COMPARISON FOR DETECTION METHODS OF VITK 2 IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF GRAM-NEGATIVE BACTERIA CLINICAL ISOLATED FROM BLOOD CULTURE

Abstract

Background: Thousands of people die each year as a result of bloodstream infections. The fast and exact identification of the causative bacteria that cause these illnesses allows for precise treatment care, which improves the patient's prognosis. In an attempt to shortening the time for microbial identification devices have been developed, including the automated or manual antimicrobial susceptibility testing in clinical laboratories. The aim of this study was to evaluate the accuracy of the Vitek 2 system in the identification and their susceptibility against six classes of antibiotics.

Methods: Based on conventional identification of the isolated was carried out by Gram staining and parallel culture on blood agar, chocolate agar and MacConkey agar, as well as on one drop blood test of Vitek 2 identification and antimicrobial susceptibility testing. The isolates included *Enterobacteriaceae*, *Acinetobacter baumannii* and selected species of non-fermenters.

Results: A total of 36 isolates were recovered from 36 blood cultures, 80% of the isolates were multiple resistant to three antimicrobial agents and more. Resistance to imipenem and meropenem, yet resistance to amikacin was observed in 60% of isolates. The mean time for detection of Gram-negative direct samples less than Statistical analysis, showed significant difference with *Klebsiella pneumoniae* (P=0.014). The finding of this study highlights the emerging trends of *Acinetobacter baumannii* and *Klebsiella pneumoniae* as potential drug resistant pathogen in hospital setting in Saudi Arabia.

Conclusions: The results of the present study show that the indirect method is high accuracy with short times, and especially when Gram-negative bacteria and determine

bacterial identification from positive blood culture without cost expenditure and additional time

Keywords*:* VITAK 2*, Klebsiella pneumoniae*, *Acinetobacter baumannii*, antimicrobial susceptibility testing

INTRODUCTION

Sepsis is the second causes of death among patients who are hospitalized in critically ill patients [1]. The morality rate due to bloodstream infections vary in different parts of the world and range from 20-70% [2,3]. Increased mortality and morbidity of bloodstream infections is often related to insufficient, delayed result and inappropriate antibiotic treatment [2]. Bacteria are well-known as the leading cause of bloodstream infections. 4. Gram-negative bacteria are the cause of 25-30% of bloodstream infections, according to research [5].

One of the main problems of this approach is that the time needed for identification too long turnaround time (3 days or more), failure to identify slow growing and pathogens other than bacteria or yeast. It has been documented that only about 34% of cases of nosocomial infection are detected with blood culture [6].

The objective of this study outlines of advantage this new method may be bring to the rapid identification of bacteria and antimicrobial agent in positive blood culture of patient with bloodstream infections.

MATERIALS AND METHOD

Study design and participants

Isolates study two hundred microorganisms isolated from positive blood cultures of patients hospitalized in intensive care units of Ohud Hospital, Medina, Saudi Arabia, who agreed to participate in this study. The Ohud hospital is a 300 beds facility with all general and subspecialty medical services. The hospital provides primary, secondary care services for Saudi and pilgrims patients. It also provides tertiary care services to all Saudi

citizens on referral bases. The blood samples were inoculated into blood culture bottles and incubated in the BACT/ALERT apparatus.

In the first method one drop taken from blood bottle was extracted in a tube containing 3 ml of sterile normal saline. In the second method, after subculture on blood agar, chocolate agar and MacConkey agar, the isolates were inoculated into the following specific identification cards of the automated Vitek 2 system using the standard protocol [7].

Gram-positive bacilli (GN), will be inoculate into the card from colonies grown on blood agar and Gram-negative bacilli from colonies grown on MacConkey agar, was extracted in a tube containing 3 ml of sterile normal saline (0.45%) to a 0.5 McFarland standard, allowed to stand for few minutes then well vortexes to give uniform inoculums-suspension. all diluted in saline (0.45 % NaCl) [7].

Data collection

Clinical and Laboratory Standards Institute (CLSI) recommendations for 2018 were adopted for culture, isolation and identification of all Enterobacteriaceae isolates and for antibiotic susceptibility testing using the Vitek 2 system (bioMerieux, Marcy L'Etoile, France) using the card for Gram-negative strains (GN cards) and AST-N291 and AST-N292 [4,5] and antibiotic susceptibility testing to beta-lactam/bate-lactamase inhibitor, cephalosporins, aminoglycosides, and carbapenems were performed by disk diffusion method [4,7]. Samples that were tested manually or against only one of the AST-cards or to different AST-cards were excluded, i.e., only samples that were tested against both AST cards were included in the study. Quality control was ensured by testing *E. coli* ATCC 25922 in every batch. The minimal inhibitory concentration (MIC) interpretive standards for *Enterobacteriaceae* were adopted from the CLSI guideline 2018 [4,5,6] for the following classes of antibiotics:

• Group I: penicillin's (ampicillin), beta-lactams/beta- lactamase-inhibitor combinations (ampicillin/sulbactam and piperacillin/tazobactam), 3rd and 4th generation cephalosporins (ceftazidime, cefotaxime ceftriaxone, and cefepime);

• Group II: carbapenems (imipenem, meropenem);

• Group III: fluoroquinolones (ciprofloxacin and levofloxacin);

• Group IV: aminoglycosides (amikacin and gentamicin);

• Group V: folate pathway inhibitors (sulfamethoxazole/trimethoprim);

• Group VII: lipopeptides (colistin), and potential antimicrobial agents;

• tigecycline

Statistical methods

Statistical analysis is performed using SPSS (Statistical Package for Social Sciences) version 17.0 (SPSS Inc., Chicago, IL) and a P-value <0.05 is assumed to be significant.

RESULTS

Of 255 hospitalized patients suspected to have BSIs, only 36 (14%) showed positive blood culture. Most of the Gram-negative bacteria identified by Vitek-2 from of 36 patients with positive blood culture, 22 (61.1%) were of age 60 years or more. Of all patients, 118 (47.2%) received empirical antibiotic treatment.

Table 1 show the bacteria isolated from our patients using direct (colony) and indirect (blood) methods. 36 isolates were Gram-negative bacilli. *Klebsiella pneumoniae* was most often encountered, followed by *Acinetobacter baumannii* , and others isolates. The mean time to detection of Gram-negative direct samples less than Statistical analysis (Chi-square test and Fishers exact test), showed significant difference with *Klebsiella pneumoniae* (P=0.014). Although the range of the mean time to detection was larger among indirect blood compared to direct colony cases, mean and standard deviation values are not significantly different (P=0.07 and P=0.082) (Table 1).

The rates of discrepancy between MIC indirect blood method and direct colony method are summarized in (Table 2, 3, and 4). The discrepancies observed with ampicillin/sulbactam, tazobactam, ceftazidime, cefepime, meropenem, ciprofloxacin, gentamicin and amikacin were within the acceptable limits, while colistin, tigecycline, imipenem, minocycline and cotrimoxazole showed discrepant results above the acceptable limit recommended by CLSI. However, discrepancy in results of Vitek-2 were

seen for five antibiotic isolates of *Klebsiella pneumoniae* which were *Acinetobacter baumannii* and were mis antimicrobial susceptibility test.

DISCUSSION

With an estimated annual occurrence rate of up to 19 million individuals worldwide, bloodstream infection is one of the top causes of death in adult intensive care and patients who have taken antibiotics before microbiological culture [{1}]. Whilst diagnosis and treatment of bloodstream infection have been improved over the last decades [{2}], and guidelines are in place to help physicians with this complex syndrome [{3}], the mortality among hospitalized patients [{7}]. In the present study, we used drop blood turnaround time identification and antimicrobial susceptibility testing diagnosis of bacteremia in patients with suspected bloodstream infections. The results obtained using direct and indirect methods were compared.

According to our report, a higher percentage of bacteremia identification has been reported in these trials compared to blood culture (8)9. In compared to the culture approach, these results suggest that indirect blood methods have higher sensitivity identification and can identify lower quantities of bacteria in blood samples. Every strategy has benefits and drawbacks. However, using a culture-based method, multiple bacterial species can be detected simultaneously, but the most important disadvantages of this method are: (1) it is time consuming more than 72 hours, (2) the bacterial species that are not cultivable are eliminated, and (3) it is incapable to identify bacteria in patients who have taken antibiotics. Although the indirect blood approaches do not have the aforementioned drawbacks, they only identify a limited number of bacterial species. Our findings revealed that prior long-term antibiotic use was a risk factor for bacteremia. This discovery is consistent with the findings of other researchers, and it can help clinicians consider a higher risk of bacteremia in hospitalised patients who have received longer antibiotic courses [{10,11}].

The use of the two drops of blood sample directly from blood culture leads to a reduction in overall time to results from15 to >48 hours (in some studies >72 hours), depending on the generation time growth rate on blood culture. This more rapid time to result is to the advantage of the patient, since, in combination with antibiotic stewardship programs,

optimized antibiotic therapy can very often be administered on the basis of a species/genus identification of the underlying microorganisms [11].

DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval :

The study was approved by the Research Ethics Committee of the General Directorate of Health Affairs in Al Madinah (IRB number: 588).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

REFERENCES

1. Martin G.S., Mannino D.M., Eaton S., Moss M., 2003. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 16, 1546-54.

2. Angus D.C., Linde-Zwirble W.T., Lidicker J., Clermont G., Carcillo J., Pinsky M.R., 2001. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 29(7): 1303-10.

3. Seifert H., 2009. The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. Clin Infect Dis.48, S238-45.

4. Wellinghausen N., Kochem A.J., Disqué C., et al., 2009. Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol. 47, 2759-65.

5. Anbazhagan D., Mui W.S., Mansor M., Yan G.O., Yusof M.Y., Sekaran S.D., 2011. Development of conventional and real-time multiplex PCR assays for the detection of nosocomial pathogens. Braz J Microbiol. 42,448-58.

6. Liesenfeld O., Lehman L., Hunfeld K.P., Kost G., 2014. Molecular diagnosis of sepsis: New aspects and recent developments. Eur J Microbiol Immunol (Bp), 4(1): 1-25**.**

7. Sheldon I.M., 2016. Detection of pathogens in blood for diagnosis of sepsis and beyond. E Bio Medicine. 9: 13-4.

8. Shang S., Chen G., Wu Y., Du L., Zhao Z., 2005. Rapid diagnosis of bacterial sepsis with PCR amplification and microarray hybridization in 16S rRNA gene. Pediatr Res, 58(1): 143-8.9.

9. El Gawhary S., El-Anany M., Hassan R., Ali D., El Gameel Q., 2016. The role of 16S rRNA gene sequencing in confirmation of suspected neonatal sepsis. J Trop Pediatr, 62(1): 75-80.

10. Nguyen M.L., Toye B., Kanji S., Zvonar R., 2015. Risk factors for and outcomes of bacteremia caused by extended-spectrum ßlactamase– producing Escherichia coli and Klebsiella species at a canadian tertiary care hospital. Can J Hosp Pharm,68(2): 136-43.

11. Tängdén T., Cars O., Melhus A., Löwdin E., 2010. Foreign travel is a major risk factor for colonization with Escherichia coli producing CTX-Mtype extended-spectrum beta-lactamases: A prospective study with Swedish volunteers. Antimicrob Agents Chemother, 54(9): 3564-8.

Table 1: The mean time to detection (TTD) of different organisms in two methods:

Organisms	Mean time to detection of identification (hours)		*P*-value
	Direct (colony)	Indirect (blood)	
	Mean ± SD Min. – Max.	Mean ± SD Min. – Max.	
Klebsiella pneumoniae	10.39 ± 1.053	11.448 ± 1.22	0.014

(n=17)	9.07 – 13.13	9.59 – 14.59	
Acintobacter baumannii complex (n=12)	9.528 ± 2.30 7.0 – 14.55	11.412 ± 2.747 7.17 – 15.05	0.082
Other isolates (n=7)	11.399 ± 1.55 9.59 – 13.35	9.369 ± 2.205 7.17 – 13.58	0.07

Table 2: Antibacterial susceptibility pattern of *Klebsiella pneumoniae* isolated from two methods.

Antibiotic	Number of susceptibilities *Klebsiella pneumoniae* at indicated concentration						P-value
	Direct (colony)			Indirect (bloody)			
	S	I	R	S	I	R	
Amp/Sulbactam			17 (100 %)			17 (100 %)	----
Tazobactam			17 (100 %)			17 (100 %)	----
Ceftazidime			17 (100 %)			17 (100 %)	----
Cefepime			17 (100 %)			17 (100 %)	----
Imipenem	1 (5.9 %)		16 (94.1	0 (0 %)		17 (100 %)	0.500

			%)				
Meropenem			17 (100 %)			17 (100 %)	----
Amikacin	5 (29.4 %)		12 (70.6 %)	6 (35.3 %)		11 (64.7 %)	0.714
Gentamicin	1 (5.9 %)	4 (23.5 %)	12 (70.6 %)	2 (11.8 %)	8 (47.1 %)	7 (41.2 %)	0.225
Ciprofloxacin			17 (100 %)			17 (100 %)	----
Minocycline		4 (23.5 %)	13 (76.5 %)		3 (17.6 %)	14 (82.4 %)	0.50
Cotrimoxazole			17 (100 %)			17 (100 %)	----
Tigecycline	9 (52.9 %)	8 (47.1 %)	0 (0 %)	6 (35.3 %)	9 (52.9)	2 (11.8 %)	0.265
Levofloxacin			17 (100 %)			17 (100 %)	----

Table 3: Antibacterial susceptibility pattern of *Acintobacter baumannii* isolated from two methods.

Antibiotics	No. of susceptibility *Acintobacter baumannii* complex at indicated concentration						P-value
	Colony			Blood			
	S	I	R	S	I	R	
Amp / Sulbactam			12 (100 %)			12 (100 %)	----
Tazobactam			12 (100 %)			12 (100 %)	----
Ceftazidime			12 (100 %)			12 (100 %)	----
Cefepime			12 (100 %)			12 (100 %)	----
Imipenem		0 (0 %)	12 (100 %)		1 (8.3 %)	11 (91.7 %)	0.50
Meropenem			12			12	----

			(100 %)			(100 %)	
Amikacin			12 (100 %)			12 (100 %)	----
Gentamicin		1 (8.3 %)	11 (91.7 %)		1 (8.3 %)	11 (91.7 %)	0.761
Ciprofloxacin			12 (100 %)			12 (100 %)	----
Minocycline	4 (33.3 %)	1 (8.3 %)	7 (58.3 %)	4 (33.3 %)	0 (0 %)	8 (66.7 %)	0.587
Colistin	11 (91.7 %)		1 (8.3 %)	12 (100 %)		0 (0 %)	0.50
Cotrimoxazole	8 (66.7 %)		4 (33.3 %)	7 (58.3 %)		5 (41.7 %)	0.50
Tigecycline	12 (100 %)	0 (0 %)		7 (58.3 %)	5 (41.7 %)		0.019

Table 4: Antibacterial susceptibility pattern of other isolated from two methods.

Antibiotics	Number of susceptibilities other isolates at indicated concentration						P-value
	Direct (colony)			Indirect (bloody)			
	S	I	R	S	I	R	
Ampicillin / Sulbactam			7 (100 %)			7 (100 %)	----
Tazobactam	1 (14.3 %)		6 (85.7 %)	1 (14.3 %)		6 (85.7 %)	0.769
Ceftazidime	1 (14.3 %)		6 (85.7 %)	1 (14.3 %)		6 (85.7 %)	0.769
Cefepime	1 (14.3 %)		6 (85.7 %)	1 (14.3 %)		6 (85.7 %)	0.769
Imipenem	7 (100 %)			7 (100 %)			----
Meropenem	7 (100 %)			7 (100 %)			----
Amikacin	7 (100 %)			7 (100 %)			----
Gentamicin	1 (14.3 %)		6 (85.7 %)	1 (14.3 %)		6 (85.7 %)	0.769
Ciprofloxacin	7 (100 %)			7 (100 %)			----
Minocycline	6 (85.7 %)		1 (14.3 %)	6 (85.7 %)		1 (14.3 %)	0.769
cotrimoxazole			7 (100 %)			7 (100 %)	----
Tigecycline	7 (100 %)			7 (100 %)			----