Original Article

TJVR 2019; 3(1): 9-12

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Comparison of Some Bacterial Identification Methods

Özgül GÜLAYDIN[1] İsmail Hakkı EKİN[1] Cihat ÖZTÜRK[1] Ziya İLHAN[2] Erdal ÖĞÜN[3]

[1] *Department of Microbiology, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey,*
[2] *Department of Microbiology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey,*
[3] *Department of Molecular Biology and Genetics, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey*

Correspondence: *İsmail Hakkı EKİN (ihekin@yyu.edu.tr)*

Received: 02.01.2019 Accepted: 11.03.2019

ABSTRACT

Objective: In this study, three different methods were compared for the identification of some Gram-positive and Gram-negative reference bacteria.

Material and Methods: For this purpose, the identification accuracy rates of *Enterococcus faecalis, Enterococcus faecium, Streptococcus agalactiae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens* were analysed by conventional bacteriological methods, commercial bacterial identification test kit (Microgen™ ID) and automated bacteria identification system (BD Phoenix 100™).

Results: As a result of analyses, the identification accuracy rates of examined cultures were 94.5%, 95.2%, 94.6%, 89.6%, 91.1%, 92.5%, 86.9%, 96.4% by conventional bacteriological methods, 93.84%, 89.2%, 98.86%, 96.55%, 97.98%, 95.43%, 86.69%, 92.39% by commercial bacterial identification test kit and 98%, 99%, 99%, 96%, 96%, 97%, 99%, 98% by automated bacteria identification system, respectively.

Discussion: In comparison of methods, the identification accuracy values obtained from the automated system were higher than the other methods. It was concluded that automated identification systems that developed for accurate, reliable and rapid identification of bacteria, could be used as an alternative to conventional methods and commercial kits. In addition, it was thought that it would be useful to evaluate the identification of biotypes obtained from clinical isolates by similar methods.

Keywords: *Bacteria, Identification, Conventional methods, Automated systems*

INTRODUCTION

Identification of microorganisms on species level is critical for epidemiological research, antimicrobial therapy and control of infections. The identification of bacteria on species level by conventional bacteriological method is a difficult and time consuming (2-7 days) process. In addition, various media, solutions, reagents and chemical agents are needed in the application of this method. The preparation of media and standardization of the reagents causes loss of time, labour force and high cost. Considering the increase in the number of samples, the conventional method is insufficient for the identification of bacteria in terms of time and labour force according to commercial identification kits, semi-automatic or automatic identification methods and molecular methods.

Commercial bacterial identification test kits consist of dehydrated media placed in small wells. After the incubation period, the test results are evaluated visually according to the colour change by dropping of the special reagents for each parameter well. These kits are easy to use and small in size. However, the necessity of various reagents for colour change is considered a disadvantage in terms of cost and evaluation (Blankenfeld-Enkvist and Brannback, 2002; Fung, 2002; Fung, 2006).

Automated identification system provides highly accurate identification, simple application, rapid and verifiable results, methodological standardization, internal and external quality control applications, delivery of results in digital environment, fast and efficient reporting, require a few numbers of qualified laboratory personnel. In addition, these systems can store the patient's data about

clinical sample, isolate and sensitivity of the antimicrobial agents for many years. Owing to software of these systems many statistical and epidemiological retrospective analysis can be done according to the determined templates (Barenfanger et al., 1999; Ferraro and Jorgenson, 1999; Felmingham and Brown, 2001; Berktaş, 2009).

However, automated identification system requires device maintenance and technical service dependency as well as calibration, control, database update and additional tests. Furthermore, because of depending on the contents of identification panels, microbiologists cannot review the results. Although these systems can reduce the labour force, they require highly qualified technical personnel (Barenfanger et al., 1999; Berktaş, 2009).

In this study, conventional bacteriological methods, commercial bacterial identification test kits and automated bacteria identification system were compared for the identification of some Gram-positive and Gram-negative reference strains. Thus, the reliability of these identification methods used in routine laboratories were evaluated.

MATERIALS and METHODS

Reference Strains: In this study, *Streptococcus agalactiae* ATCC® 13813, *Staphylococcus aureus* ATCC® 6538, *Enterococcus faecalis* ATCC® 29212, *Enterococcus faecium* ATCC® 6057, *Escherichia coli* ATCC® 8739, *Salmonella typhimurium* ATCC® 14028, *Serratia marcescens* ATCC® 14756 and *Pseudomonas aeruginosa* ATCC® 9027 reference strains were used.

Identification by conventional bacteriological methods: For this purpose; haemolysis, catalase, oxidase, coagulase, carbohydrate fermentations, urease, amino acid decarboxylation, methyl red, Voges-Proskauer, nitrate, citrate, hippurate, aesculin and gelatine hydrolysis tests, growth performance on MacConkey agar, EMB agar, TSI agar, SIM medium and 6.5% NaCl medium were used (Carter et al., 1984; Lenette et al., 1985; Koneman et al., 1988; Koneman et al., 1997; Arda et al., 2006). Test results were evaluated manually and analysed by Global Infectious & Epidemiology Network (GIDEON, Ver 2.0) program and the cultures were identified on species level.

Identification by commercial bacterial identification test kits: Three different panels (Microgen™ STREP-ID, Microgen™ STAPH-ID and Microgen™ GnA + GnB-ID) were used to identify the strains. The tests were carried out according to the manufacturer's recommendations. Results were evaluated manually and analysed by the firm's proposed MID Ver 1.2 program for identification.

Identification by automated bacteria identification system: Two different identification panels (BD Phoenix™ PMIC / D-87 and BD Phoenix™ NMIC / D-400) were used in this system. The implementations were carried out according to the manufacturer's recommendations. Results were evaluated automatically by the system's software.

RESULTS

Results of identification by conventional bacteriological methods: While Gram-positive reference strains were identified with the accuracy ratio of 86.9-95.2% (Table 1, Figure 1), Gram-negative reference strains were identified with the accuracy ratio of 86.9-96.4% (Table 2, Figure 2) by conventional bacteriological methods. Although *S. aureus* was identified as 89.6% by the standardized tests, after adding coagulase test the bacteria was identified as 100%.

Table 1. Identification rates of Gram-positive reference strains by three different identification methods.

Reference Strains	Results of Identification (%)		
	Conventional Methods	Commercial Kit	Automated System
E. faecalis ATCC® 29212	94.5	93.84	98
E. faecium ATCC® 6057	95.2	89.2	99
S. agalactiae ATCC® 13813	94.6	98.86	99
S. aureus ATCC® 6538	89.6	96.55	96

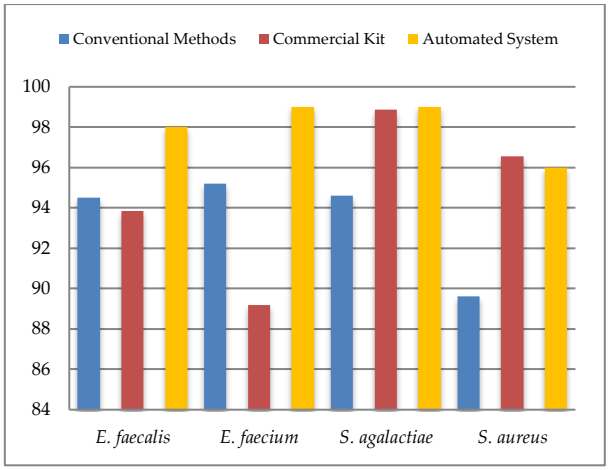

Figure 1. Identification rates of Gram-positive reference strains by three different identification methods

Table 2. Identification rates of Gram-negative reference strains by three different identification methods.

Reference Strains	Results of Identification (%)		
	Conventional Methods	Commercial Kit	Automated System
E. coli ATCC® 8739	91.1	97.98	96
P. aeruginosa ATCC® 9027	92.5	95.43	97
S. typhimurium ATCC® 14028	86.9	86.69	99
S. marcescens ATCC® 14756	96.4	92.39	98

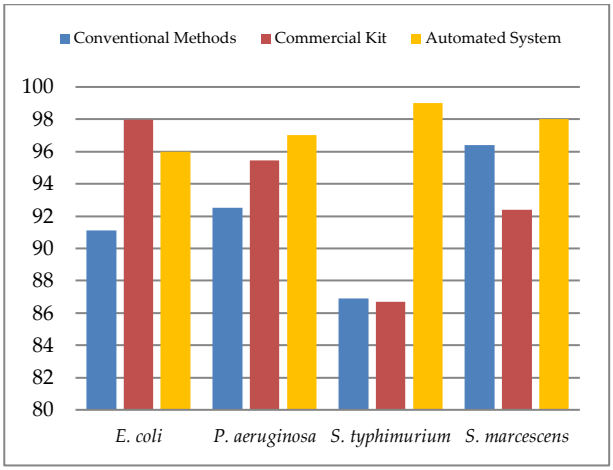

Figure 2. Identification rates of Gram-negative reference strains by three different identification methods

Results of identification by commercial bacteria identification test kit: Gram-positive and Gram-negative reference strains were identified with the accuracy ratio of 89.2-98.86% and 86.69-97.98%, respectively by commercial bacterial identification test kit (Table 1, 2; Figure 1, 2).

Results of identification by automated bacteria identification system: It was observed that all of Gram-positive and Gram-negative reference strains were correctly identified on species level, except *S. typhimurium*. This strain could be identified on genus level by automated bacteria identification system (Table 1, 2; Figure 1, 2).

DISCUSSION

Identification of bacteria by biochemical methods is a time consuming, labour and high cost process. In order to reduce these disadvantages, various commercial kits and automated systems have been developed as an alternative to conventional methods. The advantages and disadvantages of these methods are still discussed.

To date, automated methods, conventional methods and commercial kits were compared for the identification of some Gram-positive and Gram-negative reference bacteria and the reliability values of these methods were evaluated in some studies. In this study, some Gram-negative and Gram-positive reference strains were also identified by conventional bacteriological methods, Microgen bacteria identification test kit and BD Phoenix system.

Menozzi et al. (2006) reported that 384 *Enterobacteriaceae* and 110 non-fermentative isolates were identified on species level by BD Phoenix system as 98.4% and 99.1%, respectively, and the system showed a generally satisfactory performance. Similarly, 251 *Enterobacteriaceae* spp. were identified by BD Phoenix automated system and conventional bacteriological methods. It was reported that the results obtained from the both methods had 95.6% and 94.4% similarity on genus and species level, respectively (Carroll et al., 2006).

In another study, *Enterobacteriaceae* species were identified by BD Phoenix System and Microscan Walkaway system with the accuracy rate of 98.7% and 97.7%, respectively and non-fermentative Gram-negative rods were identified as 100% and 97.7%. As a result, it was concluded that Phoenix system was more reliable in identification of *Enterobacteriaceae* spp. (Snyder et al., 2008). O'Hara (2006) identified 507 *Enterobacteriaceae* isolates by BD Phoenix system. In the study, 456 (89.9%) strains were identified on genus and species level while 20 (3.9%) strains were identified only on genus level correctly. It was also reported that 29 (5.7%) strains were misidentified and the most common misidentification was found in *Salmonella* species.

Stefaniuk et al. (2003) identified a total of 260 bacterial isolates including 174 Gram-negative and 86 Gram-positive bacteria by BD Phoenix automated bacterial identification system and compared the results with conventional bacteriological test methods. In the study, it was reported that similar identification results were obtained from all of Gram-positive cocci, 96% of Gram-negative non-fermentative bacteria and 92.5% of *Enterobacteriaceae* species by both methods.

In a study evaluating the reliability of commercial bacterial identification test kits, it was reported that 95% and 60% of *P. multocida* isolates were identified correctly by API 20NE and API 20E identification kit, respectively (Lizarazo et al., 2008). In another study, it was reported that 53 *P. multocida* isolates were identified with accuracy rate of 100% by Microgen bacteria identification test kit (Gülaydın, 2018). Layer et al. (2006), confirmed 27 reference and 20 clinical staphylococcal isolates with API ID32 STAPH kit. Researchers also reported that 1 reference strain and 1 clinical isolate were identified incorrectly by this testkit.

In this study, it was observed that all the reference strains were identified correctly with different accuracy rate by all identification methods. Gram-positive reference strains were correctly identified as 89.6 - 95.2% by conventional bacteriological methods, 89.2 - 98.86% by Microgen bacteria identification test kit and 96 - 99% by BD Phoenix automated bacterial identification system. With this, the identification rates of Gram-negative reference strains were determined as 86.9 - 96.4%, 86.69 - 97.98% and 96 - 99% by conventional bacteriological methods, Microgen bacteria identification test kit and BD Phoenix bacteria identification system, respectively. According to obtained results, because of using high number of test parameters and evaluating of different enzyme activities of bacteria, BD Phoenix bacteria identification system showed higher accuracy than the other methods.

CONCLUSION

It was concluded that automated identification systems, developed for the accurate, reliable and rapid identification of bacteria, could be used as an alternative to conventional methods in the identification of both Gram-positive and Gram-negative bacterial species.

In addition, because clinical isolates may differ in biochemical properties, the reliability of automated systems should be evaluated periodically in the identification of these isolates. Also, it should be introduced biotype profiles obtained from scientific studies to databases of automated systems.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study was supported by Research Fund of the Van Yuzuncu Yil University with the project number of TSG-2017-6286.

REFERENCES

Arda M. Temel Mikrobiyoloji, 3. Baskı, 80-102, Medisan Yayınları, 2006; Ankara.

Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J Clin Microbiol*, 1999; 37 (5):1415-1418.

Berktaş M. Otomatize tanımlama sistemleri tanımlamada artıları ve eksileri. Klinik Mikrobiyoloji Uzmanlığı ve Laboratuvar Uygulamaları Sempozyumu, 23-24 Ekim 2009 Ankara.

Blankenfeld-Enkvist GV, Brannback M. Technological trends and needs in food diagnostics. *Technol Review*, 2002; 132.

Carroll KC, Glanz BD, Borek AP, Burger C, Bhally HS, Henciak S, Flayhart D. Evaluation of the BD Phoenix Automated Microbiology System for Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae*, *J Clin Microbiol*, 2006; 44 (10), 3506-3509.

Carter GR. Diagnostic Procedures in Veterinary Bacteriology and Mycology. Fourth edition. Springfield, Illinois, 1984; USA.

Felmingham D, Brown DFJ. Instrumentation in antimicrobial susceptibility testing. *J Antimicrobial Chemother*, 2001; 48 (Suppl 1): 81-85.

Ferraro MJ, Jorgenson JH. Manual of clinical microbiology, susceptibility testing instrumentation and computerized expert sysytems for data analysis and interpretation. In: Murray PR, Baron EJ, Pfallller MA, Tenover FC and Yolken RH (eds). 7th ed. Washington, DC: American Society for Microbiology, pp: 1999; 1539-600.

Fung DYC. Rapid methods and automation in microbiology. *Compreh Rev Food Sci Food Safety*, 2002; 1: 3-21.

Fung DYC. Rapid methods and automation in microbiology: 25 years of development and predictions. *Bull Tech U Ist*, 2006; 54 (4): 45-55.

Gülaydın Ö. Sığırların solunum yolundan izole edilen *Pasteurella multocida* fenotip ve genotiplerinde virülens genlerin dağılımı. Van Yüzüncü Yıl Üniversitesi Sağlık Bilimleri Enstitüsü Veteriner Mikrobiyoloji Anabilim Dalı Doktora Tezi, 2018; Van.

Koneman EW, Allen SD, Dowel VR, Janda WM, Sommers HM, Winn WC. Color Atlas and Text Book of Diagnostic Microbiology. Third edition. Lippincott Comp. Philadelphia, 1988; USA.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Guidelines for the collection, transport, processing, analysis and reporting of cultures from specific specimen sources. In: Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC (eds). Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. New York: Lippincott Philadelphia, 1997; pp:121-70.

Layer F, Ghebremedhin B, Moder KA, König W, König B. Comparative study using various methods for identification of Staphylococcus species in clinical specimens. *J Clin Microbiol*. 2006; 44 (8): 2824-2830.

Lenette EH, Balows A, Hausler JWJ, Shadomy JH. Manual of Clinical Microbiology. USA. 1985; 4, 1149.

Lizarazo YAV, Ferri EFR, Martı´n CBG. Evaluation of different API systems for identification of porcine *Pasteurella multocida* isolates. Res Vet Sci, 2008; 85, 453-456.

Menozzi MG, Eigner U, Covan S, Rossi S, Somenzi P, Dettori G, Chezzi C, Fahr AM. Two center collaborative evaluation of performance of the BD Phoenix microbiology system for identification and antimicrobial susceptibility testing of Gram-negative Bacteria. *J Clin Microbiol.* 2006; 44 (11): 4085–4094.

O'Hara CM. Evaluation of the Phoenix 100 ID/AST System and NID panel for identification of Enterobacteriaceae, Vibrionaceae, and commonly isolated nonenteric Gram-negative bacilli. *J Clin Microbiol*. 2006; 44 (3): 928–933.

Snyder JW, Munier GK, Johnson CL. 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 (7): 2327- 2333.

Stefaniuk E, Baraniak A, Gniadkowski M, Hryniewicz W. Evaluation of the BD Phoenix Automated Identification and Susceptibility Testing System in Clinical Microbiology Laboratory Practice, *Eur J Clin Microbiol Infect Dis*, 2003; 22, 479–485.