSI breakpoints in 2008.

Although, EA was acceptable for all methods, there was a trend towards gradient diffusion methods reporting a lower MICs than BMD. This was quite apparent when ATCC 51915 was tested using E-test.

Further research should be done to determine the clinical impact of these findings.

With the introduction of vaccination programs, serotype replacement has occurred. The need for accurate routine susceptibility testing is clinically important as resistant strains like 19A are becoming more prevalent.

## Acknowledgements & References

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