Ann. For. Res. 66(1): 355-370, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

IN VITRO: INVESTIGATION THE EFFECT OF BACTERIA ISOLATED FROM BACTEREMIA PATIENTS ON HEMATOLOGICAL PARAMETERS AND ITS NUMBER IN BLOOD

Abbas Hameed Al-Wandawy[1], Luma Abdulhady Zwain[2], Dalia Maher Khidher[3]
[1-2]Department of Biology /Collage of Education Pure Science Ibn Al-Haitham/University of Baghdad/Iraq.
[3]Directorate of Health of Kirkuk/ Azadi-Teaching Hospital Ministry of Health/Iraq
[1]E-mail:abbas7wandawy@gmail.com

Abstract
The bloodstream in a healthy human is generally a sterile environment and the presence of bacteria in it is a indicate of bacteremia, most body fluids in healthy people do not contain bacteria such as blood so the presence of infection can affect the patient's life. 313 blood samples were collected from bacteremia pationts, including 146 (30 patient and 116 samples of out patients) from Azadi Teaching Hospital, (36) from the dialysis Unit at Kirkuk General Hospital , 126 samples (42 samples of patients and 84 samples of out patients) from the Children's Hospital, and (5) samples from the Women's and Obstetrics Hospital in Kirkuk province, for the period from the twenty-fourth of January 2022 to the tenth of September 2022. Blood samples were cultured in the Brain heart infusion broth and incubated at 37 ° C for 24 hours, then cultured on the MacConkey agar and Blood agar and the results showed that 29(58%) isolates were gram positive gram bacteria and 21 (42%) gram negative. Twenty-eight isolates of the gram positive belonged to negative coagulase *Staphylococcus*, and most of them were 37.93% *S.hominis* bacteria, while negative gram bacteria was mostly the *Pseudomonas aeruginos* by 52.38% ,this isolated bacteria studied on the hematological parameters , by use the EDTA tubes containing blood (healthy) ,than inoculated with *S.hominis* and *P.aeruginosa*. It was noted from the results that the hematological parameters (WBC, MID%, NEUT%, LYM#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, MPV, PDW, PCT, P-LCR) were not affected at a growth density of 1.5×10^8 and 3×10^8 for *S.hominis* and *P.aeruginosa* bacteria at zero time, 4 hours and 24 hours except for the PLT and the LYM% criterion was different according to the incubation period. Then the effect of blood on the bacteria *S.hominis* and *P. aeruginosa* was studied and the results showed a decrease in the number of bacteria at (2, 4 ,24) hours of incubation with more decrease after incubation 24 hours, and the decrease in the number of gram positive bacteria was more than the gram negative bacteria at (2, 4) hours of incubation.

Keywords: Bacteremia, CBC, Bacteria, Blood.

Introduction

The bloodstream in a healthy human is generally a sterile environment and the presence of bacteria in it, refers to a signal of bacteremia (Osaiyuwu, 2021). Most body fluids in healthy people do not contain bacteria such as blood, so the presence of infection can affect the patient's life and infection is the most common cause of bacteremia, as the infection is limited to a specific place in the body, and is aided by the movement of bacteria in the blood (Garnica *et al*., 2021). A bacteremia is called

primary bacteremia if it comes from unknown source, while secondary bacteremia is the bacteremia in a patient with a previous local infection caused by the same bacteria isolated from the blood sample (Sirijatuphat *et al*., 2018).

Staphylococcal bacteria cause diseases ranging from simple dermatitis to a life-threatening bacteremia infection (Muhammad *et al*., 2014). Coagulase-negative staphylococci are one of the main opportunistic pathogens for the occurrence of increased bacteremia and as the main cause of inflammation of the bloodstream, and among the most dominant bacterial species among patients with bacteremia are the bacteria *S.hominis*, *S.epidermidis* followed by *S. capitis* (Cui *et al*., 2019). A recent study indicated that the majority of positive growth of blood cultures in newborns are *Staphylococcus* bacteria that are Coagulase-negative staphylococci (CoNS) followed by *Klebsiella* spp. , *Streptococcus agalactiae*, *Enterobacter cloacae*, *Escherichia coli*, and *Acinetobacter baumanii* (Al-Harbi, 2022). *S. hominis* bacteria are gram positive, coagulase-negative are present as a naturally coexisting organism on human skin and rarely cause native valve endocarditis (NVE) (Vasconcellos *et al*., 2022). It is the second most frequently isolated CoNS from healthy skin, and there is evidence to suggest that it plays an important role in excluding pathogens and may protect the skin barrier from opportunistic pathogens, including *S. aureus*, from colonization or injury to the skin (Severn *et al*., 2022). *S. hominis* bacteria may sometimes cause infection in patients who have a weakened immune system, and tend to colonize in areas with many secreted glands such as the axillae and pubic region (Voineagu *et al*., 2012). *P. aeruginosa* , aerobic organism, *P. aeruginosa* bacteria are bacilli gram negative and positive for oxidase and producing pigments (Sastry and Bhat, 2019); (Abdullah and Falih, 2019). commonly found in humid environments in hospitals (Riedel *et al*., 2019), is the primary human pathogen as it is widespread in nature (Cornelissen *et al*., 2013),. It causes pneumonia in hospitals, urinary tract infections, surgical site infections, severe burn infections, infections of patients undergoing either chemotherapy (for patients with malignant tumors) or antibiotic therapy and patients with abnormal host defenses (Cornelissen *et al.*, 2013; Murray *et al*., 2021). *P. aeruginosa* bacteria cause vesicular fibrosis and bacteremia (Altaai *et al*., 2014). The causes of bacteremia is skin, skin and soft tissue infections, intravenous drug use and other injections into the vascular device (Smit *et al*., 2018). As well as other infections such as endocarditis, urinary tract infection, cholangitis, cholecystitis and gastrointestinal tract infection (Ryu *et al*., 2019). The Complete Blood Count (CBC) and Differential Leukocyte Count (DC) is one of the most frequently performed tests on patients with idiopathic infections such as bacteremia, so it can be considered a predictor of the risks of bacteremia (Lien *et al*., 2022).

Materials and Methods

Samples collection

Three hundred thirty blood samples were collected, including 146 (30 patient and 116 out patients) from Azadi Teaching Hospital, (36) from the Dialysis Unit at Kirkuk General Hospital, 126 samples (42 samples of patients and 84 samples of out patients) from the Children's Hospital and (5) from the Women's and Obstetrics Hospital in Kirkuk province, for the period from January 2022 to September 2022.

Diagnosis of sample

Blood samples of patients' were cultured in Brian heart infusion broth and incubated at 37°C for 24 hours, and were cultured on the Blood agar and MacConkey agar then incubated at 37°C for 24 hours. All isolates were diagnosed depending on macroscopic, microscopic , API Staph Kit, Compact system Vitek –2, for gram positive bacteria, and API 20E kit for gram negative bacteria.

The effect of bacteria on hematological parameters

Ethylene diamine tetra-acetic acid (EDTA) tubes containing 500 µl of healthy blood were inoculated with *S. hominis* and *P. aeruginosa* after was compared with standard turbidity this about equal to (1.5×10^8) and (3×10^8) cfu/ml both alone and incubated at 37°C for (0, 2, 4, 24) hours and then placed in a Automater Hematology analyzer CBC Genex COUNT-60 to measure the hematological parameters.

Effect of blood on bacterial number

EDTA tubes containing 500 µl of healthy blood were inoculated with *S. hominis* and *P. aeruginosa* with a density of 1.5×10^8 and 3×10^8, both alone and incubated at 37°C for (0, 2, 4, 24) hours and a series of diluted was performed after the end of the incubation period and counted the number of colonies using the Tryptone soy agar

Results and Discussion

The results of the blood incubated in the Brian heart infusion broth as in Table (1): Percentage of positive growth culture according to the source of bacteremia ,as the number of positive culture were 32 (17.87%) from febrile patients, 3 (8.33%) from dialysis patients in the dialysis unit, and 15(65.21%) from burns and wounds patients.

Table (1): Percentage of positive growth culture according to the source of bacteremia.

Source of bacteremia	Total number of blood samples	Number of positive growth specimens	Percentage %
Febrile patients	179	32	17.87
Dialysis patients (dialysis unit)	36	3	8.33
Burns and wounds	23	15	65.21

Table (2) : showed number and Percentage of gram positive and negative bacterial species isolated from bacteremia patients, 29 (58%) were gram positive and 21 (42%) were gram negative bacteria .

Table (2): The number and percentage of gram positive and negative bacterial species isolated from bacteremia patients

Number of Total cultures Positive Isolates	Gram positive		Gram negative	
	Number	Percentage%	Number	Percentage
50	29	58	21	42

Table (3) shows the number and percentage of *Staphylococcus* isolates from bacteremia patients. The gram positive bacteria were presented by *Staphylococcus hominis*, *S. epidermidis* (11) 37.93% and(4)13.79%) respectively, and *S. warneri* , *S. haemolyticus* were (2)6.89% and *Enterococcus faecalis* was (1)3.44%. While the gram negative bacteria were *Pseudomonas aeruginosa* (11)52.38% and Escherichia coli (5) 14.28 %, *Enterobacter cloacae* (2) 9.52%, *Raoultella terrigena, KlebsiellaSpp.* and *Acinetobacter Spp.* were (1)4.76%.

Table (3): Number and percentage of isolated bacterial species of bacteremia patients.

Reaction with the gram stains	Genus	Species	Number	Percentage%
Gram positive bacteria	*Staphylococcus*	*warneri*	2	6.89
		haemolyticus	2	6.89
		hominis	11	37.93
		epidermidis	4	13.79
		Spp.	9	31.03
	Enterococcus	*faecalis*	1	3.44
Gram negative bacteria	*Raoultella*	*terrigena*	1	4.76
	Pseudomonas	*aeruginosa*	11	52.38
	Enterobacter	*cloacae*	2	9.52
	Escherichia	*coli*	5	14.28
	Klebsiella	*Spp.*	1	4.76
	Acinetobacter	*Spp.*	1	4.76

It is noted from the results that most of the bacterial species were gram positive and were represented by *Staphylococcus* bacteria, while the gram negative bacteria was *P.aeruginosa* . Jassim and Ameer Alash (2020) indicated in his study on patients with bacteremia that 33.3% of the bacterial isolates were Gram-negative bacteria, while the Gram-positive bacteria were 66.6%. Park *et al*. (2022) indicated in his study that included 158 patients with bacteremia, 45 (29%) were infected with gram-positive bacteria, 35 (22%) had gram-negative bacteria, 27 (17%) had fungi and 51 (32%) were growth-negative. Anwer and Husien (2007) indicated that *P.aeruginosa* bacteria are the second most common gram negative bacteria in hospital infections. While Gille *et al*. (2021) indicated that 101 of the patients had 39 blood cultures that were positive growth, 16 (41%) were gram negative, 18 (46.2%) were gram positive bacteria, and 5 (12.8%) were samples containing both positive and negative types of gram stains. Rasool (2011) noted in his study that the most common people with bacteremia among children were the age group (1 day-1 year) 64.89%.

Salah et al. (2021) noted in their study that the main bacterial pathogens of gram positive bacteria in newborn bacterimia were *S.haemolyticus* (9.1%), *S. epidermidis* (7.1%) and *S. hominis* (5.1%) noting that the bacteria negative for coagulase-negative Staphylococci (CONS) accounted for most of the types of bacteria gram positive bacteria and *S. haemolyticus* bacteria were among the most present.

Abbas *et al*. (2014) noted in their study that bacterial isolates from newborns were gram positive cocci bacteria (21.23%), followed by gram negative bacilli and yeasts, while Rasool (2011) in his study indicated that the percentage of isolated *S.epidermidis* bacteria from bacteremia patients was 54.78%. While de Oliveira *et al*. (2021) in their study which included 200 isolates of the bacterium *Staphylococcus* spp., of which 50 were isolated from *S. aureus* and 150 were CoNS that included species (50 *S. epidermidis*, 7 *S. lugdunensis*, 20 isolates each of *S.haemolyticus*, *S. warneri*, and *S.hominis*).

Effect of *Staphylococcus hominis* and *Pseudomonas aerogenosa* isolated from bacteremia patients on hematological parameters

Staphylococcus hominis: It is noted in Table (4) the effect of *S. hominis* which was adjusted to 0.5 macFarland turbidity equivalent to 1.5×10^8 cfu/ml on the hematological parameters at zero time as each hematological parameter is no different from the parameters in non-inoculated blood(control), as the bacteria did not effect on each of parameter: WBC,% LYM, %MID, %NEUT, LYM#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PLT, MPV, PDW, PCT, P-LCR.

Either at 2 hours incubation the bacteria did not affect on the hematological parameters; WBC, LYM%, MID%, NEUT%, LYM#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, MPV, PDW, PCT, P-LCR. Except for their effect on the RDW-SD and PLT standard, a decrease in them and a slight decrease in the #NEUT parameter were observed. While bacteria had an effect in a 4-hour time on the PLT parameter, a decrease was observed compared to the control treatment. The effect of bacteria in a 24-hour time on the LYM% and PLT parameters was slightly low while their effect was high in the #NEUT parameter.

Table (4): Effect of *Staphylococcus hominis* on hematological parameters at turbidity equivalent to 1.5×10^8 cfu/ml.

Hematological parameters	Whole blood	Inoculated periods (hour)							
		zero		2 hours		4 hours		24 hours	
		Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria
WBC	5.1	5.1	4.9	5.0	4.6	5.1	3.9	4.4	4.5
LYM%	23.6	22.4	24.4	25.0	32.3	34.2	38.0	37	39.1
MID%	5.9	6.7	7.5	6.8	6.8	8.1	7.7	13.2	11.7
NEUT%	70.5	70.9	68.1	68.2	60.9	57.7	54.3	26.0	49.2
LYM#	1.2	1.1	1.2	1.3	1.5	1.7	1.5	2.7	1.8
MID#	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.6	0.5
NEUT#	3.6	3.7	3.3	3.4	2.8	3.0	2.1	1.1	2.2
RBC	5.82	5.18	5.09	5.18	5.14	5.20	5.07	5.18	5.00

HGB	11.0	10.2	10.4	10.5	10.5	10.7	10.7	10.0	9.8
HCT	41.4	36.7	35.9	36.5	36.3	36.7	35.7	39.4	37.8
MCV	71.2	70.9	70.7	70.6	70.7	70.0	70.6	76.1	75.6
MCH	18.9	19.6	20.4	20.2	20.4	20.5	21.1	19.3	19.6
MCHC	26.5	27.7	28.9	28.7	28.9	29.1	29.9	25.3	25.9
RDW-SD	46.5	46.5	46.5	86.5	46.5	46.5	46.5	52.0	50.2
RDW-CV	16.1	16.2	16.2	16.3	16.2	16.2	16.2	16.9	16.4
PLT	334	282	299	231	120	227	124	172	156
MPV	11.3	11.0	11.1	11.3	11.3	11.0	11.1	11.6	11.7
PDW	17.4	16.7	17.9	17.9	15.9	17.7	20.0	16.4	19.0
PCT	0.37	0.31	0.33	0.26	0.13	0.24	0.13	0.19	0.18
P-LCR	42.0	39.8	39.4	41.2	41.9	38.4	40.2	44.3	43.5

WBC, white blood count; LYM%, Lymphocyte%; MID%, Mid-range absolute count; NEUT%, Neutrophils%; LYM#, Lymphocytes #; MID#, Mid-range absolute count#; NEUT#, Neutrophils#; RBC, Red blood cell; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular Hemoglobin; MCHC, Mean corpuscular Hemoglobin concentration; RDW-SD, Red cell distribution width-Standard Deviation; RDW-CV, Red cell distribution width – Coefficient of Variation; PLT, platelet count; MPV, mean platelet volume; PDW, Platelet distribution width; PCT, Plateletcrit; P-LCR, Platelet-large cell ratio.

It is noted from Table (5) that there is no effect of *S. hominis* which was adjusted to 1macFarland turbidity equivalent to 3×10^8 cfu/ml on the hematological parameter at zero time as it is not observed that each parameters WBC, LYM, MID%, NEUT%, lym#, neut#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PLT, MPv, PDW, PCT, P-LCR from the hematological parameters of non-inoculated blood with bacteria. At a time of 2 hours, its effect on the RDW-SD, PLT parameters was observed as a decrease was observed, while the decrease was slight in the #NEUT parameters and a slight increase in LYM. While the effect of bacteria in a time of 4 hours on the PLT parameter was observed to be lower than the control parameter, while the effect of bacteria in a time of 24 hours on the LYM parameter was observed to decrease while their effect was high in the #NEUT and PLT parameter.

Table (5): Effect of *Staphylococcus hominis* on hematological parameters at turbidity equivalent to 3×10^8 cfu/ml..

Hematological parameters	Whole blood	Inoculated periods (hour)							
		zero		2 hours		4 hours		24 hours	
		Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria
WBC	5.1	5.1	4.9	5.0	3.8	5.1	3.0	4.4	3.6
LYM%	23.6	22.4	24.4	25.0	31.7	34.2	39.3	37	40.0
MID%	5.9	6.7	7.5	6.8	7.3	8.1	7.1	13.2	10.4
NEUT%	70.5	70.9	68.1	68.2	61.0	57.7	53.6	26.0	49.6
LYM#	1.2	1.1	1.2	1.3	1.2	1.7	1.2	2.7	1.4
MID#	0.3	0.3	0.4	0.3	0.3	0.4	0.2	0.6	0.4
NEUT#	3.6	3.7	3.3	3.4	2.3	3.0	1.6	1.1	1.8
RBC	5.82	5.18	5.09	5.18	4.98	5.20	5.22	5.18	5.25
HGB	11.0	10.2	10.4	10.5	10.5	10.7	10.6	10.0	9.8
HCT	41.4	36.7	35.9	36.5	35.2	36.7	37.0	39.4	39.7
MCV	71.2	70.9	70.7	70.6	70.8	70.0	71.0	76.1	75.7
MCH	18.9	19.6	20.4	20.2	21.0	20.5	20.3	19.3	18.6
MCHC	26.5	27.7	28.9	28.7	29.8	29.1	28.6	25.3	24.6
RDW-SD	46.5	46.5	46.5	86.5	46.5	46.5	46.5	52.0	52.0
RDW-CV	16.1	16.2	16.2	16.3	16.2	16.2	16.2	16.9	17.0
PLT	334	282	299	231	180	227	187	172	196
MPV	11.3	11.0	11.1	11.3	10.9	11.0	11.4	11.6	11.5
PDW	17.4	16.7	17.9	17.9	17.7	17.7	19.0	16.4	20.0
PCT	0.37	0.31	0.33	0.26	0.19	0.24	0.21	0.19	0.22

P-LCR	42.0	39.8	39.4	41.2	38.1	38.4	41.0	44.3	41.6

WBC, white blood count; LYM%, Lymphocyte%; MID%, Mid-range absolute count; NEUT%, Neutrophils%; LYM#, Lymphocytes #; MID#, Mid-range absolute count#; NEUT#, Neutrophils#; RBC, Red blood cell; HGB, Hemoglobin ; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular Hemoglobin; MCHC, Mean corpuscular Hemoglobin concentration; RDW-SD, Red cell distribution width-Standard Deviation; RDW-CV, Red cell distribution width – Coefficient of Variation; PLT, platelet count; MPV, mean platelet volume; PDW, Platelet distribution width; PCT, Plateletcrit; P-LCR, Platelet-large cell ratio.

Pseudomonas aerogenosa: It is noted from Table (6) the effect of *P.aerogenosa* which was adjusted to 0.5 macFarland turbidity equivalent to 1.5×10^8 on the hematological parameters at zero time if the bacteria did not affect each of parameters; WBC, LYM%, MID%, NEUT%, LYM#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PLT, MPV, PDW, PCT, P-LCR. While the effect of bacteria at 2 hours of incubation was on MCV, MCH, MCHC, P-LCR hematological parameters was slightly high and each of HCT, RDW-SD, PLT hematological parameters were lower than the control parameters, while the rest of the parameters showed no difference from control parameters such as WBC, LYM%, MID%, NEUT%, RBC, LYM#, NEUT#, HGB, RDW-CV, MPV, PDW, PCT. Also, there was no effect in the 4-hour except for the PLT parameter, RDW-SD, which was lower than the control parameters. While the effect of bacteria at the time of 2 hours of incubation was on both hematological parameters

Table (6): Effect of *Pseudomonas aerogenosa* on hematological parameters at turbidity equivalent to 1.5×10^8 cfu/ml..

Hematological parameters	Whole blood	Inoculated periods (hour)							
		zero		2 hours		4 hours		24 hours	
		Blood+ saline	Blood+ Bacteria	Blood+ saline	Blood+ Bacteria	Blood+ saline	Blood+ Bacteria	Blood+ saline	Blood+ Bacteria
WBC	**5.1**	**5.1**	**4.9**	**5.0**	**5.0**	**5.1**	**4.3**	**4.4**	**4.4**
LYM%	**23.6**	**22.4**	**21.2**	**25.0**	**24.4**	**34.2**	**25.6**	**37**	**52.9**
MID%	**5.9**	**6.7**	**6.4**	**6.8**	**6.7**	**8.1**	**6.4**	**13.2**	**16.9**
NEUT%	**70.5**	**70.9**	**72.4**	**68.2**	**68.9**	**57.7**	**68.0**	**26.0**	**30.2**
LYM#	**1.2**	**1.1**	**1.0**	**1.3**	**1.2**	**1.7**	**1.1**	**2.7**	**2.3**
MID#	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.4**	**0.3**	**0.6**	**0.7**
NEUT#	**3.6**	**3.7**	**3.6**	**3.4**	**3.5**	**3.0**	**2.9**	**1.1**	**1.4**
RBC	**5.82**	**5.18**	**5.20**	**5.18**	**2.37**	**5.20**	**5.19**	**5.18**	**5.30**
HGB	**11.0**	**10.2**	**10.1**	**10.5**	**10.5**	**10.7**	**10.6**	**10.0**	**9.8**
HCT	**41.4**	**36.7**	**36.9**	**36.5**	**20.2**	**36.7**	**36.6**	**39.4**	**39.9**
MCV	**71.2**	**70.9**	**71.0**	**70.6**	**85.4**	**70.0**	**70.6**	**76.1**	**75.4**
MCH	**18.9**	**19.6**	**19.4**	**20.2**	**44.3**	**20.5**	**20.4**	**19.3**	**18.4**
MCHC	**26.5**	**27.7**	**27.3**	**28.7**	**51.9**	**29.1**	**28.9**	**25.3**	**24.5**

RDW-SD	**46.5**	**46.5**	**46.5**	**86.5**	**57.6**	**46.5**	**26.5**	**52.0**	**50.2**
RDW-CV	**16.1**	**16.2**	**16.2**	**16.3**	**16.7**	**16.2**	**16.2**	**16.9**	**16.4**
PLT	**334**	**282**	**296**	**231**	**74**	**227**	**188**	**172**	**212**
MPV	**11.3**	**11.0**	**11.3**	**11.3**	**12.0**	**11.0**	**11.0**	**11.6**	**11.5**
PDW	**17.4**	**16.7**	**17.9**	**17.9**	**13.3**	**17.7**	**18.2**	**16.4**	**18.5**
PCT	**0.37**	**0.31**	**0.33**	**0.26**	**0.08**	**0.24**	**0.20**	**0.19**	**0.24**
P-LCR	**42.0**	**39.8**	**41.5**	**41.2**	**52.1**	**38.4**	**38.9**	**44.3**	**42.2**

WBC, white blood count; LYM%, Lymphocyte%; MID%, Mid-range absolute count; NEUT%, Neutrophils%; LYM#, Lymphocytes #; MID#, Mid-range absolute count#; NEUT#, Neutrophils#; RBC, Red blood cell; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular Hemoglobin; MCHC, Mean corpuscular Hemoglobin concentration; RDW-SD, Red cell distribution width-Standard Deviation; RDW-CV, Red cell distribution width – Coefficient of Variation; PLT, platelet count; MPV, mean platelet volume; PDW, Platelet distribution width; PCT, Plateletcrit; P-LCR, Platelet-large cell ratio.

It is noted from Table (7) the effect of *P.aerogenosa* which was adjusted to 1 macFarland turbidity equivalent to 3×10^8 on the hematological parameters at zero time if there was no effect for each of parameters; WBC, LYM%, MID%, NEUT%, LYM#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PLT, MPV, PDW, PCT, P-LCR. Either at a time of 2 and 4 hours if the bacteria did not effect on each of parameters; WBC, LYM%, MID%, NEUT%, LYM#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, MPV PDW, PCT, P-LCR, except for PLT and RDW-SD parameters, they were lower than the control parameters, while the effect of bacteria in a 24-hour time on the PLT and NEUT# parameters was slightly high, while the LYM% parameter was slightly lower than the control parameter.

Table (7): Effect of *Pseudomonas aerogenosa* bacteria on hematological parameters at turbidity equivalent to 3×10^8 cfu/ml..

Hematological parameters	Whole blood	Inoculated periods (hour)							
		zero		2 hours		4 hours		24 hours	
		Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria	Blood + saline	Blood+ Bacteria
WBC	5.1	5.1	4.9	5.0	4.9	5.1	4.8	4.4	5.2
LYM%	23.6	22.4	21.2	25.0	23.4	34.2	25.7	37	40.9
MID%	5.9	6.7	6.4	6.8	6.0	8.1	6.5	13.2	20.5
NEUT%	70.5	70.9	72.4	68.2	70.6	57.7	67.8	26.0	38.6

LYM#	1.2	1.1	1.0	1.3	1.1	1.7	1.2	2.7	2.1
MID#	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.6	1.1
NEUT#	3.6	3.7	3.6	3.4	3.5	3.0	3.3	1.1	2.0
RBC	5.82	5.18	5.20	5.18	5.21	5.20	4.95	5.18	5.16
HGB	11.0	10.2	10.1	10.5	10.4	10.7	10.7	10.0	10.0
HCT	41.4	36.7	36.9	36.5	36.7	36.7	34.9	39.4	40.8
MCV	71.2	70.9	71.0	70.6	70.5	70.0	70.6	76.1	79.1
MCH	18.9	19.6	19.4	20.2	19.9	20.5	21.6	19.3	19.3
MCHC	26.5	27.7	27.3	28.7	28.3	29.1	30.6	25.3	24.5
RDW-SD	46.5	46.5	46.5	86.5	46.5	46.5	46.5	52.0	53.9
RDW-CV	16.1	16.2	16.2	16.3	16.3	16.2	16.2	16.9	16.8
PLT	334	282	296	231	170	227	172	172	193
MPV	11.3	11.0	11.3	11.3	11.0	11.0	11.4	11.6	12.3
PDW	17.4	16.7	17.9	17.9	15.6	17.7	21.3	16.4	15.9
PCT	0.37	0.31	0.33	0.26	0.18	0.24	0.19	0.19	0.23
P-LCR	42.0	39.8	41.5	41.2	39.8	38.4	41.4	44.3	52.3

WBC, white blood count; LYM%, Lymphocyte%; MID%, Mid-range absolute count; NEUT%, Neutrophils%; LYM#, Lymphocytes #; MID#, Mid-range absolute count#; NEUT#, Neutrophils#; RBC, Red blood cell; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular Hemoglobin; MCHC, Mean corpuscular Hemoglobin concentration; RDW-SD, Red cell distribution width-Standard Deviation; RDW-CV, Red cell distribution width – Coefficient of Variation; PLT, platelet count; MPV, mean platelet volume; PDW, Platelet distribution width; PCT, Plateletcrit; P-LCR, Platelet-large cell ratio.

It is noted from the results that the bacteria *Staphylococcus hominis* and *Pseudomonas aerogenosa* did not effect on the hematological parameters at a growth density of 1.5×10^8 or 3×10^8 and incubation times 0, 2, 4 and 24 hours except for some parameters such as LYM%, NEUT%, PLT and RDW-SD and this may be due to the adhesion of some white blood cells on platelets, which

leads to a change in their number or the occurrence of a aggregates of lymphocytes giving different readings (Gulati *et al*., 2022).

Effect of blood on *Staphylococcus hominis* and *Pseudomonas aerogenosa* isolated from the blood of patients with bacteremia

Table (8) shows the bacterial number of *S.hominis* bacteria in the blood at different incubation times and dilutes as the number of colonies of bacteria in dilution of 10^7 was more than (300) cfu/ml at incubation times (zero, 2, 4, 24). In dilution 10^5 it was more than (300) cfu/ml at a time of 0,2 hours, (114) cfu/ml at a time of 4 hours and (115) cfu/ml at a time of 24 hours, while the number of bacterial colonies in the dilution of 10^3 cfu/ml was more than 300 at the time of (0), and at a time of (2, 4, 24) hours was (48, 28, 23) respectively.

Table (8): The number of *Staphylococcus hominis* in the blood at different times of incubation.

Dilutes	Numbers of colonies (colony-forming unit)			
	Zero hours	2 hours	4 hours	24 hours
10^7	300‹	300‹	300‹	300‹
10^5	300‹	300‹	114	115
10^3	300‹	48	28	23

Table (9) shows the bacterial number of *P. aeruginosa* in the blood at different times of incubation as the number of bacteria was more than 300 cfu/ml at incubation times (zero, 2, 4, 24) hours. The number of bacteria in dilution 10^5 was more than 300 at a time of (0, 2, 4) hours and 115 at a time of 24 hours, while the number of bacterial colonies in dilution of 10^3 was more than 300 cfu/ml at the time of zero and was (150, 156, 23) cfu/ml at a time of (2, 4, 24) hours respectively.

Table (9): The number of *Pseudomonas aerogenosa* in the blood at different times of incubation.

Dilutes	Numbers of colonies (colony-forming unit)			
	Zero hours	2 hours	4 hours	24 hours
10^7	300‹	300‹	300‹	300‹
10^5	300‹	300‹	300‹	115
10^3	300‹	150	156	23

Through the results, it is noted that there is a decrease in the number of bacteria, especially when diluting 10^3, as the number of bacteria decreased in the time of (2, 4, 24) hours by the effect of the blood, and the decrease in the number of gram positive bacteria was more than the gram negative bacteria at the time of incubation (2, 4) hours. This is what Anderson *et al*. (2018) showed that red blood cells bind and perforate chemicals, nucleic acids and pathogens, and red blood cells may promote immune activation or maintain immune quiescence leading to the killing of bacteria. While Minasyan (2019) showed that the majority of bacterial species are killed in bacteremia by oxidation on the surface of erythrocytes and digested by local phagocytes in the liver and spleen, but it is possible that the bacteria that cause bacteremia overcome this mechanism of innate

immunity of the human body by versatile respiration, the production of antioxidant enzymes, hemolysins, exotoxin and endotoxins, exopolymers and other factors that inhibit the defenses of the host and increase the survival of bacteria alive. While Tsuiji *et al*. (2019) showed that S.*aureus* produces many external proteins that inhibit the immune system such as complement inhibitors immunoglobulin-binding proteins and chemotaxis inhibitory proteins, showing that the toxins of bacteria were more effective in monocyte cells than lymphocyte either B or T. Minasyan (2021) showed that the process of phagocytosis by white blood cells and the mechanism of capture, killing and removing bacteria from the bloodstream cannot be for many reasons, noting that the latest data led to the conclusion that in bacteremia bacteria are quickly removed from the blood and red blood cells are the main cells that capture, kill and remove bacteria, stressing that red blood cells catch bacteria by attracting the triboelectric charge and killing it with oxygen released from oxyhemoglobin, this phenomenon is called oxycytosis in a way that is comparable to or equivalent to the term phagocytosis. In addition, some of the main components of bacteria stimulate immunity such as lipopolysaccharide for gram negative bacteria and Lipoteichoic acid (LTA) in the bacterial cell wall of gram positive, and it is expressed not only in pathogenic bacteria but also in non-pathogenic bacteria positive for gram stains (Ryu *et al*., 2009). Guo and Rondina (2019) noted that in recent years it has been shown that platelets, which are cells anucleate produced by megakaryocytes cells, support research showing the evolving role of platelets as a key guardian and respondent in infectious diseases, especially in bleeding and inflammation, when the pathogen invades intravascular, platelets can directly feel viral, parasitic and bacterial infections through pattern recognition receptors and integrin receptors or the pathogen factor. Immunoglobulin complexes remain through Fc and supplement system receptors – although our understanding of these reactions persists but they are incomplete, and the constant search for areas of injury or inflammation during their circulation in blood vessels as platelets also respond indirectly to the pathogen's invasion through interactions with leukocytes and endothelium cells.

After the antigen is recognized, platelets often become activated and through a variety of mechanisms, activated platelets can isolate or kill pathogens or facilitate the removal of pathogens by activating macrophages and neutrophils, promoting the formation of neutrophil extracellular traps (NETs) and forming microthrombis platelet. Platelets are involved in preventing the spread of pathogens as they act as the so-called immunothrombosis and at the same time pathogens can work the risks of blood clotting and the occurrence of blood clots. During bacterial infections, platelets mediate by activating the response by interacting with white blood cells and containing antibacterial compounds such as platelet CTAP-3 (connective tissue–activating peptide 3), platelet basic protein, Tβ-4 (thymosin β-4) and fibrinopeptide (A and B) (Koupenova *et al*., 2018). Platelets can interact with different strains of bacteria, including the family of *Staphylococcus* bacteria, where platelets adhere and gather around the bacteria using IgG receptors, fibrinogen and fibrinktin, so after the interaction of platelets with bacteria releases antibacterial compounds and some alpha toxins work in *S.aureus* by releasing β-defensin from platelets, as it works to form (NET) (Kraemer *et al*., 2011) or platelet factor 4 or named CXCL4 binds gram negative bacteria, and then exposure to PF4/heparin-like epitopes, which increases the binding of antibodies to

bacteria, leading to the process of opsonization and ingestion of bacteria by neutrophil (Krauel *et al*., 2011; Krauel *et al*., 2012). Koupenova *et al*. (2018) showed that by knowing the components of the bacterial cell and distinguishing them, platelets can activate either the coagulation pathway or the inflammatory pathway. Other studies have indicated that platelets have a role in inducing adaptive immunity during infection with bacteria if platelets bind to bacteria and slow down the process of cellular filtering, which keeps bacteria alive for the purpose of inducing the adaptive immune response mediated by T CD8+ cells, this process mediated by platelets depends on the occurrence of the opsonization process of bacteria mediated by the complement C3, platelet binding mediated by Glycoprotein (GP1b) and also the bacteria-platelet aggregates (Broadley *et al*., 2016). Shannon (2015) showed that the main role of platelets is to maintain blood clotting by sticking and gathering at injury sites and releasing coagulants, while Semple & Freedman (2010) showed that platelet activation is that they release granules that include some substances including proinflammatory molecules, chemokines, and anti-microbial peptides.

Conclusion

It notes from the results that the bacteria causing the Bacteremia did not cause a clear effect in the blood titration when measured with a device CBC. As well as the effect of blood in reducing the number of bacteria that cause bacteremia.

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