

# Evaluation of the BD Phoenix Automated Microbiology System SMIC/ID panel for identification and antimicrobial susceptibility testing of *Streptococcus* spp

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## Abstract

The BD Phoenix Automated Microbiology System SMIC/ID panel was evaluated for identification and antimicrobial susceptibility testing (AST) of various streptococci. A group of 97 consecutive clinical isolates of *Streptococcus pneumoniae*, 23 *Streptococcus pyogenes*, 24 *Streptococcus agalactiae*, and 34 viridans streptococci were collected and comparisons made with routine manual methods used in the clinical microbiology laboratory. Overall, in 162 (91%) of 178 isolates, Phoenix identification results demonstrated agreement. For AST results for the 162 isolates that demonstrated identification concordance, the overall essential agreement rate was 98.5%; the category agreement was 94.9%; and the very major error, major error, and minor error rates were 0%, 0.15%, and 5.8%, respectively. Although relatively high minor error rates were observed with *S. pneumoniae* and  $\beta$ -lactams, 79.2% of the 77 minor errors were the result of a single log<sub>2</sub> dilution difference. The Phoenix SMIC/ID panel performed favorably and demonstrated the advantages of automation and simple methodology.

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## 1. Introduction

With increasing test volume in the face of cost constraints, clinical microbiology laboratories must continue to seek cost-effective approaches to maximize efficiency while having a significant impact on the management of infections. Several automated identification (ID) and antimicrobial susceptibility testing (AST) systems are currently available on the market. In the face of increasingly complex emerging resistance, increasingly sophisticated systems have been developed to ascertain the antimicrobial phenotype of the organism using algorithms based on kinetic analysis of data as well as

expert systems that apply a series of predetermined rules to analyze results.

The BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD [BD]), a newly developed automated instrument for ID and AST for the majority of clinically encountered bacterial isolates, has been reported to be accurate and reliable for the majority of clinical isolates encountered in the clinical microbiology laboratory (Donay et al., 2004; Fahr et al., 2003; Funke and Funke-Kissling, 2004; Spanu et al., 2004). In addition, the Phoenix system is reported to be a reliable method for laboratory detection of extended-spectrum  $\beta$ -lactamase (Leverstein-van Hall et al., 2002; Sanguinetti et al., 2003).

The evolving antimicrobial resistance of streptococci as well as taxonomic changes presents a challenge to the clinical microbiology laboratory with respect to timely and accurate ID as well as assessment of susceptibility (Facklam, 2002). For the viridans streptococci group, antimicrobial resistance

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is significant. Resistance to penicillin ranges from 30% to nearly 50% in strains in different areas of the globe (Pfaller et al., 1999). Although the precise identification of the species of viridans streptococci reported may be arguable in light of limitations in reliance on phenotypic tests, antimicrobial resistance to  $\beta$ -lactams as well as macrolides is not uncommon (Alcaide et al., 2001; Teng et al., 1998). With respect to *Streptococcus pneumoniae*, high rates of resistance to  $\beta$ -lactams, macrolides, and fluoroquinolones have been reported (Felmingham et al., 2002).

One of the major challenges for automated ID systems lies in the development of a database based on phenotypic characteristics that is discriminatory enough to be consistent, at least to the group level, with taxonomic changes based on 16S rRNA sequencing. Furthermore, AST testing without the addition of supplements and availability of results more rapidly than overnight testing methods is highly desirable.

The objective of the present study was to evaluate the newly developed Phoenix streptococci panel (SMIC/ID) for identification and AST of streptococci.

## 2. Materials and methods

### 2.1. Bacterial isolates

A total of 178 single clinical isolates of *Streptococcus* spp. isolated from various clinical specimens at the clinical microbiology laboratory, Nagasaki University Hospital, during the period from January 1999 to June 2003 were evaluated on the Phoenix in the study. The following streptococcal isolates were included: 97 *S. pneumoniae* (21 penicillin-resistant, 33 penicillin-intermediate, 2 cefotaxime/ceftriaxone-intermediate, 67 erythromycin-resistant, and 4 levofloxacin-resistant), 24 *Streptococcus agalactiae*, 23 *Streptococcus pyogenes* (5 erythromycin-resistant), and 34 viridans streptococci. Of the viridans streptococci, 22 isolates were from the *anginosus* group and 12 were from the *mitis* group.

### 2.2. BD Phoenix SMIC/ID panel design and identification

Identification with the Phoenix system was performed as described previously (Donay et al., 2004; Fahr et al., 2003). Briefly, the Phoenix System SMIC/ID panel uses identification substrates on one side and antimicrobial drugs on the other side of the panel. The ID side of the Phoenix System SMIC/ID panel contains a total of 45 dried substrates, including fluorogenic substrates, fermentation substrates, carbon sources, chromogenic substrates, esculin, and 2 fluorescent controls. All isolates were subcultured twice onto trypticase soy agar supplemented with 5% sheep blood (TSA II, BD Diagnostic Systems, Tokyo, Japan) and incubated at 35 °C under 5% CO<sub>2</sub> before evaluation on the Phoenix system. After the Phoenix ID broth (4.5 mL) was inoculated with several colonies and adjusted to 0.5–0.6 McFarland standard using a calibrated CrystalSpec neph-

lometer (BD Diagnostics, Japan), 25  $\mu$ L of the ID broth suspension was transferred to the Phoenix AST broth (8 mL); the remaining ID broth was poured into the ID side of the combo panel and loaded into the instrument.

A combination of commercial ID system and phenotypic tests was used in the identification of streptococci. The protocol routinely used in this laboratory consists of assessment of hemolytic reaction of the isolate on 5% sheep blood supplemented trypticase soy agar, catalase test reaction, size of colonies, and, where appropriate, susceptibility to optochin, bile esculin test, and Lansfield serologic grouping (for  $\beta$ -hemolytic streptococci only) was performed. In addition, the RapID STR system (AMCO Incorporated, Tokyo, Japan) (Appelbaum et al., 1986; You and Facklam, 1986) was set up from the same pure culture on optochin-resistant isolates.

Where discrepancies occurred with the results obtained with Phoenix and the laboratory's routine identification methods, streptococci isolates were further characterized according to standard biochemical methods (Facklam, 2002; Ruoff et al., 2003).

### 2.3. Antimicrobial agents

In total, the following 14 antimicrobials were tested: penicillin (0.03–8  $\mu$ g/mL), ampicillin (0.5–16  $\mu$ g/mL), cefotaxime (0.5–4  $\mu$ g/mL), ceftriaxone (0.5–4  $\mu$ g/mL), cefepime (0.5–4  $\mu$ g/mL), cefuroxime (0.5–4  $\mu$ g/mL), imipenem (0.125–4  $\mu$ g/mL), meropenem (0.125–2  $\mu$ g/mL), levofloxacin (0.5–16  $\mu$ g/mL), gatifloxacin (0.25–8  $\mu$ g/mL), erythromycin (0.0625–4  $\mu$ g/mL), linezolid (0.25–4  $\mu$ g/mL), vancomycin (0.125–1  $\mu$ g/mL), and tetracycline (0.5–16  $\mu$ g/mL).

### 2.4. Phoenix AST

The Phoenix AST method was performed using the protocol recommended by the manufacturer. Briefly, 8 mL of AST broth was supplemented with 1 drop of Phoenix AST indicator (oxidation–reduction indicator based on resazurin). From the McFarland 0.5–0.6 standardized ID broth, 25  $\mu$ L was transferred to the AST broth resulting in a final inoculum density of approximately  $5 \times 10^5$  CFU/mL. The AST broth was then poured into the AST antimicrobial side of the combo panel.

### 2.5. Reference AST

A commercial cation-adjusted Mueller–Hinton broth supplemented with 2–5% lysed horse blood microdilution assay (Eiken Chemical Co, Tokyo, Japan) prepared according to National Committee for Clinical Laboratory Standards (NCCLS, 2003a) guidelines was used as the reference method.

### 2.6. Quality control organisms

The NCCLS control strain, *S. pneumoniae* ATCC 49619, was used for quality control for both the Phoenix system and the reference microdilution panel.

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