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Original article

Evaluation of VITEK 2 system and VITEK MS system for the identification of *Haemophilus* species: a diagnostic accuracy study

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Abstract

Background: *Haemophilus* is an important pathogen in community-acquired pneumonia and invasive diseases, such as sepsis and meningitis. We aimed to evaluate the VITEK 2 system and VITEK MS system for the identification of *Haemophilus* strains isolated from clinical specimens in Korea during 2023.

Methods: In total, 118 *Haemophilus* strains isolated from respiratory specimens (n = 107) and blood samples (n = 11) from 10 sentinel hospitals in Kor-GLASS were included in this study. All *Haemophilus* strains were evaluated using the VITEK 2 and VITEK MS systems. Real-time PCR and 16S rRNA sequencing were used to identify specific species.

Results: Among the 118 *Haemophilus* isolates, 115 were identified as *H. influenzae* by real-time PCR using *hpd* gene, and the remaining three strains were identified as *H. parainfluenzae* by 16S rRNA sequencing. Eighty-eight of the 115 (76.5%) and two of three (66.7%) isolates were correctly identified as *H. influenzae* and *H. parainfluenzae*, respectively, using the VITEK 2 system. The VITEK 2 system showed low discrimination (n = 22), misidentification (n = 4),

and unidentified organisms (n = 2) in the 28 *Haemophilus* strains. The VITEK MS system achieved 100% sensitivity and specificity in identifying all 115 *H. influenzae* and three *H. parainfluenzae* isolates.

Conclusion: The VITEK MS system showed excellent performance in the identification of *H. influenzae* and *H. parainfluenzae*, whereas the VITEK 2 system showed relatively low concordance.

Keywords: *Haemophilus*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, Identification, Matrix-assisted laser desorption-ionization mass spectrometry

Introduction

Background

Haemophilus influenzae is an important pathogen that causes community-acquired pneumonia and invasive diseases such as sepsis and meningitis [1-3]. It can also cause other infections including acute otitis media, sinusitis, and acute exacerbations of chronic obstructive pulmonary disease [4,5].

Automated identification systems such as VITEK 2 system (VITEK® 2, bioMérieux), Microscan Walkaway plus Microbiology system (Siemens Healthcare Diagnostics), and BD Phoenix M50 (BD Biosciences) have a long history of use in clinical microbiology laboratories. However, these systems show limited accuracy and reproducibility for specific bacterial groups [6,7]. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a valuable tool for microbial identification in clinical microbiology laboratory for rapid, cost-effective, and highly accurate identification of the most common pathogens. Unlike traditional biochemical methods, which can be affected by variability in metabolic expression among strains, MALDI-TOF MS provides consistent and reproducible results, even for closely related species [8,9].

Objectives

We aimed to evaluate VITEK 2 system and VITEK MS system (VITEK® MS PRIME) for the identification of *Haemophilus* strains isolated from clinical specimens.

Materials and methods

Study design

This was a retrospective diagnostic accuracy study using the VITEK 2 and VITEK MS systems as index tests and *hpd* real-time polymerase chain reaction (PCR) and 16S rRNA sequencing as reference tests. This study was performed in accordance with the STARD statement available at https://www.equator-network.org/reporting-guidelines/stard/.

Clinical isolates

A total of 118 non-duplicate *Haemophilus* strains isolated from respiratory specimens (n = 107) and blood (n = 11) from the Kor-GLASS (Global Antimicrobial Resistance Surveillance System in Korea) collection network between January 2023 to December 2023 were included in this study. The Kor-GLASS, compatible with the GLASS platform, was established in 2016, with the third phase (2023–2025) involving 10 sentinel hospitals (Gangnam Severance Hospital, National Health Insurance Service Ilsan Hospital, Wonju Severance Christian Hospital, Chungbuk National University Hospital, Chonnam National University Hospital, Inje University Busan Paik Hospital, Hallym University Dongtan Sacred Heart Hospital, Jeju National University Hospital, Keimyung University Dongsan Hospital, Wonkwang University Hospital) across 10 regions [10,11]. These strains were stored at -80°C in skim milk containing 10% glycerol until use.

The sample size of 118 strains was determined by the total number of available non-duplicate isolates collected in the Kor-GLASS system during the study period. Although no formal sample size calculation was performed, the study included all available isolates in order to minimize selection bias.

Identification of *Haemophilus* by VITEK 2 system

All *Haemophilus* strains were identified using the VITEK 2 system with a VITEK 2 *Neisseria-Haemophilus* (NH) identification card according to the manufacturer's instructions. The bacterial suspension prepared in 0.45% aqueous NaCl was adjusted to a McFarland standard of 3 using the VITEK 2 DensiCheck instrument (bioMérieux). A 64-well NH card was placed on the VITEK 2 system, and VITEK 2 software version 9.02 was used for data analysis. *H. influenzae* ATCC 49247 was used as a quality control strain.

Identification of *Haemophilus* using VITEK MS system

VITEK MS system (VITEK® MS PRIME, bioMérieux) was used to identify all *Haemophilus* strains according to the manufacturer's instructions. A fresh colony was smeared onto a 48-well target plate and covered with 1 μL of α-cyano-4-hydroxycinnamic acid matrix solution. After drying, the target plate was loaded onto a VITEK MS system [12]. The results were interpreted using the VITEK MS database version 3.2. *Escherichia coli* ATCC 8739 was used as the calibrant and quality control strain. The final result was reported as good identification when one organism (or organism group) was identified with a probability of 60.0%–99.9%.

Identification of *Haemophilus* using real-time PCR and 16S rRNA sequencing

Real-time PCR of *hpd* gene was performed for all 118 *Haemophilus* strain isolates as the gold standard for identification of *H. influenzae.* Bacterial genomic DNA was extracted using Instagene matrix (Bio-Rad Laboratories). PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems). The primer and probe sequences were described in Table 1 [13]. Real-time PCR was performed under the following conditions: 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 60 s. *H.*

influenzae ATCC 49247 was used as a positive control.

When the result of real-time PCR using *hpd* was negative, we performed 16S rRNA gene sequencing to identify *Haemophilus* species other than *H. influenzae*. The 16S rRNA gene was amplified using universal primers 27F and 1492R and sequencing was performed using a total of four primers: 27F, 785F, 907R, and 1492R (Table 1).

Table 1. Primers and probe list used in the study

Target genes	Primer or probe	Sequences (5′-3′)
hpd	hpd822F	GGT TAA ATA TGC CGA TGG TGT TG
	hpd952R	TGC ATC TTT ACG CAC GGT GTA
	Pb896i	(FAM) TTG TGT ACA CTC CGT TGG T (BHQ1)
16S rRNA	27F	AGA GTT TGA TCM TGG CTC AG
	1492R	GGT TAC CTT GTT ACG ACT T
	785F	GGA TTA GAT ACC CTG GTA
	907R	CCG TCA ATT CMT TTR AGT TT

Index tests and reference standards

The investigators performing the VITEK 2 and VITEK MS tests were blinded to the results of the reference standard assays. Likewise, personnel conducting PCR and sequencing were blinded to the results of the index tests to minimize interpretation bias.

Analysis

We calculated the percentages of accurate identification, low discrimination, misidentification, and no identification results from the VITEK 2 and VITEK MS systems based on *hpd* real-time PCR and 16S rRNA sequencing. We precisely analyzed the discrimination results.

Results

Real-time PCR using *hpd* and 16S rRNA sequencing

Among the 118 *Haemophilus* isolates, 115 were identified as *H. influenzae* by real-time PCR using *hpd*. The remaining three strains were identified as *H. parainfluenzae* by 16S rRNA sequencing.

Identification by VITEK MS system

Of the 118 *Haemophilus* isolates, 115 (97.5%) were identified as *H. influenzae*, and three strains (2.5%) were correctly identified as *H. parainfluenzae* using the VITEK MS system. The VITEK MS system exhibited 100% accuracy in identifying all 115 *H. influenzae* and three *H. parainfluenzae* isolates (Table 2).

Table 2. Identification results from VITEK MS and VITEK 2 systems for 118 *Haemophilus* strains

VITEK MS (n, %)	VITEK 2 (n, %)	*hpd* real-time PCR and 16S rRNA sequencing
H. influenzae (115, 100)	*H. influenzae* (88, 76.5)	*H. influenzae*
	Low discrimination (22, 19.1)	
	H. parainfluenzae (3, 2.6)	
	Unidentified organism (2, 1.7)	
H. parainfluenzae (3, 100)	*H. parainfluenzae* (2, 66.7)	*H. parainfluenzae*
	H. influenzae (1, 33.3)	

Abbreviation: PCR, polymerase chain reaction.

Identification by VITEK 2 system

For the 115 *H. influenzae* isolates, 88 strains (76.5%) were correctly identified as *H. influenzae*, and 22 strains (19.1%) were reported as "low discrimination" by the VITEK 2 system. The remaining two strains (1.7%) showed "unidentified organism" results and three strains (2.6%) were incorrectly identified as *H. parainfluenzae*. Among the three *H. parainfluenzae* isolates, two were correctly identified as *H. parainfluenzae*, whereas one isolate was misidentified as *H. influenzae* by the VITEK 2 system. The VITEK 2 system correctly identified 76.3% of the 88 *H. influenzae* and two *H. parainfluenzae* isolates (Table 2).

All 22 strains categorized as having "low discrimination" showed mixed results with *H. influenzae* along with other *Haemophilus* and *Actinobacillus* species (Table 3). Mixed identification results were observed in six strains of *H. influenzae*/*H. haemolyticus*, five strains of *H. influenzae*/*A. ureae*, three strains each of *H. influenzae*/*H. parainfluenzae* and *H. influenzae*/*H. parahaemolyticus*, and one strain of *H. influenzae*/*A. pleuropneumoniae*. The remaining three strains exhibited mixed identification results involving three distinct bacterial species.

Table 3. Low discrimination results of 22 *Haemophilus influenzae* strains by VITEK 2 system

VITEK 2 results of identification	Strains (n)
H. influenzae / *H. haemolyticus*	6
H. influenzae / *A. ureae*	5
H. influenzae / *H. parainfluenzae*	3
H. influenzae / *H. parahaemolyticus*	3
H. influenzae / *A. pleuropneumoniae*	1
H. influenzae / *A. ureae* / *H. haemolyticus*	1
H. influenzae / *H. haemolyticus* / *H. parainfluenzae*	1
H. influenzae / *H. parahaemolyticus* / *A. pleuropneumoniae*	1
Total	22

Discussion

Key results

Although widely used in clinical laboratories, the VITEK 2 system showed a limited ability to identify *H. influenzae*. In addition, *H. influenzae* is sometimes misidentified as *H. parainfluenzae*. The use of molecular methods such as real-time PCR, 16S rRNA sequencing, and MALDI-TOF should be considered to ensure correct identification of *H. influenzae* when the VITEK 2 system yields "low discrimination" or "unidentified organisms".

Interpretation/comparison with previous studies

Automated identification systems have been used in clinical microbiology laboratories for several years. In a report by Janda et al., MicroScan using NH identification panel with 132 *Haemophilus* strains showed good performance for *H. influenzae* (98.8%) and *H. parainfluenzae* (97.1%) [14]. Munson et al. evaluated the performance of the VITEK system for identification of *Haemophilus* and obtained accuracies of 98.2% and 85.7% for 174 *H. influenzae* and 154 *H. parainfluenzae*, respectively [15].

Valenza et al. reported that the VITEK 2 system showed correct identification results for 84% and 92.5% for 25 *H. influenzae* and 27 *H. parainfluenzae* isolates, respectively [16]. Low discrimination was observed for two *H. influenzae* isolates (*H. influenzae-H. haemolyticus*), reflecting uncertainty in distinguishing between these closely related species. The other two *H. influenzae* isolates were labeled as unidentified organisms. Incorrect results for the two *H. parainfluenzae* isolates showed low discrimination (*H. parainfluenzae-H. segnis*) and unidentified results. Rennie et al. reported higher identification accuracies of 93% for *H. influenzae* and 83% for *H. parainfluenzae* using 90 *H. influenzae* and 41 *H. parainfluenzae* strains evaluated using the VITEK 2 system [17]. In contrast, our study showed a lower correct identification rate (76.5%) for *H. influenzae* using this system, with a large proportion of low discrimination (19.1%), misidentification as *H. parainfluenzae* (2.6%), and unidentified organisms (1.7%). However, all results considered as low discrimination by the VITEK 2 system still included *H. influenzae* as a possible organism.

Unlike the VITEK 2 system, which uses traditional biochemical methods, VITEK MS is a laboratory instrument based on MALDI-TOF MS. Currently, VITEK MS is widely adopted in clinical laboratories because of its high accuracy and rapid identification capabilities [18,19]. Bruin et al. evaluated the Bruker MALDI Biotyper® System (Bruker Daltonics) using 244 *H. influenzae* and 33 *H. haemolyticus* [20]. The accuracy rates were 100% for *H. influenzae* and 87.9% *H. haemolyticus*. Frickmann et al. [21] reported that 88% of *H. influenzae* isolates were correctly identified using the Bruker system. In our study, all *H. influenzae* and *H. parainfluenzae* strains were correctly identified at the species level using the VITEK MS system. Another report by Nürnberg et al. showed similar results in identifying *H. influenzae* [22]. However, they reported that 42% of *H. haemolyticus* strains were identified as *H. influenzae*. These findings highlight the persistent difficulties in differentiating closely related species, particularly *H. haemolyticus*, even when using VITEK MS. In our study, only a few *H. parainfluenzae* and no *H. haemolyticus* were included; thus, further evaluation of other *Haemophilus* species is necessary.

Limitation

This study had several limitations. There were only a few *H. parainfluenzae* except *H. influenzae*. No other *Haemophilus* species were examined. We evaluated only the VITEK 2 and VITEK MS systems, although other commercial identification systems are used in clinical laboratories.

Conclusion

The VITEK 2 system showed relatively low performance in identifying *H. influenzae*, although results

were generally satisfactory. The VITEK MS system is appropriate for identifying *H. influenzae* in clinical laboratories.

Ethics statement

This study was approved by the Institutional Review Board of Inje University Busan Paik Hospital (BPIRB NON2024-002), and the requirement for patient consent was waived.

Conflict of interest

Soo Hyun Kim has been an editorial board member since 2011 and Young Uh has been a statistical editor of the *Annals of Clinical Microbiology* since 2024. However, they were not involved in the review process of this article. No potential conflict of interest relevant to this article was reported.

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Data availability

The data sets generated in this study are available from the corresponding author upon request.

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