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Performance of VITEK 2 in the routine identification of bacteria from positive blood cultures in Sulaimani pediatrics' hospital

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Abstract

Sepsis is a major cause of death worldwide among hospitalized patients, however, an accurate and advanced identification method is associated with improved sepsis patient survival. This Retrospective study carried out in Sulaimani pediatric teaching hospital from January 2014 to July 2015 and aimed to compare the effectiveness of VITEK 2 system with traditional manual procedures for identification of pathogenic bacteria in patients with a serious disease like sepsis. The positive blood cultures were divided into two groups; 138 positive cultures identified by conventional manual methods and 104 positive cultures identified by automated VITEK 2 system. The results showed that VITEK 2 system identified 16 genera and 30 species whereas only nine genera with seven species diagnosed by using routine method. The most important result during this study was the identification of five uncommon bacterial genera *Kocuria*, *Leuconostic*, *Cedecea*, *Pantoea* and *Burkhoderia* which have never been diagnosed in the microbiology laboratory of the hospital until the modern automated system VITEK 2 use. In conclusion, using VITEK 2 is required to enhance the performance of hospital's microbiology laboratory which is essential for accurate diagnosis and prompt effective treatment of blood stream infections.

Keywords: Sepsis, VITEK 2, Blood culture

أداء جهاز VITEK2 في الفحوصات الروتينية لتشخيص البكتريا من مزارع الدم الموجبة في مستشفى الاطفال في السليمانية

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الخلاصة

يعد تسمم الدم سببا رئيسا للوفاة بين المرضى الراقدين في المستشفى وان دقة التشخيص باستعمال طرق حديثة يكون مرتبطا بتحسين فرصة المريض للنجاة. اجريت هذه الدراسة في مستشفى الاطفال في السليمانية للفترة من بداية شهر كانون الثاني 2014 والى نهاية شهر حزيران 2016 .هدف الدراسة هوالمقارنة بين الجهاز الالي VITEK2 و الطرق اليدوية التقليدية المستعملة لتشخيص البكتريا المرضية للمرضى المصابين بتسمم الدم. شملت الدراسة تسجيل نتيجة التشخيص لكل الحالات التي اظهرت نتيجة زرع دم موجبة والتي قسمت الى مجموعتين: الاولى تضمنت 138 عينة شخصت بواسطة الطرق الروتينية والثانية تضمنت

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104عينة شخصت بواسطة الجهاز الالي VITEK2. اسفرت نتائج التشخيص باستعمال VITEK2 عن تشخيص 16 جنس بكتيري و 30 نوع بينما لم تشخص الا 9 اجناس و 7 انواع باستعمال الطرق التقليدية.

ان اهم نتيجة استحصلت من هذه الدراسة كانت تشخيص 5 اجناس لبكتريا غير شائعة (*Kocuria*, *Leuconostic, Cedecea, Pantoea* and *Burkhoderia*) والتي لم تشخص سابقا في مختبر المايكروبايولوجي ولكن شخصت فقط عندما بدأ العمل بنظام التشخيص الالي. نستنتج من هذه الدراسة ان استعمال جهاز VITEK2 ضروري لتحسين الاداء في قسم المايكروبايولوجي والذي يعد اساسيا للحصول على تشخيص دقيق يؤدي الى معالجة فعالة لحالات تلوث مجرى الدم.

Introduction

Sepsis is a major cause of death worldwide among hospitalized patients. Sepsis is different than any other type of microbial infection, early appropriate antibiotic therapy is crucial for patient survival [1]. The first 24 h of patient care is critical for determining and administering the appropriate antimicrobial therapy. The mortality rates increase by approximately 7% for every hour a septic patient remains untreated or receives inappropriate antimicrobial therapy [2]. Patients with positive blood culture are 12 times more likely to die during hospitalization than those with negative blood culture [3] .Initial therapy must be empirically based on likely pathogens and typical patterns of antimicrobial susceptibility. Therefore, the microbiology laboratory plays its most important role when the actual pathogen and the antimicrobial susceptibility deviate from that predicted by the clinician [2].

Traditionally, bacterial identifications in clinical microbiology laboratories are mainly performed according to phenotypic characteristics, gram stain and various biochemical reactions. All of these methods cannot achieve high accuracies of identification at the level of species and it takes at least one day or longer to complete the whole identification process [4].

In the last 30 years, a variety of automated systems for the identification and antimicrobial susceptibility testing of microorganisms has been developed based on automated interpretation of the results of biochemical tests or using microdilution trays following overnight incubation and photometric determination of growth [5-7].

One of these automated instruments is VITEºK system which was originally designed as an onboard system for the detection and identification of urinary tract pathogens from astronauts in spacecraft. It was first introduced in clinical laboratories in 1979 and has since been evaluated extensively [8]. Recently, the new VITEK 2 system was introduced and it's widely used in clinical laboratories all over the world but more recently it became available in our hospital's laboratories. This system automatically performs all of the steps required for identification and antimicrobial susceptibility testing after a primary inoculum has been prepared and standardized [9]. VITEK 2 system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals [10] .This advanced technology which provide rapid bacterial identification and antimicrobial susceptibility testing are now recognized as having both clinical and financial benefits .Using automated technique in conventional laboratory provide easier, faster and more accurate bacterial identification which is important especially in cases when patients are suffering from infectious diseases and where therapeutic intervention is urgently needed[11]

Thus, this study aimed to compare the effectiveness of VITEK2 system with traditional manual procedures for identification of pathogenic bacteria in patients with a serious disease like sepsis.

Materials and methods

This study was retrospectively conducted at Sulaimani pediatrics' hospital in Kurdistan region of Iraq from January 2014-July 2015. During 18 months, the received blood culture bottles were loaded into the automated system BacT/ALERT® 3D (bioMerieux), positive blood bottles were sub-cultured on three type of culture media: blood agar, MacConkey and chocolate agar. Blood agar and MacConkey plates were incubated at 37°C under aerobic condition while chocolate agar incubated under microaerophilic condition by using anaerobic jar with a GasPak at the same temperature. The plates were examined for growth after 24-48 hours of incubation and the result of bacterial identification was recorded.

This study was done by dividing the results of the identification of positive cultures in to two groups; the first group included 138 positive blood cultures collected during the first nine months and

identified by the conventional methods, and the second group included 104 positive blood cultures collected during the next nine months and identified by the automated system VITEK 2 (bioMerieux). The conventional method includes the standard microbiology technique, namely: Gram staining, catalase test, coagulase test (slide method), growth on mannitol salt agar, hemolytic activity on blood agar plate, susceptibility to Novobiocin, Optochin and Bacitracin disk diffusion test for gram-positive bacteria .On the other hand, oxidase test, urease test, triple sugar iron and hydrogen sulphide production were performed for gram-negative bacteria. The identification with VITEK 2 includes ID-GN card for gram-negative bacilli and ID-GP card for gram-positive bacteria.

Results

A total of 242 positive blood culture specimens were tested. Of the 138 positive cultures tested and identified by conventional manual methods, nine genera with seven species were recorded (Table-1). While among the 104 positive cultures tested and identified by automated VITEK2 system, 16 genera and 30 species were registered Table-2.

Table 1-Results of 138 positive blood culture identified by conventional method.

Organism	Number of identified isolates
Gram-negative bacteria	
Escherichia coli	15
Proteus spp	3
Klebsiella spp	2
Enterobacter spp	13
Pseudomonas aeruginosa	6
Acinetobacter spp	4
Salmonella typhi	3
Gram-positive bacteria	
Staphylococcus aureus	67
Staphylococcus saprophyticus	2
Staphylococcus spp	6
Streptococcus pneumoniae	3
Streptococcus pyogenes	2
Streptococcus spp	12

The most important result with VITEK2 is the identification of three uncommon Gram-negative genus; *Burkholderia cepacia, Cedecia lapagei* and *Pantoea agglomerans* and two genera of Gram-positive bacteria with four species; *Kocuria varians, Kocuria kristinae, Leuconostoc mesenteroides, Leuconostoc seudomesenteroides* Table-2.

Table 2- Results of 104 positive blood culture identified by VITEK2.

Organism	Number of identified isolates
Gram-negative bacteria	
Escherichia coli	12
Proteus mirabilis	1
Klebsiella pneumoniae	6
Klebsiella spp	1
Enterobacter aerogenes	1
Enterobacter cloacea	2
Enterobacter faecium	3
Enterobacter spp	1
Pseudomonas aeruginosa	2
Pseudomonas luteola	1
Acinetobacter radioresistens	1
Acinetobacter banmannii	2
Salmonella typhi	3
Uncommon Gram-negative	
Burkholderia cepacia	1
Cedecia lapagei	1
Pantoea agglomerans	1
Gram-positive bacteria	
Staphylococcus aureus	21
Staphylococcus saprophyticus	1
Staphylococcus hominis	2
Staphylococcus haemolyticus	3
Staphylococcus xylosus	1
Staphylococcus lentus	9
Staphylococcus spp	5
Streptococcus pneumoniae	2
Streptococcus pyogens	2
Streptococcus mutant	1
Streptococcus spp	6
Enterococcus faecium	2
Enterococcus faecalis	2
Micrococcus luteus	1
Uncommon Gram-positive	
Kocuria varians	1
Kocuria kristinae	2
Leuconostoc mesenteroides	3
Leuconostoc seudomesenteroides	1

Also the results showed a difference in the variation of the species among coagulase negative staphylococci species with the identification of only one species by conventional method versus five species identified by VITEK 2.

Discussion

Sepsis is a global health problem that carries a high risk of death. UNICEF announced that more than 40% of under-five deaths globally occur in the neonatal period, resulting in 3.1 million newborn deaths each year [12], moreover the World Federation of Pediatric Intensive Care and Critical Care Societies recorded that In the developing world, sepsis accounts for 60-80% of lost lives per year, affecting more than 6 million newborns and children annually [13].

The type of organism causing severe sepsis is an important determinant of outcome [14]. An epidemiological study on Sepsis done in the United States from 1979 through 2000 proved that Bacteria are the most common causative microorganisms in sepsis [15]. Rapid and reliable species

identification of these organisms is essential for accurate diagnosis and prompt effective treatment of these infections [11, 16-19]. Traditional methods of bacterial identification which rely on phenotypic identification (differential staining, culturing on selective media and some biochemical methods) is not sufficient to reach the final identification of many uncommon genera and most of the species because some strains require more specific culture media and biochemical tests which are not available in our laboratories. Besides, there are some strains which exhibit unique biochemical characteristics that do not fit into the identification chart that are usually used in our laboratories as a guide for the identification of bacterial genera and species. That was obvious in the results of Table-1 when only nine genera with seven species that fit the chart were identified .Laboratories have no difficulty in identifying typical strains of common bacteria using commonly available tests. Problems arise when atypical strains or rare or newly described species are isolated and need to be identified, such situation lead to the result of misidentified or unidentified strains. For this reason, a number of automated commercial systems have been evaluated for routine laboratory use. Many peer-reviewed publications demonstrated that automated VITEK 2 technology and VITEK 2 ID cards provide reliable and accurate results for clinically important Gram-positive cocci and Gram-negative bacilli [9, 20-24]. In this study, the automated system VITEK2 (Table-2) successfully identified 16 genera and 30 species. That noticeable difference between the two methods in the diagnosis at the level of genus and species among the isolates due to the ability of VITEK2 to identify more than 150 fermentative and non-fermentative Gram-negative bacilli, and up to 120 organisms significant non-spore-forming Gram-positive bacteria with a high discrimination between species and low rate of multiple choice or misidentification. In this study, VITEK 2 shows advantage with the identification of five uncommon genera; *Kocuria, Leuconostic, Cedecea, Pantoea* and *Burkhoderia* which have never been diagnosed before in the microbiology laboratory of pediatric teaching hospital. Chris Higgins [25] mentioned that all known pathogenic bacterial species and many usually non-pathogenic (opportunistic) species have been isolated from blood in particular cases. The opportunistic species represent a threat to immunosuppressed patients, therefore it must be recognized as a potential pathogen for sepsis. Another advantages of VITEK 2 system is identification of a verity of *Staphylococcus* species which cannot be identified by conventional method. VITEK 2 shows a verity of coagulase negative Staphylococci genus and species (Table-2). Many researches have mentioned that the increased use of broad-spectrum antibiotics during the last two decades paired with the growing number of immunocompromised and seriously ill patients, has led to the emergence of coagulase-negative staphylococci particularly, and these organisms plays a prominent role in nosocomial bloodstream infections [26, 27]. Coagulase-negative staphylococci, which were once considered simple commensal organisms of human skin and mucous membranes, are now common opportunistic pathogens in intensive care units [28-30]. In clinical laboratories that use limited manual tests alone, the organism responsible for the Staphylococci infection can be misidentified.

In conclusion, accurate identification of bacterial isolates is crucial for the correct management of bloodstream infections. The diversity of organisms causing sepsis varies from region to another and changes over time even in the same place [31, 32] so it is very important to correctly identify the Gram-positive and Gram-negative bacteria at the level of genus and species (especially the new and uncommon bacteria) which is essential for providing a reliable epidemiological surveillance of the bacterial causative agents of sepsis.

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