Antibiotic Susceptibility Testing of *Stenotrophomonas maltophilia* with Trimethoprim/Sulfamethoxazole Using the Phoenix™ System

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**OBJECTIVES:** To evaluate a new automated antibiotic susceptibility test system, Phoenix™ BD Biosciences, Sparks, MD, USA, for the determination of MICs, when testing *Stenotrophomonas maltophilia* (Sm) with trimethoprim/sulfamethoxazole (SXT).

**METHODS:** A newly developed rapid AST system, Phoenix, was evaluated for testing the susceptibility of Sm with SXT. The PASCO® System, BD Biosciences, Sparks, MD, USA, was used as a reference method. Isolates of Sm were pre-screened using a NCCLS recommended standard broth microdilution test, in order to include more strains with higher MICs to SXT in the study. A total of 97 strains of Sm and 4 NCCLS recommended QC strains were tested with the Phoenix and PASCO in parallel using a single inoculum preparation. Discrepant results between the two systems were resolved by the agar dilution method (AD), performed per NCCLS procedures. Agar dilution was selected based on the results from a separate study in which an essential accord (EA) between PASCO and AD was 91%. A total of 31 isolates (27 Sm and 4 QC) were tested twice independently to evaluate reproducibility.

**RESULTS:** Of the 97 strains tested, 12 strains showed a MIC value higher than 4 µg/ml and 7 strains showed a MIC value between 1 to 2 µg/ml by the Phoenix or PASCO system. The EA between the two systems was 96% with no very major errors. There were 2 strains which tested resistant (MIC ≥ 4 µg/ml) by Phoenix but susceptible by PASCO (confirmed by AD) resulting in 2.3% major errors. The time to result of the Phoenix system for Sm/SXT combination was 7-12 h. Reproducibility of the system was 90%.

**CONCLUSION:** These data suggest the feasibility of testing susceptibility of Sm with SXT in the Phoenix system. We believe that further enhancements to performance and reproducibility could be achieved through a more comprehensive and diverse database.

**INTRODUCTION AND PURPOSE**

*Stenotrophomonas maltophilia* (Sm) an increasingly recognized nosocomial pathogen and an important opportunistic pathogen in immunocompromised patients in the past decade, is frequently intrinsically resistant to many antibiotics, including b-lactams and aminoglycosides.

The *in vitro* susceptibility results of certain groups of antibiotics to Sm and their clinical association are not yet established. The combination drug, trimethoprim/sulfamethoxazole (SXT) has been regarded as the agent of choice for the treatment of Sm. However, the management of Sm infection is often jeopardized by the difficulties associated with the methodological problems of routine *in vitro* susceptibility testing in clinical laboratories.

A standardized method for testing susceptibility of Sm has not yet been established. Trailing end points of microbial growth are often observed when performing antibiotic susceptibility test with agar or microdilution method. The presence of small resistant colonies or a haze growth inside the zone of inhibition...
have been demonstrated to result in inconsistency in the E-test® (AB Biodisk, Piscataway, NJ) and disk diffusion testing.

Several studies have documented and addressed the performance of commercial systems in antibiotic susceptibility testing of Sm. Poor correlation was observed between the Sensititre microdilution system (Alamar/Sensititre, Sacramento, CA) and agar dilution methods when testing eight b-lactams and ciprofloxacin with Sm. The MicroScan® (Dade International Corp., Sacramento, CA), Alamar® colorimetric broth microdilution method (Alamar/Sensititre) and an in-house microbroth dilution methods demonstrated inconsistent results. The Vitek® system (bioMerieux, Hazelwood, MO) was poorly correlated with microbroth and diffusion methods.

The objective of this study was to evaluate a new automated antibiotic susceptibility test system, Phoenix, for the determination of MICs when testing Sm with SXT as compared to reference methods, the PASCO System (Difco Laboratories, Detroit, MI).

**METHODS**

**BACTERIAL STRAINS:** A total of 96 clinical strains of Sm from the BD Biosciences internal culture collection and Sm ATCC13637 were included in the studies. Four National Committee for Clinical Laboratory Standards (NCCLS) recommended QC strains, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus faecalis* ATCC29212, and *Staphylococcus aureus* ATCC29213 were also included in every experiment performed.

**MEDIA:** Trypticase® soy agar plates with 5% defibrinated sheep blood (BBL, BDB) were used to grow overnight cultures of test isolates. Mueller-Hinton II® agar (BBL) was used for antibiotic susceptibility testing with ET, DD and AD methods.

**Antibiotics and Concentrations Tested:** Standard laboratory trimethoprim and sulfamethoxazole powders were obtained from Hoffmann LaRoche (Nutley, NJ). SXT was prepared with appropriate solvents and diluents and used freshly for the preparation of in-house agar dilution plates according to the NCCLS procedures. The concentrations of SXT tested were: Phoenix panel, 0.5/9.5 – 16/304 µg/ml; PASCO panel, 0.5/9.5 – 4/76 µg/ml; and agar dilution, 0.25/4.75 – 16/304 µg/ml. Antibiotic containing plates were stored at 4°C and used within 3-4 days. QC was performed to ensure the quality and accuracy of Phoenix or PASCO panels manufactured. Phoenix panels tested were stored at room temperature before use. PASCO panels were stored at -70°C before use.

**PHOENIX AST METHOD:** A newly developed rapid AST system, Phoenix, was evaluated for testing the susceptibility of Sm with SXT. The PASCO System was used as a reference method. Isolates of Sm were pre-screened using a NCCLS recommended standard broth microdilution test in order to include more strains with higher MICs to SXT in the study. A total of 97 strains of Sm and 4 NCCLS recommended QC strains were tested with the Phoenix and PASCO in parallel. Bacterial suspension from overnight culture on BAP were prepared and adjusted to a 0.5 McFarland standard in sterile normal saline; aliquots of the preparation were used for each system and inoculated within 30 min of initial preparation. For the PASCO System, procedures suggested by the manufacturer were followed. For testing the Phoenix system, a final inoculum density equivalent to 5 x 10⁶ cfu/ml in Phoenix AST Broth was used. The inoculated panels were then placed into the Phoenix Instrument for incubation and continuous reading. After 18 and 24 h of incubation at 35±1°C in ambient air, the PASCO test result was interpreted by at least 2 trained technologists. Twenty-six strains that exhibited trailing growth were tested twice subsequently with both systems. Discrepant results between the two systems were resolved by the agar dilution method, performed according to the NCCLS procedures. Agar dilution was selected based on the results from a separate study in which an essential accord (EA) between PASCO and AD was 91%. Twenty-seven strains of Sm and 4 QC strains were tested twice independently to evaluate the reproducibility of the Phoenix system. The NCCLS breakpoints were used for categorical interpretation (susceptible ≤ 2/38 and resistant > 4/76 µg/ml).
RESULTS

CONCLUSIONS

- The EA between the two systems was 96% with no very major errors. There were 2 strains which tested resistant (MIC $\geq 4 \mu g/ml$) by Phoenix but susceptible by PASCO (confirmed by agar dilution method) resulting in 2.3% major errors.
- The time to result of the Phoenix System for Sm/SXT combination was approximately 7-12 h (mean ± standard deviation = 10.98 ± 1.13 h).
- Reproducibility of the Phoenix System was 90%, where the PASCO System showed a higher reproducibility.
- The data suggests the feasibility of testing susceptibility of Sm with SXT in the Phoenix system. We believe that further enhancements to performance and reproducibility could be achieved through a more comprehensive and diverse database.