**Verification of Gradient Strip Susceptibility Testing Methods for Resistant Gram-negative Organisms**

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**INTRODUCTION**

Gram-negative bacteria are increasingly displaying multi-drug resistance. While new antibiotics have been approved to tackle the challenge posed by MDR GN organisms, many automated routine laboratory susceptibility testing methods do not include them. While supplemental manual methods may be used, they are often not well-verified against gold standard methods. The objective of this project is to verify gradient strips for new antibiotics targeting resistant GN organisms against gold-standard methods.

**METHODS**

An array of multi-drug resistant gram-negative bacteria (44 Enterobacterales, 20 Pseudomonas aeruginosa, 19 Acinetobacter, 7 Aeromonas, 10 non-fermenters, 6 Burkholderia cepacia, and 9 Stenotrophomonas maltophilia) were tested to determine very major errors (VME), major errors (ME), minor errors (MinE), and categorical/essential agreements (CA/EA) using bioMérieux’s ETEST and Liofilchem’s gradient strips against BMD. Acceptability was determined using Cumitech31A thresholds (≤90% EA/CA/VME/MinE with Acinetobacter, and ≤7% MinE/ME) and calculating 95%CI using CLSI or EUCAST breakpoints.

**RESULTS**

Table 1. Table showing results of each supplemental test compared to the in-house agar. A green cell indicates point estimate agreement within the threshold, yellow cell indicates point estimate disagreement with the threshold but agreement within the confidence interval, and red cells indicate disagreement with the threshold and confidence intervals. Grey cells show where there was not enough isolates for that category (e.g. grey VME means no resistant bacteria) or where the antibiotic was not evaluated.

**DISCUSSION AND CONCLUSIONS**

- All strips other than tigecycline had acceptable EA/CA/VME/MinE with Enterobacteriades, and acceptable EA/CA/ME/MinE with Aeromonas (except Aeromonas VME due to lack of resistant isolates). Only meropenem/vaborbactam and piperacillin/tazobactam had acceptable EA/CA/VME/MinE for both B. cepacia and S. maltophilia and cefotizone/tazobactam strips and TMP/SXT ETEST had acceptable EA/CA/VME/MinE with B. cepacia. A summary of EA/CA/ME/MinE results for Enterobacteriades, Pseudomonas, and Acinetobacter within range (green), within range taking Cl into account (orange) and out of range (red) (table 1)

**CONCLUSION**

Laboratories should be aware of the limitations of gradient strip supplemental testing of resistant Gram-negative organisms and should ideally perform verification testing prior to implementation.

**UPDATED ABSTRACT**

**Objectives:** Routine susceptibility testing methods do not include newer agents for resistant Gram-negative organisms (MDRGN). Laboratories are testing novel agents with methods that may not have been well verified against gold standard methods. We verified bioMérieux’s ETEST strips and Liofilchem’s gradient strips against gold-standard custom broth microdilution (BMD).

**Methods:** MDRGN (44 Enterobacteriades, 20 Pseudomonas aeruginosa, 19 Acinetobacter, 7 Aeromonas, 10 non-fermenters, 6 Burkholderia cepacia, and 9 Stenotrophomonas maltophilia) were tested to determine very major errors (VME), major errors (ME), minor errors (MinE), and categorical/essential agreements (CA/EA) using bioMérieux’s ETEST and Liofilchem’s gradient strips against BMD. Acceptability was determined using Cumitech31A thresholds (≤90% EA/CA/VME/MinE with Acinetobacter, and ≤7% MinE/ME) and calculating 95%CI using CLSI or EUCAST breakpoints.

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<th>MinE</th>
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<td>B. cepacia</td>
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