**Original Research Article**

# Comparison for detection methods of Vitek 2 identification and antimicrobial susceptibility testing of gram-negative bacteria clinical isolated from blood culture



## Abstract:

Background: Bloodstream infections are responsible for thousands of deaths each year. The rapid identification of the microorganisms causing these infections permits correct therapeutic management that will improve the prognosis of the patient. In an attempt to reduce the time spent on this step, microorganism identification devices have been developed, including the automated or manual antimicrobial susceptibility testing in clinical laboratories. This study was to evaluate the accuracy of the Vitek 2 system in the identification of microorganisms isolate from blood cultures and compare the results to those obtain with direct from blood culture and from colony in MacConkey agar.

Methods: Primary identification of the isolates was carried out by Gram stain and parallel culture on blood agar, chocolate agar and MacConkey agar, as well as on one drop blood of Vitek 2 identification and antimicrobial susceptibility testing. They include *Enterobacteriaceae, Acintobacter baumannii* and selected species of non-fermenters. Results will be compared to identification and antimicrobial susceptibility testing by MICs determined using the CLSI broth microdilution method. 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 60% of isolates. The mean time to detection 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 *Acintobacter baumannii* and *Klebsiella pneumoniae* as potential drug resistant pathogen in hospital setting in Saudi Arabia, the indirect method is a rapid and sensitive technique specially for diagnosing sepsis among patients in whom bacteremia is difficult to diagnose with culture method including neonates intensive care, adult intensive care and patients who have taken antibiotics before microbial culture.

Keywords: *Klebsiella pneumoniae*, *Acintobacter baumannii*, antimicrobial susceptibility testing



## Introduction:

Sepsis is a global health problem and an estimated 17 million cases of sepsis occur each year in the world (1). The early initiation of appropriate antibiotic therapy is determinant for the prognosis and survival of patients with bloodstream infections (2). Patients receiving antibiotic therapy that is adapted based on the susceptibility profile of the infectious agent isolated from blood.

cultures exhibit lower mortality than those treated initially with inadequate antibiotic therapy (3). In addition, technological advances that permit the rapid and reliable identification of most pathogens involved in infectious diseases have long been recognized to have clinical benefits, including shorter hospital stays and lower mortality, as well as financial benefits by reducing healthcare costs (4). The objective of this study will be evaluating the sensitivity of the Vitek 2 system, a system that automatically performs the processes required for microorganism identification and for the determination of antimicrobial susceptibility using a standard primary inoculum isolated from subcultures of positive blood cultures. Although classical identification methods are still considered the gold standard, these methods are slow, time consuming and prone to subjective interpretations. On the other hand, the Vitek 2 system reduces the time necessary for identification and permits the standardization of inter- and intra-laboratory results, the storage of results, issuing rapid epidemiological reports, and simultaneous identification and antimicrobial susceptibility testing. Studies using direct inoculation of Vitek 2 cards from blood culture bottles have been conducted in an attempt to further reduce the time of identification of microorganisms that cause bloodstream infections, but the results were acceptable only for Gram-negative bacilli (5, 6). For this reason, the present study use inocula of microorganisms cultured previously on solid media for 24 h.

## Materials and Methods:

### Study design and participants

Isolates study one 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 bottles were inoculated into blood culture bottles and incubated in the BACT/ALERT apparatus.

Identification of microorganisms in positive blood cultures Automated phenotypic identification Samples exhibiting microbial growth were submitted to Gram staining, cultured on solid media and directly from the blood culture bottles. The first method one drop taken from blood bottle was extracted in a tube containing 3 ml of sterile normal saline. Second method, after subculture on blood, chocolate and MacConkey agar, the isolates were inoculated into the following specific identification cards of the automated Vitek 2 system using the standard protocol:

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

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. 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 (5,6,7) for the following groups of antibiotics:

- group I: penicillins (ampicillin), beta-lactames/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.

## Result:

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 included 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 direct (colony) and indirect (blood). Of the 36 isolates was Gram-negative bacilli. *Klebsiella pneumoniae* was most often encountered, followed by *Acinobacter baumannii complex,* and others isolates. The mean time to detection 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).



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<br>Min. – Max. | Mean ± SD<br>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<br>7.0 – 14.55 | 11.412 ± 2.747<br>7.17 – 15.05 | 0.082 |
| Other isolates (n=7) | 11.399 ± 1.55<br>9.59 – 13.35 | 9.369 ± 2.205<br>7.17 – 13.58 | 0.07 |

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 5 antibiotic isolates of *Klebsiella pneumoniae* which were *Acintobacter baumannii* and were misantimicrobial susceptibility test.



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 | | | 17 | ---- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | (100 %) | | | (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 | | | 12 | ---- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | (100 %) | | | (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 (100 %) | | | 12 (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 %) | | | ---- |

## 4. DISCUSSION

Bloodstream infection is one of the most important causes of mortality among hospitalized patients (7). Rapid diagnosis and proper treatment of this infection could be effective in reducing the mortality rate associated with bloodstream infections (7). In the present study, we used drop blood for rapid identification and antimicrobial susceptibility testing diagnosis of bacteremia in patients with suspected bloodstream infections. The results obtained were compared between direct and indirect methods to the results of blood culture.

In these studies, in accordance with our report, a higher rate of identification of bacteremia in comparison to blood culture has been reported (8&9). These results show that the indirect blood methods have higher sensitivity identification and can detect lower levels of bacteria in the blood samples in comparison to culture method. All techniques have advantages and disadvantages. However, using a culture 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 methods do not have the mentioned limitations, these techniques specifically identify only a group of desired bacterial species. Our results showed that the previous long-term antibiotic use was a risk factor associated with bacteremia. This finding is similar to other researcher findings and can

help clinicians to consider a higher probability of bacteremia in hospitalized patients who have received longer courses of antibiotic therapy (10 &11).

Finally, the findings of the present survey revealed that the indirect blood method is a rapid and sensitive technique specially for the diagnosis of sepsis among patients in whom bacteremia is difficult to diagnose with culture method including neonates intensive care, adult intensive care and patients who have taken antibiotics before microbial culture.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.



## 5. References

1. Instituto Latino Americano para Estudos da Sepse. Sepse: um problema de saúde pública. rasília: CFM; 2015.
2. Diament D, Salomão R, Rigatto O, Gomes B, Silva E, Carvalho NB, Machado FR. Guidelines for the treatment of severe sepsis and septic shock—management of the infectious agent—diagnosis. Rev Bras Ter Intensiva. 2011;23(2):134–44.
3. Weinstein MP, Murphy JR, Reller LB, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev Infect Dis. 1983;5(1):54–70.
4. Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J Clin Microbiol. 1999;37(5):1415–8.
5. Gherardi G, Angeletti S, Panitti M, Pompilio A, Di Bonaventura G, Crea F, Avola A, Fico L, Palazzo C, Sapia GF, Visaggio D, Dicuonzo G. 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.
6. Prod'hom G, Durussel C, Greub G. A simple blood-culture bacterial pellet preparation for faster accurate direct bacterial identification and antibiotic susceptibility testing with the VITEK 2 system. J Med Microbiol. 2013;62(5):773.
7. Sheldon IM. Detection of pathogens in blood for diagnosis of sepsis and beyond. EBioMedicine 2016; 9: 13-4.

8. Shang S, Chen G, Wu Y, Du L, Zhao Z. Rapid diagnosis of bacterial sepsis with PCR amplification and microarray hybridization in 16S rRNA gene. Pediatr Res 2005; 58(1): 143-8.9.
9. El Gawhary S, El-Anany M, Hassan R, Ali D, El Gameel Q. The role of 16S rRNA gene sequencing in confirmation of suspected neonatal sepsis. J Trop Pediatr 2016; 62(1): 75-80.
10. Nguyen ML, Toye B, Kanji S, Zvonar R. 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 2015; 68(2): 136-43.
11. Tängdén T, Cars O, Melhus A, Löwdin E. 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 2010; 54(9): 3564-8.