

Comparison of Four Commercially Available Systems for Antimicrobial Susceptibility Testing (AST) of *Streptococcus pneumoniae*

R. RENNIE¹, C. BROSNIKOFF¹, L. TURNBULL¹, S. SHOKOPLES¹, M. LOVGREN², J. GALBRAITH³, C. BROWN³, L. KIPKE³ AND N. HART³

Medical Microbiology Laboratory, University of Alberta Hospital¹, National Center for *Streptococcus*²,
Dynacare Kasper Medical Laboratories³, Edmonton, Alberta, Canada

ABSTRACT (REVISED)

■ **OBJECTIVE.** The objective of this study was to evaluate four commercially available systems for AST with a challenge set of *Streptococcus pneumoniae* (SPN) of known serotypes and antimicrobial resistance patterns.

METHODS. One hundred and eight (108) clinical strains of SPN from 36 different serotypes were included in this study. All strains were tested using two manually read dried panel systems, the Sensititre *Haemophilus influenzae*/*Streptococcus pneumoniae* (TREK Diagnostic Systems, Cleveland, OH), and Microscan MICroSTREP plus 1 (Dade Behring, Mississauga, ON), and two fully automated systems: Vitek 2 AST-P506 card (bioMérieux, St. Laurent, Quebec) and Phoenix SMIC (BD Diagnostics, Sparks, MD). Testing was performed according to the manufacturers guidelines and all required quality control tests were included. SPN ATCC 49619 was included as a common quality control strain in all systems. Reference broth microdilution testing (MIC) was performed by the National Centre for Streptococcus, Edmonton, AB on all strains according to NCCLS recommendations and was considered the gold standard.

RESULTS. By reference MIC, 57% were penicillin-S, 24% were penicillin-I and 19% were penicillin-R; 36% were erythromycin-R; 7 strains (6.4 %) were ceftriaxone-R (meningitis breakpoints) and 7 strains (6 %) were ofloxacin-R. The antimicrobials tested were not identical for all systems. For penicillin, there was 90% agreement within interpretive categories for all systems with one major error for Sensititre and Microscan, and the remainder were minor errors (4 – 10%). For erythromycin, there were 5 major errors (3-Phoenix, 1-Microscan, 1-Sensititre) and the remaining were minor errors (1 – 7%). For ceftriaxone there was one very major error (Vitek 2), 1 major error (Microscan) and the remaining were minor errors (7.4 – 12%). For quinolones, ofloxacin (MIC & Vitek 2) and levofloxacin (Microscan, Sensititre and Phoenix) were tested. Results for these agents showed that there were only minor errors for all systems (5.5–7.4%) compared to reference MIC.

CONCLUSIONS. All systems performed favorably when compared to the reference MIC method. The isolates grew equally well in the dried panels and the automated systems. For those agents commonly used to treat SPN infections, these commercial systems are easy to set up, read and provide equivalent results. The automated systems also provide expert interpretation of AST according to NCCLS guidelines.

OBJECTIVE

The objective of this study was to evaluate four commercially available systems for antimicrobial susceptibility testing with a challenge set of *Streptococcus pneumoniae* isolates. This set of strains included 36 different serotypes with several resistance mechanisms.

INTRODUCTION

Antimicrobial resistance in *Streptococcus pneumoniae* has become a significant clinical issue. In the past the major consideration was resistance to penicillin which was easily tested for by disk diffusion, agar dilution screening or MIC tests. Higher level resistance to penicillin carried the development of decreased susceptibility to second and third generation cephalosporins. With the emergence of strains resistant to the macrolides and then to fluoroquinolones, it has become important to test at least invasive *S. pneumoniae* against all these agents in a timely manner.

In house prepared broth microdilution panels are acceptable for this purpose, although recently, the major manufacturers of automated identification and antimicrobial susceptibility testing systems have developed panels specifically for *S. pneumoniae* that include all these important agents, as well as some of the newer agents such as new fluoroquinolones. We have evaluated these systems against a series of challenge strains of *S. pneumoniae* that encompass the significant resistance determinants.

MATERIALS AND METHODS

Strains: One hundred and eight (108) clinical strains of *Streptococcus pneumoniae* were included in this study. All strains were of known serotypes, including the following: 1 (4), 3 (5), 4 (4), 5 (2), 6A (6), 6B (11), 7F, 8 (3), 9N (3), 9V (6), 10A, 12F (2), 13 (2), 14 (7), 15, 15A, 15B, 16F, 17F, 18B, 18C (4), 19A (5), 19F (6), 20, 22A, 22F, 23A, 23F (10), 25F, 31, 33A, 33F, 34 (2), 35B (3), 37 and 38 (3). There were also three non-typeable strains included. Each strain was a unique patient isolate from epidemiological unrelated sites. As shown in Table 1, the collection also encompassed a range of serotypes within each resistance determinant.

The minimum inhibitory concentrations (MIC) to penicillin of all strains was previously established by broth microdilution (NCCLS) methods. Fifty-seven percent (57%) of the strains had penicillin MIC's of ≤ 0.06 mg/L, 24% had MIC's of 0.12 – 1 mg/L and the remaining strains (19%) had MIC's of ≥ 2 mg/L.

Streptococcus pneumoniae ATCC 49619 was included as a common quality control strain for all methods.

Manual read panels: The two methods were Sensititre *Haemophilus influenzae/Streptococcus pneumoniae* (TREK Diagnostic Systems, Cleveland, OH) and MicroScan MICroSTREP plus 1 (Dade Behring, Mississauga, ON). Both methods involve making an organism suspension in Mueller-Hinton broth with laked horse blood and inoculating the trays according to the manufacturer's guidelines. The trays are incubated for 20 – 24 hours at 35°C in a non-CO₂ incubator, and read manually with no visible growth as the endpoint.

Automated Systems: The two automated methods were Phoenix (BD Diagnostics, Oakville, ON) and the Vitek 2 (bioMerieux Inc., St. Laurent, Que.). The panels for both systems were inoculated following the manufacturer's instructions and placed on the appropriate instrument for incubation and reading of the panels.

Antibiotics: The antimicrobials tested were not identical for all systems and the antimicrobial agents compared in this study were those that were common to all four systems: ceftriaxone, erythromycin, penicillin, ofloxacin, cefotaxime, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin.

RESULTS

By standard broth microdilution, the range of MICs for erythromycin resistant strains was 1 - 128 mg/L suggesting that these strains had both *erm* and *mef* resistance mechanisms. All ceftriaxone and cefotaxime resistant strains had high penicillin MICs. None of the ofloxacin/levofloxacin resistant strains had elevated penicillin MICs. These strains were high level (MICs of 16 - >32 mg/L) double mutation resistant mutants (*gyrA* and *parC*).

The comparison of the systems to NCCLS broth microdilution results for comparable antimicrobial agents are shown in Tables 2-5. By reference MIC, 57% were penicillin-S, 24% were penicillin-I and 19% were penicillin-R; 36% were erythromycin-R; 7 strains (6.4 %) were ceftriaxone-R (meningitis breakpoints) and 7 strains (6 %) were ofloxacin-R. For penicillin, there was 90% agreement

within interpretive categories for all systems with one major error for Sensititre and Microscan, and the remainder were minor errors (4 - 10%). For erythromycin, there were 5 major errors (3-Phoenix, 1-Microscan, 1-Sensititre) and the remaining were minor errors (1 – 7%). For ceftriaxone there was one very major error (Vitek 2), 1 major error (Microscan) and the remaining were minor errors (7.4 – 12%). For quinolones, ofloxacin (MIC & Vitek 2) and levofloxacin (Microscan, Sensititre and Phoenix) were tested. Results for these agents showed that there were only minor errors for all systems (5.5–7.4%) compared to reference MIC. For cefotaxime, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin, there were no qualitative errors; MICs of all strains did not vary by more than one dilution between any system for these agents.

RESULTS (CONTINUED)

Table 1. Challenge Organism Characteristics

	#	Range (mg/L)	Serotypes (#)
Penicillin Susceptible	62 (57%)	0.004 - 0.06	1 (4), 3 (5), 4 (4), 5 (2), 6A, 6B (6), 7F, 8 (3), 9N (4), 10A, 12F (2), 13 (2), 14, 15A, 16F, 18B, 18C (4), 19A, 20, 22A, 22F, 23A, 23F (3), 25F, 31, 33A, 33F, 34, 35B, 37, 38 (2), NT
Penicillin Intermediate	26 (24%)	0.12 - 1	6A (5), 6B (4), 9V (2), 14, 15A, 15B, 17F, 19A (4), 19F (2), 23F (2), 38, NT (2)
Penicillin Resistant	20 (19%)	≥ 2-8	6B, 9V (3), 14 (5), 19F (4), 23F (5), 35B (2)
Erythromycin Resistant	38 (35%)	≥ 1 - ≥16	1, 3, 5, 6A (5), 6B (9), 9V, 12F, 14 (4), 15A, 17F, 19A, 19F (5), 23F (5), 38, NT
Ceftriaxone Resistant**	7 (6.4%)	≥ 2 - 8	6B, 14 (2), 19F (2), 23F (2)
Ofloxacin/Levofloxacin Resistant	7 (6.4%)	≥ 8 - 32	4, 6B, 9V, 18C, 19F, 23F (2)

** Meningitis criteria used.

Table 2. Ceftriaxone Errors

	Very Major	Major	Minor
MicroScan	0/108	1/108	8/108 (7.4%)
Phoenix	0/108	0/108	13/108 (12%)
Trek	0/108	0/108	11/108 (10%)
Vitek2	1/108	0/108	12/108 (11%)

Table 4. Penicillin Errors

	Very Major	Major	Minor
MicroScan	0/108	1/108	4/108 (4%)
Phoenix	0/108	0/108	11/108 (10%)
Trek	0/108	1/108	10/108 (9%)
Vitek2	0/108	0/108	10/108 (9%)

Table 3. Erythromycin Errors

	Very Major	Major	Minor
MicroScan	0/108	1/108	1/108 (1%)
Phoenix	0/108	3/108	0/108
Trek	0/108	1/108	3/108 (2.7%)
Vitek2	0/108	0/108	8/108 (7.4%)

Table 5. Ofloxacin/Levofloxacin Errors

	Very Major	Major	Minor
MicroScan	0/108	0/108	8/108 (7%)
Phoenix	0/108	0/108	7/108 (6.5%)
Trek	0/108	0/108	8/108 (7%)
Vitek2	0/108	0/108	6/108 (5.6%)

DISCUSSION

All systems performed favorably when compared to the reference MIC method. The isolates grew equally well in the dried panels and the automated systems. For those agents commonly used to treat SPN infections, these commercial systems are easy to set up, read and provide equivalent results. The automated systems also provide expert interpretation of AST according to NCCLS guidelines.

ACKNOWLEDGEMENTS

We would like to acknowledge TREK Diagnostic Systems, Dade Behring Inc., BD Diagnostics and bioMerieux Inc for their support of this project.