



The comparative survey of phenotypic methods and the BD Phoenix M50 automated microbiology system for detecting the genus of non-fermenting gram-negative bacteria isolated from blood samples in Isfahan, Iran

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Abstract

Introduction: A comparative study was conducted to identify the genus and species of non-fermenting gram-negative bacilli isolated from blood culture samples using phenotypic methods and the Phoenix method.

Objectives: This study aimed to evaluate the Phoenix system compared to the currently available phenotypic process.

Materials and Methods: This descriptive-analytical cross-sectional study evaluated 30 samples collected from 2019 to 2020 from patients at AL Zahra and Kashani hospitals who required blood cultures. The specimens were injected into BACTEC™. Positive cultures identified as non-fermenting gram-negative bacteria by the phenotypic method were included in the study and then evaluated using the Phoenix method to determine the genus and species of non-fermenting gram-negative bacteria. A comparison was then conducted.

Results: The study identified 30 non-fermenting gram-negative bacteria. The Phoenix method revealed that 78.5% of the diagnoses were *Acinetobacter*, while the phenotypic approach identified 86.7% as *Acinetobacter* spp.

Conclusion: The present study demonstrated a significant difference between the Phoenix and phenotypic methods in identifying the type of bacteria

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Introduction

The “non-fermenting” group includes gram-negative bacilli that do not ferment glucose and other sugars. They constitute about 15% of gram-negative bacilli isolated from hospitalized patients. Although many non-fermenting genera are known, 75% of those of clinical relevance are *Pseudomonas aeruginosa*, and the majority of the remaining 25% are *Acinetobacter*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia*. As a group, they are environmental bacteria and are not usually considered members of the normal flora of the human body, except as colonizers in hospitalized patients (1,2). Gram-negative bacilli include Enterobacteriaceae, many of which are normal flora in the digestive tract. Gram-negative non-fermenting bacilli (such as *Pseudomonas aeruginosa* and *A. baumannii*) are found in the environment and cause human infection when

Key point

A comparative study was conducted to evaluate the effectiveness of the Phoenix method in identifying non-fermenting gram-negative bacilli from blood culture samples. The study compared the Phoenix system with the phenotypic process currently in use. Results showed a significant difference between the two methods, with the Phoenix method identifying 78.5% of the diagnoses as *Acinetobacter*, while the phenotypic approach identified 86.7% as *Acinetobacter* spp.

host defenses are compromised. In laboratory diagnosis, unsuccessful and slow diagnosis of gram-negative non-fermentative disorders is complicated by treatment failure and patient death (1,2).

Usually, bacteria are identified by morphological and biochemical tests and determined by additional specialized tests such as antibiotic sensitivity and resistance



patterns on solid culture medium. The traditional and standard methods of detecting the bacterial cause of infection include microscopic observation (such as gram staining), phenotypic examination of bacterial characteristics, identification of antibodies against bacterial structures, and sensitivity determination. Antimicrobial (antibiogram) may be time-consuming and need more accurate identification and distinguishing between bacteria, especially at the species level (3).

With higher certainty, the Phoenix™ method can provide faster and more reliable detection of bacteria at the genus and species level. In the Phoenix method, various commercial kits such as API 20 NE, VITEK 2, and Phoenix are used for the routine detection of bacteria. The Phoenix system has high sensitivity and automatically identifies bacteria quickly and accurately (4-6).

Considering the importance of non-fermenting gram-negative bacillus bacteria and the risks associated with these bacteria, this comparative study of phenotypic methods with the BD Phoenix M50 automated microbiology system in detecting the genus and species of non-fermenting Gram-negative bacilli isolated from blood in Al-Zahra (S) and Ayatollah Kashani hospitals in Isfahan was conducted.

Objectives

This study evaluated the Phoenix system compared to the currently available phenotypic process.

Materials and Methods

Study design

In this descriptive-analytical cross-sectional study, 30 samples were evaluated from all the patients from AL Zahra and Kashani hospitals who needed blood cultures (2019 -2020). The samples were cultured in Castaneda and BACTEC™ culture medium and then in Eosin-methylene blue (EMB) agar culture medium, blood agar, and chocolate agar accordingly. In the laboratory for seven days, they were checked daily for turbidity, hemolysis, and colony formation. Subculture was prepared too. The characteristics of the microorganism and the time when the sample became positive in each method were recorded separately

in the laboratory. Each positive result was matched with the history and clinical symptoms of the patient, and if it fits, it is valuable, and if it does not match, it is considered as pollution. Grown colonies are coded for each colony after preliminary investigations such as Gram staining, movement, and differential tests such as examination of triple sugar iron (TSI) medium, squalene, oxidation of sugars glucose, lactose, maltose, mannitol, dextrose, and sucrose. In oxidative-fermentative (OF) medium, nitrate reduction, gelatin, and citrate test were performed. If the surface and depth of the TSI medium were red (alkaline/alkaline), other complementary differential tests such as (oxidase, OF and lysine), and deoxyribonuclease (DNase) were performed at a temperature of 44 degrees Celsius (to determine the genus and species). AP120NE biochemical tests were used to confirm the identity of some isolates. In addition to using the standard phenotypic method, the samples were analyzed by the Phoenix™ method to detect non-fermenting gram-negative bacillus bacteria at the genus and species level. These methods were compared consequently.

Statistical analysis

The chi-square test was conducted to compare and find the relationship between the two methods. All the investigations are conducted by SPSS version 27. The significance level was considered as $P < 0.05$.

Results

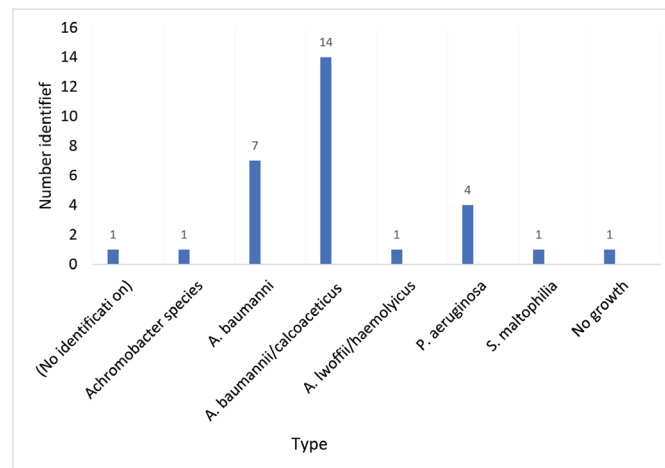
In this study, 30 bacteria were identified. In the Phoenix method, it is observed that 78.5% of the diagnoses were *Acinetobacter*, while in the phenotypic process, 86.7% were *Acinetobacter*. In the Phoenix method, *Acinetobacter* species 1 (3.5%), *A. baumannii* 7 (25%), *A. baumannii/calcoaceticus* 14 bacteria (50%), *Acinetobacter lwoffii/haemolyticus* 1 (3.5%), *P. aeruginosa* 7 (14.2%) and *Stenotrophomonas maltophilia* 1 (3.5%) were detected; however in Phenotypic method 24 (78.5%) *Acinetobacter* and 4 (14.2%) *Pseudomonas* were seen. All descriptive data are shown in Figures 1 and 2.

As shown in Table 1, a statistically significant difference exists between Phoenix and phenotypic methods in

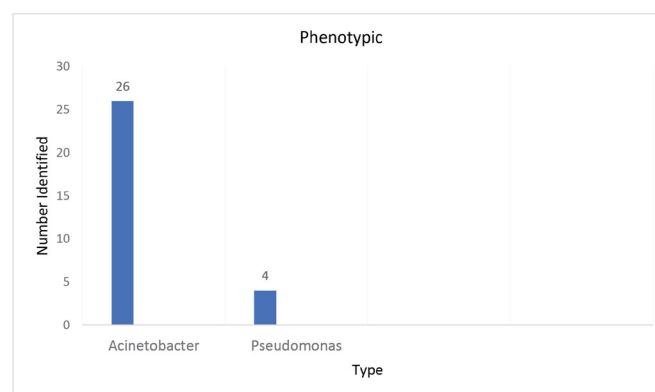
Table 1. Comparison of phenotypic and sequence methods

	Phenotypic		P value*
	<i>Acinetobacter</i>	<i>Pseudomonas</i>	
Phoenix			<0.001
	<i>Achromobacter</i> species	1	
	<i>Acinetobacter baumannii</i>	7	
	<i>Acinetobacter baumannii/calcoaceticus</i>	14	
	<i>Acinetobacter lwoffii/haemolyticus</i>	1	
	<i>Pseudomonas aeruginosa</i>	0	
	<i>Stenotrophomonas maltophilia</i>	1	
	No growth	1	
	No identification	1	

*Pearson's chi-square and likelihood ratio.



Figures 1. Frequency and type of microorganism detected by Phoenix.



Figures 2. Frequency and type of microorganism detected by the phenotypic method.

identifying the type of bacterium ($P < 0.001$).

Discussion

In the current healthcare environment, it is increasingly essential for rapid and accurate diagnosis of infectious diseases. The BD Phoenix™ automated microbiology system was introduced into clinical microbiology laboratories several years ago and its reliability in identifying bacteria from clinical isolates has been well-established (6). The Phoenix system is currently under development at BD Biosciences (7). This study aimed to compare the Phoenix™ to the phenotypic method detection of non-fermenting gram-negative bacteria. In this study, we examined 30 samples, the highest of which was *A. baumannii/calcoaceticus* in the Phoenix method and *Acinetobacter* in the phenotypic process.

The present study is a significant difference between the Phoenix and phenotypic methods in examining the type of bacteria. Several studies have been conducted to compare the BD Phoenix M50 automated microbiology system with conventional or commercial methods for identifying different groups of essential bacteria. In general, several studies have been conducted to compare the BD Phoenix M50 automated microbiology system with conventional

or commercial methods for identifying different groups of essential bacteria, which referred to a number of them.

A comparative study of the Phoenix and Vitek 2 method on 141 samples showed that the overall performance of the Phoenix system was excellent in terms of basic agreement, interpretive agreement, and the rate of significant interpretive errors (ME) or false positives. The Phoenix system showed a slightly higher than expected rate of very major error (false sensitivity), although this error rate was only 1/4 the frequency observed in the Vitek 2 system. The overall agreement for identifying non-fermenting gram-negative bacteria was similar to that reported by others in these reported studies; the performance of the Phoenix instrument was compared directly to the conventional-based identification system (8,9).

A previous study on 741 samples showed that the overall identification accuracy for tested gram-negative isolates was 98.5% at the genus level and 96.9% at the species level (10). Most of the Phoenix detections in this study were obtained between 2 and 4 hours, which should have been investigated in our study.

Another study on 195 samples demonstrated the efficiency of the BD Phoenix™ automated system for all non-fermentative gram-negative organisms (4). All studies

confirm the results of the present study. Studies comparing the Phoenix and phenotype methods in identifying gram-negative bacteria are very limited, and there is a need for further studies in this field.

Conclusion

The present study showed a significant difference between the Phoenix and phenotypic methods in order to determine the type of bacteria. Still, more research is needed due to the limited studies in this field.

Limitations of the study

The limitation of the study was and limited study population. We also suggest that more studies with extended follow-up periods should be performed.

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Authors' contribution

Conceptualization: AB, SE, EA.

Methodology: All authors.

Validation: AB, SE.

Formal analysis: EA.

Investigation: All authors.

Resources: AB, SE, EA.

Data curation: AB, SE, EA.

Visualization: AB, SE, EA.

Supervision: AB, SE.

Project administration: AB, SE, EA.

Funding acquisition: AB, SE, EA.

Writing—original draft: AB, SE, EA

Writing—review and editing: AB, SE.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Isfahan University of Medical Sciences approved this study. (Ethical code#IR.MUI.MED.REC.1399.627). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from the M.D., thesis of Elham Amini (Thesis#399297) at this university. The authors have completely observed ethical issues (including plagiarism, data fabrication, and double publication).

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References

1. Wojewoda CM, Stempak LM; Medical Bacteriology. In: McPherson RA, Pincus MR. HENRY'S Clinical Diagnosis AND Management BY Laboratory Methods, Philadelphia, Elsevier Saunders, 2022; 57; 1130-1148.
2. Gerace E, Mancuso G, Midiri A, Poidomani S, Zummo S, Biondo C. Recent Advances in the Use of Molecular Methods for the Diagnosis of Bacterial Infections. *Pathogens*. 2022 Jun 8;11:663. doi: 10.3390/pathogens11060663. PMID: 35745518; PMCID: PMC9229729.
3. Tang YW, Ellis NM, Hopkins MK, Smith DH, Dodge DE, Persing DH. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. *J Clin Microbiol*. 1998;36:3674-9. doi: 10.1128/jcm.36.12.3674-3679.1998.
4. Snyder J, Munier G, Johnson C. Direct comparison of the BD phoenix system with the MicroScan WalkAway system for identification and antimicrobial susceptibility testing of Enterobacteriaceae and nonfermentative gram-negative organisms. *J Clin Microbiol*. 2008;46:2327-33. doi: 10.1128/JCM.00075-08.
5. Menozzi MG, Eigner U, Covan S, Rossi S, Somenzi P, Dettori G, et al. Two-center collaborative evaluation of performance of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of gram-negative bacteria. *J Clin Microbiol*. 2006;44:4085-94. doi: 10.1128/JCM.00614-06
6. Thomson KS, Robledo IE, Vázquez GJ, Moland ES. KPC screening by updated BD Phoenix and Vitek 2 automated systems. *J Clin Microbiol*. 201;49:3386-7. doi: 10.1128/JCM.00772-11. doi: 10.1128/JCM.00772-11.
7. Muñoz-Cobo B, Sancho-Tello S, Costa E, Bravo D, Torregrosa I, de Lomas JG, et al. Differences in vancomycin minimum inhibitory concentrations for *Staphylococcus aureus* obtained with the automated Phoenix™ system, the Clinical and Laboratory Standards Institute broth microdilution method and the standard Etest. *Int J Antimicrob Agents*. 2011;3:278-9. doi: 10.1016/j.ijantimicag.2010.12.004.
8. Hejna J, Votta M, Rosenberger B, Holliday D, Wiles T. Comparison of Phoenix™ and Vitek® Automated Susceptibility Test Systems for Performance with *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* isolates. 101st General Meeting of the American Society for Microbiology, May; 2001. <https://legacy.bd.com/ds/technicalCenter/whitepapers/LR661.pdf>.
9. Wiles T, Brasso W, Turner D, Holliday D, Fischbein K. Rapid Susceptibility Testing of Gram-Positive Organisms: A Preliminary Study of the Phoenix™ System. 101st General Meeting of the American Society for Microbiology; 2001. <https://legacy.bd.com/ds/technicalCenter/whitepapers/LR641.pdf>.
10. White V, Fischbein K, Dunk T, Williams W, Reuben J, Salomon J, editors. Identification accuracy of Gram-negative bacilli in the BD Phoenix™ automated microbiology system. 101st General Meeting of the American Society for Microbiology, May; 2001. <https://legacy.bd.com/ds/technicalCenter/whitepapers/LR686.pdf>.