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IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF CLINICAL ISOLATES OF NONFERMENTING GRAM-NEGATIVE BACTERIA BY THE PHOENIX™ AUTOMATED MICROBIOLOGY SYSTEM

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SUMMARY

The Phoenix™ Automated Microbiology System (Becton Dickinson, Sparks, MD) was evaluated for its ability to identify nonfermenting gram-negative pathogens and measure their drug susceptibility. Isolates producing rare extended-spectrum β-lactamases (PER-1, IMP-2, VIM-1, and VIM-2) were included in the study. Species identification was compared to that given by the ATB System (bioMérieux, Marcy l'Étoile, France), whereas susceptibility results were compared to those produced by a reference broth microdilution test (panels manufactured by Pasco Laboratories, Becton Dickinson). The Phoenix™ system consistently identified all isolates of *Pseudomonas aeruginosa* (n=55) and *Stenotrophomonas maltophilia* (n=28), while in other cases species agreement was obtained for 47/53 isolates (*Acinetobacter baumannii*, 29/31; *Pseudomonas putida*, 10/11; *Burkholderia cepacia*, 6/7; and *Pseudomonas fluorescens*, 2/4). Overall, the Phoenix™ and ATB systems gave equal results in 130/136 cases (95.6%). For two isolates, consistent identification was obtained at the genus level, thus bringing the cumulative agreement to 97.1%. MIC values (interpreted according to NCCLS guidelines) gave essential and categorical agreement in 94.2% and 93.1% of cases, respectively. Minor and major errors were 5.1% and 5.2%, respectively. No very major errors were produced. The mean time to results (TTR) for the Phoenix™ system was 14.8 ± 1.6 h (mean ± SD), with the shortest TTR being observed for *A. baumannii* (13.0 ± 1.8 h) and the longest one for *P. aeruginosa* (15.6 ± 1.2 h).

In conclusion, the Phoenix™ system performed rapidly and correctly in the identification of clinical isolates of important opportunistic pathogens and in measuring their susceptibility to antipseudomonal drugs.

KEY WORDS: *Pseudomonas, Stenotrophomonas, Acinetobacter, Burkholderia*, automated systems

INTRODUCTION

Nonfermenting gram-negative bacteria are a group of aerobic, non-spore-forming, microorganisms that either do not utilize carbohydrates as a source of energy or degrade them through non-fermentative metabolic pathways (Kiska and Gilligan, 1999; Schreckenberger and von Graevenitz, 1999; Gilligan and Whittier, 1999). Among them, *Pseudomonas aeruginosa* is a well-known pathogen that is frequently isolated from clinical specimens (Arruda *et al.*, 1999; Luzzaro *et al.*, 2001), whereas *Acinetobacter*, *Stenotrophomonas*, and *Burkholderia* have recently emerged as important causes of morbidity and mortality in compromised patients (Quinn, 1998). The increasing prevalence of infections due to these pathogens is explained by several factors, one of the most important being their ability to produce enzymes capable of hydrolyzing broad-spectrum β-lactams (PER-1 and PER-2 enzymes, metallo-β-lactamases of IMP- or VIM-type). Production of these enzymes is often responsible for therapeutic failures (Rasmussen and Bush, 1997; Nordmann and Guibert, 1998; Cornaglia *et al.*, 1999; Galleni *et al.*, 2001; Docquier *et al.*, 2002). Prompt diagnostic procedures and susceptibility tests are therefore of the utmost importance for the management of serious infections.

Over the last few years, automated identification and susceptibility testing methods have become the mainstay of clinical microbiological laboratories (Craft, 2000). Due to the increasing relevance of infections caused by nonfermenting gram-negative pathogens, it is important that automated diagnostic methods be capable of correctly identifying these bacteria and measuring their susceptibility to antimicrobial drugs.

This study was designed to evaluate the ability of the new Phoenix™ Automated Microbiology System (Becton Dickinson Diagnostic Systems, Sparks, MD) to correctly identify clinically important nonfermenting gram-negative bacteria and measure their susceptibility to antipseudomonal drugs. Time to results was also studied in order to evaluate the instrument's ability to provide prompt critical diagnostic results.

MATERIALS AND METHODS

Identification of clinical isolates

One hundred and thirty-six non-duplicated clinical isolates of nonfermenting gram-negative bacteria were studied: *P. aeruginosa* (n=55), *Acinetobacter baumannii* (n=31), *Stenotrophomonas maltophilia* (n=28), *Pseudomonas putida* (n=11), *Burkholderia cepacia* (n=7), and *Pseudomonas fluorescens* (n=4). The isolates were obtained over the last two years at the Microbiology Laboratory of the Ospedale di Circolo in Varese (Italy). Identification (ID) of isolates was routinely achieved by the Sceptor™ system (breakpoint/ID gram-negative panels; Becton Dickinson), confirmed by the ATB System (ID32GN strips; bioMérieux, Marcy l'Étoile, France) and subsequently re-evaluated by the Phoenix™ system (NMIC/ID-5 panels; Becton Dickinson).

Clinical isolates producing rare extended-spectrum β-lactamases (ESBL) were included in the study: *P. aeruginosa* producing the PER-1 ESBL (n=5) and the IMP-2 metallo-β-lactamase (n=1), *P. putida* producing the VIM-1 metallo-β-lactamase (n=3), a *P. aeruginosa* isolate that produced both the PER-1 and the VIM-2 enzyme. All isolates were frozen at –70°C and passed two times on Mueller-Hinton agar (Oxoid, Milan, Italy) before testing.

Antimicrobial susceptibility testing

Reference method
Antimicrobial susceptibility testing (AST) was evaluated with frozen gram-negative MIC panels manufactured by Pasco™ (Pasco Laboratories, Becton Dickinson). Panels were frozen at –20°C until use. They were inoculated according to the manufacturer's instructions and incubated overnight at 35°C aerobically. Twelve different antimicrobial agents were studied: aztreonam, cefotaxime, ceftazidime, cefepime, piperacillin, piperacillin plus tazobactam, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and levofloxacin. The Pasco™ Reader System (Becton Dickinson) was used to read MIC panels.

Phoenix™ method
Isolated colonies from Mueller-Hinton agar plates were suspended in Phoenix™ ID broth to a 0.5-0.6 McFarland standard using the CrystalSpec™ nephelometer (Becton Dickinson). One drop of Phoenix AST indicator solution was added to each Phoenix™ AST broth tube prior to inoculation with 25 μl of the above suspension (final bacterial concentration was 5 x 10^5 CFU/ml). NMIC/ID-5 Phoenix™ panels were inoculated within 30 minutes of initial preparation. Panels were scanned and placed into the Phoenix™ instrument for incubation at 35°C and continuous reading. Phoenix™ panels were stored at room temperature until use.

Quality control strains
Escherichia coli ATCC 25922 and *P. aeruginosa* ATCC 27853 were included in each run as quality controls.

Interpretation of ID and AST results
The "consistent ID" category implies that bacteria were equally identified at the genus and species level by both the ATB and the Phoenix™ systems. In the event of discordant ID results, the assay was repeated with both systems. Results of ID tests obtained with the ATB system were used as a reference. Antimicrobial susceptibility results are expressed as raw data, i.e. without interpretation by the computerized expert Phoenix™ system. Results of susceptibility tests were categorized as susceptible (S), intermediate (I), or resistant (R) according to criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2000). MIC values obtained by Phoenix™ panels (TEST) were compared to those obtained with the reference method (REF). The following definitions were adopted: 1) Essential agreement (MIC values of TEST panel equal to or within ± 1 dilution of the REF value); 2) Categorical agreement (TEST and REF MIC values agree using the interpretative NCCLS criteria); 3) Minor errors (REF is S or R and TEST is I; alternatively, REF is I and TEST is S or R); 4) Major errors (REF is S and TEST is R; the percentage of major errors was calculated only for susceptible isolates); 5) Very major errors (REF is R and TEST is S; the percentage of very major errors was calculated only for resistant isolates).

RESULTS

Biochemical identification of clinical isolates
As compared to the ATB method, the Phoenix™ system correctly identified to the species level 123/136 test organisms (90.4%). After repeating inconsistent ID assays, accuracy to the species level increased to 95.6% (130/136). At the genus level, accuracy was 97.4% (132/136). In particular, all *P. aeruginosa* (n=55) and *S. maltophilia* (n=28) isolates were consistently identified to the species level. In the case of other species (*A. baumannii*, *P. putida*, *B. cepacia*, and *P. fluorescens*), correct ID was obtained in 47/53 cases (88.7%). All isolates producing rare ESBLs were correctly identified. Data are shown in Table 1.
Inconsistent identifications given by the Phoenix™ system included: two isolates of *A. baumannii* (both reported as *Moraxella* spp.), two of *P. fluorescens* (reported as *Pseudomonas oryzihabitans* and as a "not specified organism"), one isolate of *B. cepacia* (reported as CDC group Vb-3), and one of *P. putida* (reported as *Pseudomonas pseudoalcaligenes*).

Antimicrobial susceptibility testing
Tests were performed with 12 different antipseudomonal drugs. The following results are reported without interpretation by the Phoenix™ expert system. The overall essential agreement between the Phoenix™ system and the reference microdilution method was 94.2%. Depending on different drugs, the essential agreement ranged from 91.2 to 97.1%. The overall categorical agreement (i.e., S, I, R)

TABLE 1

Results of biochemical identification (ID): agreement of the Phoenix™ system with the ATB system used as reference method

Organism	No. of strains	Consistent ID No. (%)	Inconsistent ID No. (%)
Pseudomonas aeruginosa	55	55 (100)	0 (0.0)
Acinetobacter baumannii	31	29 (93.5)	2 (6.5)
Stenotrophomonas maltophilia	28	28 (100)	0 (0.0)
Pseudomonas putida	11	10 (90.9)	1 (9.1)
Burkholderia cepacia	7	6 (85.7)	1 (14.3)
Pseudomonas fluorescens	4	2 (50.0)	2 (50.0)
Total	136	130 (95.6)	6 (4.4)

was 93.1%, ranging from 83.8 to 97.1% for different drugs. Minor errors accounted for 5.1% of cases, major errors were 5.2%. No very major errors were produced. Results are summarized in Table 2.

Time to results (ID plus AST)

Time to results (TTR) was calculated with regard to consistently identified species only. The overall mean TTR (± SD) was 14.8 ± 1.6 h. Particularly, *A. baumannii* showed the lowest TTR (13.0 ± 1.8), whereas TTR values for *P. aeruginosa* (15.6 ± 1.2) and *S. maltophilia* (15.1 ± 0.6) were more prolonged. The cumulative TTR data are given in Figure 1.

TABLE 2

Antimicrobial susceptibility testing (AST): agreement of the Phoenix™ system with the Pasco™ reference method

Antimicrobial agent	Pasco™ method: Susceptibility results S	I	R	Phoenix™ system: Essential agreement No. (%)	Categorical agreement No. (%)	Minor errors No. (%)	Major errors No. (%)	Very major errors No. (%)
Aztreonam	21	13	102	131 (96.3)	126 (92.6)	8 (5.9)	2 (9.5)	0 (0.0)
Cefotaxime	20	24	92	129 (94.9)	129 (94.9)	5 (3.7)	2 (10.0)	0 (0.0)
Ceftazidime	62	13	61	124 (91.2)	121 (89.0)	9 (6.6)	6 (9.7)	0 (0.0)
Cefepime	57	27	42	125 (91.9)	114 (83.8)	14 (10.3)	9 (15.8)	0 (0.0)
Piperacillin	59	10	67	125 (91.9)	125 (91.9)	8 (5.9)	3 (5.1)	0 (0.0)
Piperacillin-tazobactam	74	10	52	127 (93.4)	127 (93.4)	8 (5.9)	1 (1.4)	0 (0.0)
Meropenem	59	22	55	132 (97.1)	128 (94.1)	8 (5.9)	0 (0.0)	0 (0.0)
Gentamicin	52	4	80	128 (94.1)	131 (96.3)	4 (2.9)	1 (1.9)	0 (0.0)
Tobramycin	59	1	76	131 (96.3)	131 (96.3)	4 (2.9)	1 (1.7)	0 (0.0)
Amikacin	100	4	32	130 (95.6)	129 (94.9)	5 (3.7)	2 (2.0)	0 (0.0)
Ciprofloxacin	79	8	49	128 (94.1)	126 (92.6)	6 (4.3)	4 (5.1)	0 (0.0)
Levofloxacin	80	4	52	128 (94.1)	132 (97.1)	4 (2.9)	0 (0.0)	0 (0.0)
Overall percentage				94.2	93.1	5.1	5.2	0 (0.0)

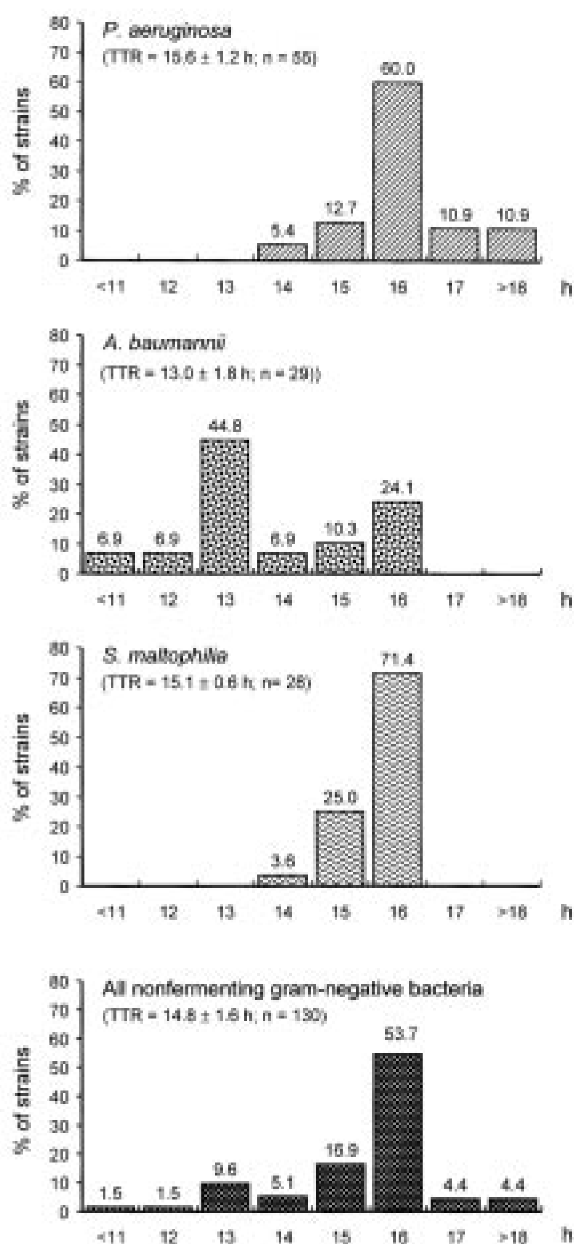

FIGURE 1 - Phoenix™ system: cumulative percentage of time to results for clinical isolates of nonfermenting gram-negative bacteria (identification plus antimicrobial susceptibility testing).

DISCUSSION

The Phoenix™ system appeared to perform satisfactorily in identifying clinical isolates of important opportunistic nonfermenting gram-negative pathogens and in determining their susceptibility to a variety of clinically relevant antipseudomonal drugs. The overall accuracy rate of identification was 95.6%, whereas the average time to results (ID plus AST) was 14.8 ± 1.6 h. When compared to ID results produced by widely used automated microbiology systems in the case of nonfermenting gram-negative organisms, results obtained with the new instrument were very satisfactory (Tenover *et al.*, 1990; Bourbeau and Heiter, 1998; Sung *et al.*, 2000; Joyanes *et al.*, 2001). This is particularly true when considering that the investigated species are notoriously difficult to identify because of slow growth (Kiska and Gilligan, 1999; Schreckenberger and von Graevenitz, 1999; Gilligan and Whittier, 1999). Notably, species identification was 100% accurate in the case of *P. aeruginosa* and *S. maltophilia* that represent the most common isolates among clinically relevant opportunistic pathogens (Quinn, 1998).

With regard to AST results, it has been recommended that an overall category error rate of <10% should be obtained for accepting the performance of susceptibility tests, with up to 3.0% major errors and up to 1.5% very major errors (NCCLS, 1994). With regard to the above guidelines, no very major errors were found, whereas major errors were 5.2%. It should be noted, however, that these data were markedly influenced by the difficulty of measuring MIC values for some expanded-spectrum β-lactams (e.g., cefepime). This may be due to the complexity of resistance mechanisms expressed by *P. aeruginosa* and other nonfermenting bacteria (Livermore, 2002). In addition to the loss of specific porins (i.e., alterations of the permeability barrier), over-expression of AmpC β-lactamases and/or efflux pumps may greatly influence MIC values for penicillins, cephalosporins, and carbapenems (Nikaido, 1998; Poole, 2000).

In the case of aminoglycosides (gentamicin, tobramycin, and amikacin), results were fully satisfactory, with minor and major errors ranging from 1.7 to 3.7%. With regard to fluoroquinolones, remarkable precision was obtained for levofloxacin with no major errors, and only 2.9% minor errors. In contrast, tests

for ciprofloxacin gave 4.3% and 5.1% minor and major errors, respectively.
The correlation between MIC values obtained by the Phoenix™ system and the reference method was highly significant, with essential and categorical agreements of 94.2% and 93.1%, respectively. Overall, the AST results obtained in this study were better or equal to those obtained with the best-performing automated systems (Joyanes *et al.*, 2001; Chandler *et al.*, 2002). However, AST results reported in this study represent pure raw data. In the clinical setting, AST results would have been interpreted by the computerized expert system of the instrument, thus further reducing minor and major errors.
In conclusion, this study demonstrates that the Phoenix™ system may represent an accurate diagnostic tool for infections caused by nonfermenting gram-negative bacteria. High-level correlations were obtained with the ID and AST methods used for comparison. Due to its rapid, convenient and consistent performance, the instrument will become an important device for the detection and therapy of life-threatening infections due to drug-resistant opportunistic pathogens. Its application may lead to a more appropriate use of antimicrobial agents, a shorter hospital stay, and decreased hospital costs (Doern *et al.*, 1994; Barenfanger *et al.*, 1999).

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