Suitability Tests for Bacterial Identification and Antibiotic Sensitivity Tests using Microscan Walkaway on Vitek 2

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Abstract

Culture or bacterial culture is the gold standard for diagnostic examination to detect the presence of microorganisms in the patient's body. The development of culture technology and culture-based automatic devices for diagnostic examination are widely researched. At present, there are several types of automatic blood culture instruments in Indonesia, namely Vitek 2 and Microscan Walkaway. This research aims to analyze the suitability of bacterial identification and test antibiotic sensitivity of Microscan Walkaway device from Beckman Coulter and Vitek 2 device from Biomerieux as a gold standard. The research conducted was an observational cross-sectional study. Sampling was done consecutively. The study sample consisted of 202 samples obtained from the results of positive isolates, during January-July 2019 at Dr. Soetomo hospital. Positive isolates were examined for bacterial identification and antibiotic sensitivity using Vitek 2 and Microscan Walkaway. The results were statistically analyzed using SPSS. Examination of bacterial identification using the Microscan device showed 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, whereas with Vitek 2, results showed 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria. Both of these instruments showed identification accuracy of 98.56% for Gram positive bacteria and 100% for Gram negative bacteria with Kappa value: 0.814 and p <0.0001. The results of the accuracy test for antibiotic sensitivity of multidrug resistance bacteria showed compatibility with *p* value <0,0001. There is very good agreement between Vitek 2 and Microscan Walkaway in the identification of bacteria and antibiotic sensitivity.

Keywords: *Identification of bacteria, antibiotic sensitivity, Microscan WalkAway, Vitek 2*

Introduction

Bacterial infection is one of the ten most common causes of death in the world[1]. Microbal floral that is found in clinical specimens obtained from different parts of the human body consists of a variety of different organisms, both pathogenic and non-pathogenic. Traditionally, the diagnosis of bacterial or fungal infection relies solely on culture-based techniques and culture is considered as the gold standard for pathogen diagnosis and detection. However, some organisms may not be easily detected by conventional culture methods used in most laboratories due to various factors. In a conventional clinical microbiology laboratory setting, the majority of microbial cultures of specimens are carried out under aerobic conditions. Standard culture techniques heavily rely on morphological and biochemical characterization for identification, which can lead to decreased specificity. In addition, only a small portion of organisms can successfully be cultured in multi-pathogenic samples. This is mostly caused by various factors, such as rapid growth requirements, non-viable organisms or inhibition of pathogenic organisms due to the production of bacteriocins by other microbes present in clinical specimens[2,3]. These factors make accurate diagnosis and treatment of infections a challenge.

For proper initial empiric therapy, it is important to ensure correct identification of microorganisms for

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accurate diagnosis[4]. Proper antimicrobial therapy has shown to save costs and to help prevent the spread of antimicrobial resistance[5,6].

Automatic culture technique uses a large number of biochemical reactions for the identification of bacteria and broth microdilution for antibiotic sensitivity. In Indonesia, there are currently two automatic culture devices, namely Vitek 2 and Microscan WalkAway. Vitek 2 uses colorimetric technology coupled with the use of three longitudinal waves to provide a general profile of organisms which is important for clinical needs. Antibiotic sensitivity test uses a test card containing standard dilutions of different antibiotics according to the cutoff points established by the Clinical and Laboratory Standards Institute (CLSI). On the other hand, the MicroScan WalkAway utilizes fluorescent technology. MicroScan panels are conventional, consisting of 40 microdilution plate wells[7]. In this study, we aim to determine the suitability of the automated systems from two different manufacturers, BioMérieux (VITEK 2) and Beckman Coulter (MicroScan WalkAway).

Method

The study design used in this research is observational cross-sectional. Research samples in this study consist of the results of positive isolates from January-July 2019 at Dr. Soetomo Hospital. The samples were obtained from the results of each patient's culture, swabs, tissue, pus and peritoneal fluid that met the inclusion criteria. The sampling technique carried out in this research was consecutive sampling. Bacterial identification and sensitivity test were performed using the Vitek 2 and Microscan WalkAway devices. The data obtained was analyzed using SPSS version 24.0.

Result

The number of research isolates was 202 samples. In this study, there were 91 men (45%) and 106 women (52.5%) with ages ranging from 15 days (youngest) until 93 years old (oldest) with overall mean age of 52.72 and median of 58 years old. Research sample data is shown in Table 1.

Table 1. Research sample data with positive culture results (isolates).

Specimen	N	%
Peritoneum Fluid	1	0.5
Blood	22	10.8
Tissue	3	15
Pus	26	12.9
Sputum	56	27.7
Gaster Swab	1	0.5
Nose Swab	2	1
Underarm Swab	1	0.5
Wound Swab	4	2
Pharynx Swab	1	0.5
Between Finger Swab	1	0.5
Throat Swab	5	2.5
Swab urethra	1	0.5
Swab vagina	1	0.5
Urine	76	37.6
Total	202	100

Each sample was examined for bacterial identification and antibiotic sensitivity using the Vitek and the Microscan WalkAway devices. Results of the bacterial identification test were obtained automatically using the GP-67 and GN-93 cards with the Vitek 2 device and the PC-34 and NUC-73 cards with the Microsscan WalkAway device. Data of bacterial identification can be seen in Table 2.

Table 2. Data of bacterial identification on Vitek 2 and Microscan WalkAway devices.

Bakteri Vitek	N	%	Bakteri Microscan	N	%
Achromobacter dentrificans	1	0.5	Achromobacter xylosoxidan	2	1
Achromobacter xylosidan	1	0.5	Acinetobacter baumannii	11	5.4
Acinetobacter baumannii	15	7.4	Aeromonas hydrophila	1	0.5
Aerococcus urinae	1	0.5	Burkholderia cepacian	3	1.5
Burkholderia cepacia	2	1	Cedecea davisae	1	0.5
Citrobacter freundii	1	0.5	Chromobacterium violaceum	1	0.5
Citrobacter koseri	5	2.5	Chryseobacterium indologe	1	0.5
Elizabeth meningoseptica	1	0.5	Citrobacter koseri (ESBL)	1	0.5
Enterobacter aerogenes	1	0.5	Citrobacter koseri	4	2
Enterobacter gallinarum	1	0.5	Elizabethkingia meningose	1	0.5
Enterobacter cloacae	1	0.5	Enterobacter aerogenes (ESBL)	1	0.5
Enterococcus faecalis	14	6.9	Enterobacter cloacae (ESBL)	2	1
Eschericia coli (ESBL)	24	11.9	Enterobacter cloacae	3	1.5
Eschericia coli	29	14.4	Enterococcus durans/hirae	1	0.5
Ewingella americana	1	0.5	Enterococcus faecalis	8	4
Klebsiella oxytoca	1	0.5	Enterecoccus gallinarum	2	1
Klebsiella pneumoniae	20	9.9	Eschericia coli (ESBL)	22	10.9
Klebsiella pneumoniae (ESBL)	10	5	Eschericia coli	22	10.9
Kocuria kristinae	3	1.5	Klebsiella oxytoca (ESBL)	1	0.5
Proteus mirabilis	1	0.5	Klebsiella oxytoca	1	0.5
Providencia rettgeri	1	0.5	Klebsiella ozaenae	1	0.5
Pseudomonas puttida	2	1	Klebsiella pneumoniae (ESBL)	8	4
Pseudomonas aeruginosa	7	3.5	Klebsiella pneumoniae	17	8.4
Salmonella spp	1	0.5	Klebsiella rhinoscleromatis	1	0.5
Serratia fonticola	1	0.5	Proteus mirabilis (ESBL)	1	0.5
Serratia marcescens	1	0.5	Ochrobactrum anthropi	1	0.5
Sphingomonas paucimobilis	1	0.5	Providencia rettgeri	1	0.5
Staphylococcus aureus (MRSA)	5	2.5	Pseudomonas aeruginosa	6	3
Staphylococcus gallinarum	1	0.5	Raoultella ornithinolytic	1	0.5
Staphylococcus hominis	2	1.0	Salmonella enterica	1	0.5
Staphylococcus aureus	18	8.9	Salmonella enterica serot	2	1
Staphylococcus epidermidis	6	3	Serratia fonticola	1	0.5
Staphylococcus haemolyticus	12	5.9	Serratia marcescens	1	0.5
Stenophomonas maltophilia	1	0.5	Shigella sonnei	1	0.5
Streptococcus agalactiae	2	1	Sphingobacterium spiritivorum	2	1
Streptococcus alactolyticus	1	0.5	Staphylococcus aureus (MRSA)	6	3
Streptococcus mitis/oralis	2	1	Staphylococcus aureus	14	6.9
Streptococcus pneumoniae	1	0.5	Staphylococcus epidermidis	5	2.5
Streptococcus pyogenes	1	0.5	Staphycoccus haemolyticus	7	3.5
Streptococcus salivarius	2	1	Staphylococcus hominis	3	1.5
Streptococcus sanguinis	2	1	Staphylococcus hyicus	1	0.5
TOTAL	202	100	Staphylococcus capitis	1	0.5
			Stenophomonas maltophilia	1	0.5

Cont... **Table 2. Data of bacterial identification on Vitek 2 and Microscan WalkAway devices.**

			Staphylococcus sciuri	5	2.5
			Streptococcus mitis/oralis	2	1
			Streptococcus pneumoniae	1	0.5
			Streptococcus pyogenes	1	0.5
			Streptococcus salivarius	2	1
			Streptococcus sanguinis	2	1
			Vibrio species group	1	0.5
			Wautersiella falsenii	1	0.5
			TOTAL	202	100

Results revealed that the identification of bacteria using the Microscan WalkAway device showed 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, whereas with Vitek 2, results showed 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria.

The top three Gram-positive bacteria identified by the Vitek 2 device were *Staphylococcus aureus* (18), *Staphylococcus haemolyticus* (12) and *Staphylococcus epidermidis* (6), while on the Microscan WalkAway the top three bacteria were *Staphylococcus aureus* (14), *Enterococcus faecalis* (8) and *Staphylococcus haemolyticus* (7).

The top three Gram-negative bacteria identified by the Vitek 2 device were *Eschericia coli* (29), *Eschericia coli* (ESBL) (24), *Klebsiella pneumoniSa* (20), while on the Microscan WalkAway the top three bacteria were *Eschericia coli* and *Eschericia coli* (ESBL) (22), *Klebsiella pneumoniae* (17), and *Acinetobacter baumannii* (11).

Antibiotic sensitivity analysis in this study was done to detect the presence of bacteria that cause multidrug resistance (MDR), namely: *Extended-spectrum beta-lactamase* (ESBL), *methicillin-resistant Staphylococcus aureus* (MRSA), and *methicillin-resistant Staphylococcus spp* (MRSS). Results are shown in Table 3, Table 4, Table 5 and Table 6. The p value <0.05 indicates a significant similarity. The suitability of antibiotic sensitivity between the Vitek 2 and Microscan WalkAway devices was analyzed according to the card type, namely AST GP-67 and AST GN-93 cards for the Vitek 2 device and ID/AST PC-34 and ID/AST NUC-73 cards for the Microscan WalkAway device.

Table 3. ESBL phenotype detection.

Device/Antibiotic	CTX	ATM	CAZ	CRO	CTX/CLA and CAZ/CLA
Vitek 2	34	34	34	34	34
Microscan WalkAway	36	36	36	36	36

According to the data in Table 3, it can be seen that the Microscan WalkAway device has the ability to detect more ESBL bacteria with value of 36, while the Vitek 2 device only detected a value of 34.

Table 3. MRSA detection.

Device	MRSA	Interpretation of MIC Oxacillin (>4 μg/l)	Cefoxitin screen result (Resistant)
Vitek 2	5	5	5
Microscan WalkAway	6	6	6

According to the data in Table 4, it can be seen that the Microscan WalkAway device has the ability to detect more MRSA bacteria with value of 6, while the Vitek 2 device only detected a value of 5.

Table 5. MRSS detection.

Device	MRSS	Interpretation of MIC Oxacillin (>4 μg/l)	Cefoxitin screen result (Resistant)
Vitek 2	20	20	20
Microscan WalkAway	22	22	22

According to the data in Table 5, it can be seen that the Microscan WalkAway device has the ability to detect more MRSS bacteria with value of 22, while the Vitek 2 device only detected a value of 20.

Table 6. Antibiotic sensitivity test of ESBL, MRSA and MRSS bacteria between Microscan WalkAway and Vitek 2.

Bacterium	Kappa	p-value
ESBL	0.732	0.0001
MRSA	0.720	0.0001
MRSS	0.688	0.0001

Statistical analysis results showed moderate suitability of ESBL, MRSA and MRSS between Microscan WalkAway and Vitek 2 devices, where *p* value was <0.05.

Discussion

As clinical microbiology laboratories have become increasingly dependent on automated systems, accuracy can be evaluated clinically as well as with reference samples. In this study, there were discrepancies in genus and species. Sensitivity test of various strains between the two systems that are most often used in hospital settings was performed. Other studies have evaluated bacterial identification and antimicrobial sensitivity performance of specific isolates of these systems. However, there are only a few studies with various clinical samples[8,9,10]. The result of bacterial identification using the Microscan device was 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, while Vitek 2 device identified 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria. Both of these devices showed identification suitability of 98.56% for Gram-positive bacteria and 100% for Gram negative bacteria. It can be concluded that results were suitable and there was no significant difference, with Kappa value: 0.814 and *p* <0.0001. Results obtained in this study were align with a research conducted by Jin *et al*. (2011), where out of the 142 isolates identified up to the species level, Vitek 2, Microscan and Phoenix devices had accuracy of

93.7%, 82.4% and 93%. Microscan-Phoenix devices had p value of $p < 0.05$ and Vitek 2-Phoenix devices had p value of $p < 0.05$[10]. According to Hernandez et al (2017), the identification accuracy of Vitek 2 and Microscan devices can be influenced by differences in the number and distribution of organisms tested, software versions, including the number of bacteria tested by the device and the bacteria listed in the device database[7]. This could also be due to the lack of incubation time. Because of this, in a research conducted by Jossart and Courcol (1999), 24 hours of incubation time for non-fermenter bacteria was suggested[11].

Difference of bacteria identification was found between the two devices. *Chromobacterium violaceum* was identified with the Microscan WalkAway device, whereas *Staphylococcus haemolyticus* was detected with the Vitek 2 device. This could be due to the misidentification of bacteria during Gram staining, differences in the number of biochemical tests used and type of bacteria that can be identified by the two devices. *Chromobacterium violaceum* is a Gram-negative bacterium in the *Neisseriaceae* family. Meanwhile, *Staphylococcus haemolyticus* is a Gram-positive bacterium in the *Staphylococcaceae* family.

Phenotypic confirmation of bacteria that produces ESBL, namely positive isolates on the ESBL screen results, must be confirmed with cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM) and a combination of CTX and CAZ with clavulanate (CLA) antibiotics. Results showed value of 34 for the Vitek 2 device and 36 for the Microscan WalkAway device. These results are similar to a study by Linscott et al (2004), where 49 isolates were tested using 4 commercial methods to confirm ESBL. The four devices had different sensitivity values, namely 100% for Microscan, 99% for Vitek, 97% for ESBL Etest and 96% for BD BBL Sensi-Disk ESBL[12].

Antibiotic sensitivity test results between the Microscan WalkAway device and the Vitek 2 device in detecting ESBL showed kappa value of 0.732 and p value of 0.0001. Kappa value of 0.720 and p value of 0 0.001 was obtained for MRSA, and kappa value of 0.688 and p value of 0.0001 was obtained for MRSS. According to these results, it can be said that there is an intermediate suitability between the two devices. Results obtained in this study are parallel to a research conducted by Gherardi et al (2012) where the categorical agreement for antibiotic resistance of Gram-negative bacteria was 97.5% and 98.1% between Vitek and Phoenix devices, whereas the categorical agreement for *methicillin resistant staphylococci* was 94.6% and 100% between the two devices[13].

Conclusion

The results of this study reveal that the Microscan WalkAway device shows equally good ability in bacterial identification and antibiotic sensitivity test with the Vitek 2 device. Further research using PCR to detect genes that cause resistance as a reference method is highly suggested.

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References

1. World Health Organization. The top 10 causes of death. Available from: http:// www.who.int/ mediacentre/factsheets/fs310/en/. Accessed on 16 November 2018.
2. Stewart EJ. Growing unculturable bacteria. J Bacteriol. 2012; 194(16): 4151–60.
3. Nishie M, Nagao J-I, Sonomoto K. Antibacterial peptides "Bacteriocins": an overview of their diverse characteristics and applications. Biocont Sci. 2012; 17(1):1–16
4. Beekmann SE, Diekema DJ, Chapin KC, *et al*. Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. J Clin Microbiol. 2003; 41(7): 3119–3125.
5. Fraser A, Paul M, Almanasreh N, *et al*. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. Am J Med. 2006; 119(11): 970–976.
6. Tumbarello M, Spanu T, Di Bidino R, *et al*. Costs of bloodstream infections caused by *Escherichia*

coli and influence of extended-spectrum-lactamase production and inadequate initial antibiotic therapy. Antimicrob Agents Chemother. 2010; 54(10): 4085–4091.

7. Rhoads S, Marinelli L, Imperatrice CA, *et al*. Comparison of MicroScan WalkAway system and Vitek system for identification of gram-negative bacteria. J Clin Microbiol. 1995; 33(11): 3044-6.
8. Juretschko S, Labombardi VJ, Lerner SA, Schreckenberger PC; Pseudomonas AST Study Group. Accuracies of beta-lactam susceptibility test results for *Pseudomonas aeruginosa* with four automated systems (BD Phoenix, MicroScan WalkAway, Vitek, and Vitek 2). J Clin Microbiol. 2007; 45(4): 1339-1342.
9. Woodford N, Eastaway AT, Ford M, *et al*. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mechanisms responsible for carbapenem resistance in Enterobacteriaceae. J Clin Microbiol. 2010; 48(8): 2999-3002.
10. Jin WY, Jang SJ, Lee MJ, *et al*. Evaluation of VITEK 2, MicroScan, and Phoenix for identification of clinical isolates and reference strains. Diagn Microbiol Infect Dis. 2011; 70(4): 442-447.
11. Jossart MF, Courcol RJ. Evaluation of an automated system for identification of Enterobacteriaceae and nonfermenting bacilli. Eur J Clin Microbiol Infect Dis. 1999; 18(12): 902-907.
12. Linscott JA, Brown JW. Evaluation of four commercially available extended-spectrum beta-lactamase phenotypic confirmation tests. J Clin Microbiol. 2004; 43(3): 1081-1084.
13. Gherardi G, Angeletti S, Panitti M, *et al*. Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. Diagn Microbiol Infect Dis. 2012; 72(1): 20-31.