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Research Article

Evaluation of complete blood count, c-reactive protein, and lactate dehydrogenase in culturable and unculturable bacteremia for early diagnosis of sepsis

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Abstract
Bacteremia is a bacterial infection that enters the bloodstream. This study was designed to investigate if culture methods could detect the bacterial infection of the bloodstream and to assess certain criteria for bacterial sepsis in culturable and non-culturable blood samples from hospitalized patients, like C-reactive protein (CRP), lactate dehydrogenase (LDH), and complete blood count (CBC). A total of 100 blood samples from patients with symptoms of sepsis who resided in the Hilla City hospital were collected as well as 25 samples as a healthy control group without disease or inflammation. Each sample was divided into three containers; 2ml in an EDTA tube for the CBC test, 3ml in a centrifuged tube for the CRP and LDH test, and 3-7ml in brain heart infusion broth for blood culture. The study result showed that 65 (65%) of 100 samples had abnormal tests, whereas only 25 (25%) of 100 were culture positive. There was a statistically significant difference between patients and control regarding all parameters CRP, LDH, white blood cell (WBC), lymphocyte (LYM) and granulocyte (GRA) (P= 0.000). No significant association in studied parameters was observed between culture-positive and culture-negative patients. Significant strong positive correlation was observed between WBC and LDH (r= 0.332) (p= 0.007), LYM and GR) (r= -0.983) (p= 0.000), LYM and CRP (r= 0.257) (p= 0.03), and between GRA and LDH (r= 0.254) (p= 0.04). Therefore, the estimation of WBC, granulocytes, lymphocytes count, CRP and LDH values and blood culture results may help in the early identification of the causative agent of sepsis.

Keywords: Bacteremia, Blood culture, C-reactive protein, Lactate dehydrogenase, Sepsis

INTRODUCTION

Bacteremia is a bacterial infection that spreads to the bloodstream. It may be transient, intermittent or continuous. This is serious since it can lead to a variety of diseases in the body (Corey, 2009). Humans usually acquire bacteria either as a result of normal activities or from infection due to the use of indwelling genitourinary or IV catheters, for example, urinary tract infection. Bacteremia that occurs during ordinary activities does not result in infections because bacteria are present in small numbers and are quickly cleared by the immune system. However, it can lead to other infections and occasionally induce a fatal body response called sepsis if bacteria are present long enough and in large enough quantities, especially among elderly patients or those who have a weakened immune system (Mammen *et al*., 2018).

Bacteremia can be caused by a wide range of bacterial species that might be able to spread from the primary site of infection and reach the bloodstream. Isolation of this organism from blood cultures could indicate actual bacteremia (Kleinschmidt *et al.,* 2015). Sepsis is a pattern of immunological response to damage. The severity of this disease varies depending on the pathogen, the host, and how quickly it is detected and treated (Singer *et al*., 2016).

Despite the fact that Blood culture represented the gold standard and most commonly used diagnostic method for detecting bacteremia (Nannan Panday *et*

al., 2019), this method is insufficiently sensitive when the patient has already received antibiotics, or in the presence of fastidious organisms that cannot grow in normal conditions, there are more specific approaches for detecting bacteria have evolved (Peker *et al*., 2018). Since bacterial infection trigger both innate and adaptive immune system in response to the pathogen-associated pattern, sepsis may emerge as a hyper-inflammatory state. In this situation, it has been proved that a number of biomarkers can confirm bacterial infection with more confidence than a blood culture and evaluating numerous potential biomarkers over time may enable the early identification and management of sepsis (Conway-Klaassena *et al*., 2020). The assessment of some markers, including acute phase protein (CRP), cells (WBC), and enzyme (LDH), may support the recognition of patients to reduce the mortality rate associated with severe sepsis. The aim of the present study was the early diagnosis of sepsis by evaluation of complete blood count (CBC), C-reactive protein (CRP) and lactate dehydrogenase (LDH) in culturable and unculturable bacteremia.

MATERIALS AND METHODS

Sample collection

The first step was to take consent, in which a patient agreed to participate in a study and authorized collecting information and a patient's history without fear of compulsion. A total of 100 blood samples from diverse patients were collected from different hospitals in the Babylon health directorate, Babylon city, Iraq, from January to June 2022. In addition, 25 samples were collected from volunteers who served as a control group without diseases, cancers, or autoimmune inflammations. Blood samples were drawn after disinfecting the injection sites with alcohol 70% and iodine, and around 8–12 ml of blood was drawn from the patients by a sterile syringe.

Sample processing

Blood samples were drawn from patients as soon as possible before they were admitted for antibiotic treatment. Each blood sample was divided into three parts: 2ml was placed into an EDTA tube for the screening test, 3ml into a centrifuged tube for using the serum in the immunological procedures, and 3-7ml into brain heart infusion broth (BHI) for blood culture for identification of different bacterial species by manual methods and confirmed by full automated VITEK device. All of the samples were accurately labelled with the known information before being transported to the laboratory.

Parameters diagnosis

A complete blood count is a common screening test for certain illnesses. It was detected by a full automated hematology analyzer (Sysmex, Jaban) to detect white blood cell (WBC), granulocytes (GRA), and lymphocyte (LYM). C- reactive protein (CRP) was detected by the slide agglutination method (Spinreact, Spain). The sensitivity of CRP in serum was detected by the kit when its concentration was <20mg/L. LDH was measured by using FUJI DR-CHEM NX500 automated clinical chemistry analyzer (FUJIFILM, Jaban).

Blood culture

The collection of samples is an important part of the blood culture procedure. Throughout the procedure, standard measures are performed, and stringent aseptic conditions are maintained. Blood cultures were obtained according to guidelines (Towns et al., 2010) to increase the quality and clinical relevance of blood culture investigations while lowering the risk of sample contamination. Brain heart infusion vials from (Himedia, India) were filled with 5-7mlbloodfrom 65 patients whose gave abnormal complete blood count CBC and incubated in an incubator at 37C° from one to 7 days with continuous monitoring through the incubation periods due to the long-time requirement of many bacterial species for activation and proliferation (Wilson *et al*., 1993). Positive blood culture (turbid vial) was directly transferred and streaked onto sheep blood agar, chocolate, MacConkey, mannitol salt, xylose lysine deoxycholate, and eosin methylene blue agar for overnight incubation at37C°. Gram stains were performed for each culture positive and then transferred to the VITEK®MS device (bioMérieux) for rapid and accurate species identification. In addition to the identity of the microbiological organism, clinical symptoms (e.g., fever, leukocytosis) were used to distinguish real infections from contaminants in blood cultures (Weinstein *et al*., 2011).

Statistical analysis

Statistical package for social science (SPSS), version 23 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Culture-positive and culture negative group with the count of WBC, lymphocyte and granulocyte were analyzed as well as CRP and LDH. The outcomes were presented as mean± SD. Independent t-test was used to compare the two groups. Additionally, Pearson's correlation test was used to explain the correlation. p-value < 0.05 was taken into account to denote statistical significance.

RESULTS

During the period of study, 125 blood samples were collected and analyzed, 25 samples served as control groups while 100 samples as patients' group for detecting the presence of bacterial sepsis in the bloodstream of hospitalized patients. The screening test revealed

Table 1. Parameters comparison between patients of sepsis and control

Parameters	Groups	No.	Mean	Std. Deviation	P value
CRP	Patients (Sepsis)	65	13.45	4.748	0.000
	Control (Without sepsis)	25	4.32	1.520	
WBC	Patients (Sepsis)	65	16.511	4.6542	0.000
	Control (Without sepsis)	25	7.740	1.6335	
LYM	Patients (Sepsis)	65	8.546	8.6089	0.000
	Control (Without sepsis)	25	26.108	3.6466	
GRA	Patients (Sepsis)	65	87.128	8.8623	0.000
	Control (Without sepsis)	25	62.656	4.8527	
LDH	Patients (Sepsis)	65	550.77	291.543	0.000
	Control (Without sepsis)	25	158.36	73.802	

Table 2. Distribution of bacterial isolates according to gender of the patients

Bacterial species	No.	Gender of patients	
		Male	Female
Staphylococcus aureus	6 (24%)	3 (12%)	3 (12%)
Staphylococcus epidermidis	4 (16%)	-	4 (16%)
Stenotrophomonas maltophilia	2 (8%)	2 (8%)	-
Escherichia coli	2 (8%)		2 (8%)
Streptococcus pneumonia	2 (8%)	1 (4%)	1(4%)
Salmonella typhi	1(4%)	1(4%)	-
Staphylococcus hemolyticus	1(4%)	-	1(4%)
Klebsiella pneumonia	1(4%)	1(4%)	-
Morganella morganii	1(4%)	-	1(4%)
Pseudomonas aeruginosa	1(4%)	1 (4%)	-
Staphylococcus hominis	1(4%)	-	1(4%)
Kocuriakristinae	1(4%)	1 (4%)	-
Enterobacter aerogenes	1(4%)	-	1 (4%)
Pseudomonas stutzeri	1(4%)	-	1(4%)
Total	25 (100%)	10 (40%)	15 (60%)

that 65 (65%) of the 100 samples had abnormal WBC, lymphocytes, granulocytes, and LDH values. In addition, 58 (89.2%) of the 65 samples had positive CRP levels, while the remaining 7 (10.8%) samples had negative CRP levels. However, 35 (35%) of the 100 samples had invaluable results from all screening tests.

All samples from abnormal screening test results (65 samples) were cultivated directly under standard bacterial growth conditions and their results revealed that 25 (38%) of the 65 samples were culture positive, whereas 40 (62%) of the 65 samples were culture negative.

Comparison of patients' group with the healthy group showed significant differences (p= 0.000) regarding all investigated parameters (WBC, GRA, LYM, CRP, and LDH) (Table 1).

When the culture-positive samples (25 samples) were grown on differential and selective media and the biochemical test was carried out using a manual methodology for identification, various species of bacteria observed. These isolates were confirmed later by VITEK®MS. From 25 culture-positive samples, 10 (40%) were males and 15 (60%) were females. The bacterial isolates identified on the basis of the morphological and biochemical features were: *Staphylococcus aureus* 6 (24%), *St. epidermidis* 4 (16%), *Stenotrophomonas maltophilia* 2 (8%), *Escherichia coli* 2 (8%), *Streptococcus pneumonia* 2(8%), *Salmonella typhi* 1(4%), *St. hemolyticus* 1 (4%), *Klebsiella pneumonia* 1(4%), *Morganella morganii* 1(4%), *Pseudomonas aeruginosa*1(4%), *St. ococcus hominis* 1(4%), *Kocuria kristinae* 1(4%), *Enterobacter aerogenes* 1(4%), and *P. stutzeri* 1(4%). However, these isolates were distributed between 15 (60%) gram-positive and 10 (40%) gram-negative bacteria (Table 2).

Statistically analysis of the results revealed that there are no significant differences between the two groups

Table 3. Comparison between culture positive and culture negative patients

Characteristics	Normal value	Culturable Mean ±SD	Non-culturable Mean ±SD	P-value
WBC	4.0-11.0*10^g/L	16.476 ± 6.2218	16.533 ± 3.4240	0.96
Lymphocyte	20.0-50.0 %	6.556±3.5385	9.790±10.4781	0.14
Granulocyte	40.0-70.0 %	89.084 ±5.1489	85.905 ±10.4204	0.16
CRP (mg/L)	<10	12.22± 2.21	13.56±3.56	0.08
LDH	12.5-220 µmol/L	594.12 ±377.099	523.68 ±223.642	0.34
Total	35	25	40	100

WBC: White blood cell; CRP: C-Reactive protein; LDH: Lactate dehydrogenase

Table 4. Comparison between males and females in culture positive patients

Characteristics of culture positive	Normal value	Culturable male Mean ±SD	Culturable female Mean ±SD	P- value
WBC	4.0-11.0 *10^g/L	13.780 ± 6.7141	18.273 ± 5.2345	0.07
Lymphocyte	20.0-50.0 %	6.290 ± 2.0223	6.733 ± 4.3302	0.76
Granulocyte	40.0-70.0 %	89.590 ± 2.2698	88.747 ± 6.4677	0.69
CRP(mg/L)	<10	12.6544±3.4784	12.9659±2.782	0.80
LDH	12.5-220 µmol/L	589.00 ± 271.711	597.53 ± 443.042	0.95

WBC: White blood cell; CRP: C-Reactive protein; LDH: Lactate dehydrogenase

of hospitalized patients (culture positive and culture negative) regarding WBC, lymphocytes, granulocytes, LDH, and CRP results (P> 0.05) (Table 3).

In addition to that, there were no significant differences between males and females regarding WBC, LYM, GRA, LDH, and CRP for those who gave culture positive (P> 0.05) [Table 4]. There were no significant differences between males and females that gave culture negative (P> 0.05) (Table 5).

Significant strong positive correlation between WBC and LDH was detected (r= 0.332) (p= 0.007). That meant when WBC increased, and the LDH also increased significantly. A significant strong negative correlation was detected between LYM and GRA (r= -0.983) (p= 0.000). That meant when LYM was increased, the GRA was decreased and vice versa.

A significant positive correlation between LYM and CRP was detected (r= 0.257) (p= 0.03). That meant when LYM was increased, the CRP was increased and vice versa. A significant positive correlation was detected between GRA and LDH (r= 0.254) (p= 0.04). That meant when GRA was increased, and the LDH also increased significantly. No significant differences were observed between other parameters (Table 6).

DISCUSSION

Sepsis is a complex inflammatory condition that is a leading cause of morbidity and mortality worldwide, although it is largely under-recognized. Sepsis kills one person every three to four seconds due to the estimated 50 million cases that occur worldwide each year (Novak-Weekley *et al*., 2016 , Egi *et al*., 2021). One of the simplest and most frequently employed investigations to determine the origin of bloodstream infections is the laboratory detection of bacteremia using blood cultures (Schwarzenbacher *et al*., 2019). A precise diagnosis of the bacteria causing bloodstream infections gives essential clinical data to identify and treat sepsis (Zhang *et al*., 2022). However, a blood culture alone is not sensitive enough to detect bacterial sepsis, especially when the patient has already taken antibiotics or there is fastidious organisms present that cannot grow under normal conditions (culture gives false negatives). Furthermore, the most challenging interpretation is determining if the organism recovered from the blood culture is an actual pathogen that causes bloodstream infection or a contaminant (false positive). If it is a contaminant, the patient can end up getting antibiotics when they are not necessary, which would put the patient at more risk (Nielsen *et al*., 2022).

Since there is no specific treatment for sepsis, it is difficult to understand the primary etiology. Therefore, it is critical to recognize it right away. There is not a single sepsis parameter that is perfect, but there are numerous such as body temperature, WBC, CRP, and LDH, along with blood culture, that can at least help identify severely ill patients so that the condition can be identified and treated. This research utilized a few diagnostic criteria, divided into two parts: a screening test and a

Table 5. Comparison between males and females in culture negative patients

Characteristics of Culture Negative	Normal value	Non-culturable male Mean ±SD	Non-culturable female Mean ±SD	P- value
WBC	4.0-11.0 *10^g/L	17.387 ±3.2930	15.376 ±3.3462	0.06
Lymphocyte	20.0-50.0 %	10.743 ±12.6070	8.500 ±6.7826	0.51
Granulocyte	40.0-70.0 %	84.948 ±12.1732	87.200 ± 7.6039	0.50
CRP(mg/L)	<10	13.6784±3.4784	12.9659±2.782	0.53
LDH	12.5-220 µmol/L	578.043 ±240.6821	442.375 ±182.5603	0.06

WBC: White blood cell; CRP: C-Reactive protein; LDH: Lactate dehydrogenase

Table 6. Correlation between diagnostic parameters of sepsis

Parameters		Correlation (r)	P value
WBC	LDH	0.332	0.007
LYM	GRA	0.983	0.000
LYM	CRP	0.257	0.03
GRA	LDH	0.254	0.004
GRA	CRP	-0.22	0.07
LDH	LYM	-0.22	0.06
LDH	CRP	-0.01	0.87
WBC	LYM	0.05	0.65
WBC	CRP	0.21	0.09

WBC: White blood cell; LYM: Lymphocyte; GRA: Granulocyte; CRP: C-Reactive protein; LDH: Lactate dehydrogenase

cultural method.

During the screening test, in addition to measuring the patient's body temperature, the parameters viz. WBC count, CRP, and LDH were evaluated. The presence of acute inflammation caused by unidentified causative agents was indicated by an increase or decrease in normal body temperature (37.5 C°) and normal WBC count (4.0-11.010^g/L) from their natural ranges. CRP was estimated as another diagnostic parameter for further progress in present investigations. CRP is one of a set of acute-phase proteins whose synthesis is in the liver. Positive CRP responses indicate the presence of infection and inflammation (Sproston and Ashworth, 2018). Although it is used to screen for early sepsis, CRP has low specificity in the case of bacterial sepsis detection. However, LDH is another potent parameter for sepsis detection. It is an enzyme that anaerobically converts pyruvate to lactate during Glucose metabolism. In individuals with sepsis, lactate levels are thought to increase because of reduced tissue perfusion, which results in hypoxia and anaerobic glycolysis (Kang and Park., 2016).

Statistical analysis revealed no significant differences in screening test results between culture-positive and culture-negative samples. This conclusion proves the vital role of WBC, CRP, LDH estimation, and blood culture in detecting bacterial sepsis.

Concerning blood culture, from 25 culture-positive samples, 6 (24%), 4 (16%), and 2 (8%) were given the morphological and biochemical characteristics of coagulase -positive *Staphylococcus aureus, coagulase-negative S. epidermidis and Stenotrophomonas maltophilia* respectively when were identified by manual and automated methods. These three bacterial isolates might be infectious agents or correlate with hospitalized patients' secondary infection through admission to a healthcare facility (hospital-acquired infection). This interpretation is related to the location and time of sample collection, as the sample was collected from Intensive care unit and Respiratory care unit within 2 weeks of hospitalization. Previous research observed bloodstream infections with gram-positive and gram-negative organisms in hospitalized and ICU patients with viral infections like influenza (Giacobbe *et al*., 2020, Khatri *et al*., 2021).

Other isolates from all 25 culture-positive samples, including *Escherichia coli* 2 (8%), *E. aerogenes* 1 (4%), *Morganella morganii* 1 (4%), *Salmonella typhi* 1 (4%), *Streptococcus pneumonia* 2 (8%), and *Klebsiella pneumonia* 1 (4%) might reflect the origin of primary site of infection because the samples were collected from hos-

pitalized patients who had history of urinary tract infections and of respiratory disease. One (4%) of the 25 culture-positive samples contained *Kocuria kristinae*, a rare pathogenic bacterium. *K. kristinae*is a part of normal skin flora. However, it can only cause serious ills in a small number of patients, including catheter-related bacteremia, especially those with defects in the immune system or debilitated patients (Dunn *et al*., 2011). Finally, *P. stutzeri* is another unique bacterium that was isolated from 1 (4%) of all 25 culture-positive samples. The nonfluorescent bacteria *P. stutzeri* is commonly present in the environment and has also been isolated from patients as an opportunistic disease. Several researchers described the isolation of these bacteria from clinical samples, particularly those that were collected from bacteremic patients (Halabi *et al*., 2019).

Conclusion

Although blood culture is the most common method for identifying bacteremia, various medical laboratory criteria can be used for early diagnosis, especially when the blood culture fails to detect the etiologic agent of sepsis (negative culture) when the patient has clinical symptoms. This investigation has proved that estimating certain markers (WBC, CRP, and LDH) along with the result of blood culture may help to identify the causative agent of sepsis earlier. The findings succeeded in translating the overall idea, which relates to constantly updating the methods of sepsis diagnosis, which may lower the death rate and help patients recover quickly.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Conway-Klaassena, J., Tilleb, P. & Azizc, H. (2020). The Use of Biomarkers in the Diagnosis and Management of Sepsis. *International Journal of Biomedical Laboratory Sciences,* 9 (1), 23-31.
2. Corey, G. R. (2009). Staphylococcus aureus bloodstream infections: definitions and treatment. *Clinical Infectious Diseases,* 48(Supplement_4). https://doi.org/10.1086/598186.
3. Dunn, R., Bares, S. & David, M. Z. (2011). Central venous catheter-related bacteremia caused by *Kocuria kristinae*: case report and review of the literature. *Annals of Clinical Microbiology and Antimicrobials*, 10(1), 1-5. https://doi.org/10.1186/1476-0711-10-31.
4. Egi, M., Ogura, H., Yatabe, T., Atagi, K., Inoue, S., Iba, T. ... & Kimura, S. (2021). The Japanese clinical practice guidelines for management of sepsis and septic shock 2020 (J-SSCG 2020). *Journal of Intensive Care*, 9(1), 1-144. https://doi.org/10.1186/s40560-021-00555-7.
5. Giacobbe, D. R., Battaglini, D., Ball, L., Brunetti, I., Bruzzone, B., Codda, G. ... & Bassetti, M. (2020). Bloodstream infections in critically ill patients with COVID□19. European J*ournal of Clinical Investigation,* 50(10), e13319. https://doi.org/10.1111/eci.13319.
6. Halabi, Z., Mocadie, M., El Zein, S., & Kanj, S. S. (2019). *Pseudomonas stutzeri* prosthetic valve endocarditis: a case report and review of the literature. Journal of infection and public health, 12(3), 434-437. https://doi.org/10.1016/j.jiph.2018.07.004.
7. Kang, H. E. & Park, D. W. (2016). Lactate as a biomarker for sepsis prognosis? *Infection & Chemotherapy*, 48(3), 252-253. DOI: https://doi.org/10.3947/ic.2016.48.3.252.
8. Khatri, A., Malhotra, P., Izard, S., Kim, A., Oppenheim, M., Gautam-Goyal, P., ... & Farber, B. (2021). Hospital-acquired bloodstream infections in patients hospitalized with severe acute respiratory syndrome coronavirus 2 infection (coronavirus disease 2019): association with immunosuppressive therapies. In: Open forum infectious diseases (8 (7), p. of ab339). US: Oxford University Press. https://doi.org/10.1093/ofid/ofab339.
9. Kleinschmidt, S., Huygens, F., Faoagali, J., Rathnayake, I. U. & Hafner, L. M. (2015). *Staphylococcus epidermidis* as a cause of bacteremia. *Future Microbiology*, 10(11), 1859-1879. https://doi.org/10.2217/fmb.15.98.
10. M Novak-Weekley, S., & Dunne Jr, W. M. (2016). Blood culture a key investigation for diagnosis of bloodstream infections. http://hdl.handle.net/123456789/1030.
11. Mammen, J., Choudhuri, J., Paul, J., Sudarsan, T. I., Josephine, T., Mahasampath, G., ... & Peter, J. V. (2018). Cytomorphometric neutrophil and monocyte markers may strengthen the diagnosis of sepsis. *Journal of Intensive Care Medicine,* 33(12), 656-662. https://doi.org/10.1177/0885066616682940.
12. Nannan Panday, R. S., Wang, S., Van De Ven, P. M., Hekker, T. A. M., Alam, N. & Nanayakkara, P. W. B. (2019). Evaluation of blood culture epidemiology and efficiency in a large European teaching hospital. *PLoS One*, 14(3), e0214052. https://doi.org/10.1371/journal.pone.0214052.
13. Nielsen, L. E., Nguyen, K., Wahl, C. K., Huss, J. L., Chang, D., Ager, E. P. & Hamilton, L. (2022). Initial Specimen Diversion Device® reduces blood culture contamination and vancomycin use in academic medical centre. *Journal of Hospital Infection*, 120, 127-133. https://doi.org/10.1016/j.jhin.2021.10.017.
14. Peker, N., Couto, N., Sinha, B. & Rossen, J. W. (2018). Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. *Clinical Microbiology and Infection,* 24(9), 944-955. https://doi.org/10.1016/j.cmi.2018.05.007.
15. Schwarzenbacher, J., Kuhn, S. O., Vollmer, M., Scheer, C., Fuchs, C., Rehberg, S., ... & Gründling, M. (2019). On-site blood culture incubation shortens the time to knowledge of positivity and microbiological results in septic patients. *Plos one,* 14(12), e0225999. https://doi.org/10.1371/journal.pone.0225999.
16. Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., ... & Angus, D. C. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *Jama,* 315(8), 801-810. doi:10.1001/jama.2016.0287.
17. Sproston, N. R. & Ashworth, J. J. (2018). Role of C-

reactive protein at sites of inflammation and infection. *Frontiers in Immunology,* 9, 754. https://doi.org/10.3389/fimmu.2018.00754.

18. Towns, M. L., Jarvis, W. R. & Hsueh, P. R. (2010). Guidelines on blood cultures. *Journal of Microbiology, Immunology, and Infection,* 43(4), 347-349. https://doi.org/10.1016/S1684-1182(10)60054-0.
19. Weinstein, M. P. & Doern, G. V. (2011). A critical appraisal of the role of the clinical microbiology laboratory in the diagnosis of bloodstream infections. *Journal of Clinical Microbiology,* 49(9_Supplement), S26-S29. https://doi.org/10.1128/JCM.00765-11.
20. Wilson, M. L., Mirrett, S., Reller, L. B., Weinstein, M. P. & Reimer, L. G. (1993). Recovery of clinically important microorganisms from the BacT/Alert blood culture system does not require testing for seven days. *Diagnostic Microbiology and Infectious Disease,* 16(1), 31-34. https://doi.org/10.1016/0732-8893(93)90127-S.
21. Zhang, J., Yang, F., Sun, Z., Fang, Y., Zhu, H., Zhang, D., ... & Zhao, H. (2022). Rapid and precise identification of bloodstream infections using a pre-treatment protocol combined with high throughput multiplex genetic detection system. *Future Microbiology*, 12(10), 368-374. https://doi.org/10.21203/rs.3.rs-1784956/v1