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# Identification of Coagulase-Negative Staphylococci by Using the BD Phoenix System in the Low-Inoculum Mode[▽]

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**The new "low-inoculum" mode of the Phoenix system was evaluated to identify clinical coagulase-negative staphylococci. API ID32 Staph panels were used as comparators, and discrepancies were resolved by 16S rRNA and *tuf* gene analysis. The system correctly identified 90.5% of isolates, with a mean time of 10.2 h. Accuracy was satisfactory for *Staphylococcus epidermidis*, *S. saprophyticus*, and *S. haemolyticus*.**

Coagulase-negative staphylococci (CoNS) have long been considered members of the saprophytic flora and are rarely pathogens. Over the last 2 decades, CoNS have emerged as significant pathogens, especially among compromised hosts and patients undergoing complex medical procedures. In many instances, CoNS are involved in nosocomial infections related to indwelling medical devices and prostheses (6, 16).

It should be noted that in addition to the most frequent CoNS species isolated from humans (i.e., *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus saprophyticus*), at least nine additional CoNS species have been isolated from clinical specimens and from food (11, 17). Thus, comprehensive and accurate identification (ID) of *Staphylococcus* species is of great importance, together with drug susceptibility testing. Several ID methods for gram-positive, catalase-positive cocci are commercially available, including manual, semiautomated, and automated systems. Among these, the API ID32 Staph system (bioMérieux, Marcy l'Étoile, France) is highly reputed for its accuracy (2, 4, 7, 8, 14).

Becton Dickinson (Sparks, MD) has developed recent software and algorithms for identifying clinically relevant pathogens for its Phoenix (PHX) automated bacterial ID system, using a low-inoculum mode (low mode) that uses a 0.25 instead of the standard 0.5 McFarland inoculum size. The new low mode was developed to increase ID accuracy by using already available Becton Dickinson panels. Results of ID plus drug susceptibility testing are produced within 18 h.

Four Italian microbiology laboratories (in Catania, Parma, Rome, and Varese) performed a multicenter study designed to assess the ability of the PHX system to identify clinical CoNS isolates by use of the new low mode. Isolates consecutively collected during the first 6 months of 2006 were investigated; isolates were obtained from blood-, catheter-, and wound-associated infections. The following 10 CoNS reference strains of different species were obtained from the American Type Culture Collection (ATCC) and used for proficiency tests: *S. aureus*, *S. epidermidis*, *S. cohnii* subsp. *urealyticum*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. sciuri*, *S. schleiferi* subsp. *coagulans*, *S. simulans*, and *S. xylosus*.

Overall, 200 isolates were biochemically identified using the new PHX low mode. The API system was used as a comparator. In the case of disagreement between the two phenotypic methods, both assays were repeated. When discrepancies persisted, ID was first confirmed by direct DNA sequencing of the 16S rRNA gene (15). If no reliable ID was produced by this method, the *tuf* housekeeping gene was sequenced (8). With regard to staphylococcal species, the *tuf* gene is considered more discriminatory than the conserved *rrn* genes (15).

Twelve different species were identified among the investigated isolates. Concordant IDs between the API and PHX systems were obtained in 88.5% of cases (data not shown). Discordant IDs were obtained for 23/200 isolates (11.5%). Highly concordant results were obtained for *S. epidermidis* and *S. haemolyticus* isolates (96% and 93%, respectively). Seven isolates were not identified by the PHX system. Isolates producing discordant results were identified by molecular methods. Table 1 shows the resolution of discrepancies by molecular methods. Sequencing of the 16S rRNA gene succeeded in identifying 11/23 strains of three different species (*S. epidermidis*, *S. hominis*, and *S. lugdunensis*). The IDs of the remaining 12 strains were obtained by *tuf* gene sequencing (*S. epidermidis*, *S. capitis*, *S. haemolyticus*, and *S. warneri*). Compared with molecular ID results, the API system correctly identified 16/23 discordant isolates (for *S. epidermidis*, $n = 4$; for *S. capitis*, $n = 2$; for *S. warneri*, $n = 2$; for *S. hominis*, $n = 7$; and for *S. lugdunensis*, $n = 1$). The PHX system correctly identified 4/23 strains (for *S. haemolyticus*, $n = 2$; for *S. warneri*, $n = 1$; and for *S. hominis*, $n = 1$).

As shown in Table 2, after discrepancy resolution by molecular tests, the PHX system gave an overall correct ID in 90.5% of cases. An incorrect ID or no ID was obtained in 6% and 3.5% of isolates, respectively. The API system correctly identified 96.5% of isolates. Incorrect ID was obtained in 3.5% of cases. The performance of the PHX system was particularly

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TABLE 1. Resolution by molecular assays of discordant ID results obtained for CoNS isolates ($n = 23$)[a]

| Result with PHX system | Result with API panels | Result by molecular assay | | Definitive ID | Concordance (API or PHX method) |
|---|---|---|---|---|---|
| | | 16S rRNA sequencing | *tuf* gene sequencing | | |
| NI | *S. epidermidis* | *S. epidermidis/S. caprae* | *S. epidermidis* | *S. epidermidis* | API |
| NI | *S. epidermidis* | *S. epidermidis/S. caprae* | *S. epidermidis* | *S. epidermidis* | API |
| *S. capitis* | *S. epidermidis* | *S. epidermidis* | — | *S. epidermidis* | API |
| *S. haemolyticus* | *S. epidermidis* | *S. epidermidis* | — | *S. epidermidis* | API |
| NI | *S. capitis* | *S. epidermidis/S. capitis* | *S. capitis* | *S. capitis* | API |
| *S. haemolyticus* | *S. caprae* | *S. epidermidis/S. capitis* | *S. capitis* | *S. capitis* | None |
| *S. auricularis* | *S. caprae* | *S. epidermidis/S. capitis* | *S. capitis* | *S. capitis* | None |
| *K. varians* | *S. capitis* | *S. aureus/S. simiae* | *S. capitis* | *S. capitis* | API |
| *K. varians* | *S. capitis* | *S. pasteuri/S. epidermidis* | *S. haemolyticus* | *S. haemolyticus* | None |
| *S. haemolyticus* | *S. warneri* | *S. aureus/S. simiae* | *S. haemolyticus* | *S. haemolyticus* | PHX |
| *S. pasteuri* | *S. simulans* | *S. aureus/S. haemolyticus* | *S. haemolyticus* | *S. haemolyticus* | PHX |
| *S. hominis* | *S. warneri* | *S. pasteuri/S. warneri* | *S. warneri* | *S. warneri* | API |
| *S. auricularis* | *S. warneri* | *S. pasteuri* | *S. warneri* | *S. warneri* | API |
| *S. warneri* | *S. equorum* | *S. pasteuri* | *S. warneri* | *S. warneri* | PHX |
| NI | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| NI | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| NI | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| *S. saprophyticus* | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| *S. saprophyticus* | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| *S. haemolyticus* | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| *S. haemolyticus* | *S. hominis* | *S. hominis* | — | *S. hominis* | API |
| *S. hominis* | *S. epidermidis* | *S. hominis* | — | *S. hominis* | PHX |
| NI | *S. lugdunensis* | *S. lugdunensis* | — | *S. lugdunensis* | API |

[a] NI, no ID produced by the phenotypic method. —, not performed.

good with regard to the following species: *S. epidermidis* (96%), *S. haemolyticus* (97.5%), and *S. saprophyticus* (100%). Low ID accuracy was obtained with regard to *S. hominis* isolates (69.6%).

The increasing isolation rate of CoNS in modern clinical medicine makes these agents interesting emerging pathogens. Thus, accurate and rapid CoNS ID is clinically relevant for patient management and for the prompt detection of nosocomial outbreaks (10). In addition, it should be noted that for staphylococci, species ID is essential for interpreting antimicrobial susceptibility tests (3).

A few studies have been published on the ability of the PHX system to identify CoNS. In a two-center study, Fahr and colleagues (5) tested the PHX performance with regard to 275 staphylococcal isolates that included several *S. aureus* strains. Good ID concordance with the comparators (Vitek and API ID32 Staph systems) was obtained for 153/161 CoNS isolates (95.1%). In contrast to the conclusions of Fahr (5), two recent papers (9, 14) have reported on the poor performance of the PHX system with regard to CoNS for the 0.5 McFarland inoculum mode. In both studies, discordant IDs were solved using molecular methods. The data underlined the difficulties encountered in identifying *S. epidermidis* and *S. hominis* isolates.

The present ID results, obtained using the recently developed PHX low mode, show improved overall performances. After resolution of discrepancies, the system gave a correct ID for 90.5% of isolates. The API panels provided a correct ID in

TABLE 2. Identification of CoNS isolates by use of API ID32 Staph panels and the PHX low-mode system[a]

| Species | No. of isolates | API system results (no. of isolates) | | % Concordance with molecular ID | PHX system results (no. of isolates) | | | % Concordance with molecular ID |
|---|---|---|---|---|---|---|---|---|
| | | Correct ID | Incorrect ID | | Correct ID | Incorrect ID | No ID | |
| *S. epidermidis* | 100 | 100 | 0 | 100 | 96 | 2 | 2 | 96 |
| *S. haemolyticus* | 41 | 38 | 3 | 93 | 40 | 1 | | 97.5 |
| *S. hominis* | 23 | 22 | 1 | 96 | 16 | 4 | 3 | 69.6 |
| *S. capitis* | 13 | 11 | 2 | 85 | 9 | 3 | 1 | 69 |
| *S. lugdunensis* | 7 | 7 | 0 | 100 | 6 | 0 | 1 | 86 |
| *S. warneri* | 5 | 4 | 1 | 80 | 3 | 2 | | 60 |
| *S. saprophyticus* | 3 | 3 | 0 | 100 | 3 | 0 | | 100 |
| *S. schleiferi* | 3 | 3 | 0 | 100 | 3 | 0 | | 100 |
| *S. cohnii* | 2 | 2 | 0 | 100 | 2 | 0 | | 100 |
| *S. cohnii* subsp. *cohnii* | 1 | 1 | 0 | 100 | 1 | 0 | | 100 |
| *S. kloosii* | 1 | 1 | 0 | 100 | 1 | 0 | | 100 |
| *S. simulans* | 1 | 1 | 0 | 100 | 1 | 0 | | 100 |
| Total ($n$) | 200 | 193 | 7 | | 181 | 12 | 7 | |
| Total % | 100.0 | 96.5 | 3.5 | | 90.5 | 6.0 | 3.5 | |

[a] Concordance of results produced by the API and PHX methods was determined by resolution of discrepancies by molecular assays.

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96.5% of cases. Particularly good performances of the PHX system were observed with regard to *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus*. In contrast, difficulties were encountered in identifying *S. hominis* isolates (69.6%). This problem may be attributed to missing ID keys in the database for one of the two varieties of this species (*S. hominis* subsp. *novobiosepticus*), a recently recognized pathogen (12). As a final note, the study also revealed that the PHX low mode produced ID and drug susceptibility results within a time frame satisfactory for prompt patient management. With regard to CoNS, the measured mean time to results was 10.2 h (range, 2.2 to 18.5 h; standard deviation, 4.7 h).

With regard to genotypic methods, several targets have been exploited for identifying species belonging to the *Staphylococcus* genus (7, 13, 15). Because of the large amount of *rrs* sequence data available in public databases, it is not surprising that the 16S rRNA gene has been the favorite choice. *rrs* gene sequences are highly significant at the genus level, but their role in staphylococcal species ID has been questioned (1, 8). Thus, *tuf* gene sequences have emerged as a reliable species identifier (7, 9, 15). The present results confirm that incorrect ID of 12/23 isolates was obtained through 16S rRNA sequencing. In these cases, sequences of the *tuf* gene were discriminatory at the species level.

In conclusion, the PHX low mode correctly identified 90.5% of CoNS isolates, including those most frequently encountered in the clinical setting (*S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*). Poor performances were produced with regard to *S. hominis.* In the clinical setting, new technology appears to be closing the gap between bacterial isolation and accurate ID plus drug susceptibility testing of hard-to-define pathogens, such as the many staphylococcal species. We look to the day when automatic, reliable, and rapid systems will be commonplace in medical microbiology laboratories.

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