ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

TJPS

TIKRIT JOURNAL OF PURE SCIENCE

Journal Homepage: http://main.tu-jo.com/ojs/index.php/TJPS/index

Bacteriological and Physiological Study of bacterial infection of the blood stream

Muhsin H. Edham[1] , Nasreen K. Kamel[2]

[1] College of Sciences , University of Kirkuk , Kirkuk , Iraq.
[2] College of Medicine , University of Kirkuk , Kirkuk , Iraq.

DOI: http://dx.doi.org/10.25130/tjps.23.2018.124

ARTICLE INFO.

Article history:
-Received: 11 / 12 / 2017
-Accepted: 14 / 3 / 2018
-Available online: / / 2018

Keywords:
Bacteremia, physiological effects bacteria , Antibiotic sensitivity.

Corresponding Author:
Name: Muhsin H. Edham
E-mail:
muhsinaljuboory@gmail.com
Tel:

Abstract

The study was included to collect one hundred blood samples of peoples suspected with Bacteremia at different ages and both sex Kirkuk General Hospital and Azadi Hospital. The results indurated to found positive bacterial growth at 16 blood samples, while 86 samples found negative bacterial growth. The 11 isolates was appear as gram positive bacteria, while Ten of them were as *Staphylococcus species*, one was *Clostridium perfringens*, and Five isolates Gram negative bacteria, *Burkloderia cepacia, Pseudomonas alcaligenese, Stenotrophonas maltiphilia, Klebsiella pneumonia, and Escherichia – coli*. all *Staphylococcus* isolates were appear are sensitive to imipenem, vancomycin, followed by the amoxicillin - clavulanic acid for 8 isolates and Ceftriaxone, Chloramphenicol and Amikacin were sensitive to 7 isolates while all *Staphylococcus* isolates were resistance to cefixime followed by azithromycin, erythromycin. The study showed *Stenotrophomonas mltophilia* were the most resistant antimicrobial isolates used in the study. The results of the physiological tests showed that the PCV values were lower in most blood samples of patients with bacteremia, whereas increased values for Clotting time, WBC due to toxic effects of invasive bacteria.

Introduction

Blood is sterile, free of germs and bacteria. When it reaches the bloodstream for any reason, it will cause Bacteremia. When immune defenses fail to contain the bacteria and prevent them from multiplying, they will reveal toxic bacteria such as LPS in the Gram-negative bacteria and release their toxins. Exotoxin in the negative and positive bacteria of chromium, which works to change the normal blood parameters and dysfunction of tissues and organs of the body, we call this case septicemia [1]. It affects adults, young people and newborns and is one of the most important causes of injury, cancer, AIDS, spleen eradication and all injuries and accidents that lead to the inhibition and weakening of the immune system. Children may also be born during childbirth due to contamination from maternal blood, Infertility The child may become infected after the birth through the arrival of germs from the external environment and can also be injured when the catheterization of his kidneys or washing the industrial kidney and during surgery and surgery of the systemic or deep burns or infection with typhoid and Maltese fever [2,3]. Signs and symptoms of Bacteremia are caused by tiredness, nausea, hyperhidrosis, sweating, impaired blood clotting, high or low blood pressure and blood pressure, rapid breathing, impaired immune function, loss of appetite and cramps, especially in children. Dizziness, jaundice, toxic shock, and changes in primary blood standards such as PCV, WBC, and clotting time [4]. The aim of this study was to isolate and diagnosis of microbial that causing the septicemia in patient blood samples and determine the effects of microbes founded on some blood values of septicemia patients.

Methods

100 blood samples were collected for people suspected of infected with Bacteremia after observation and diagnosis of symptoms by the specialist doctor at Kirkuk General Hospital and Azadi Hospital. Blood samples are collected directly by a sterilized needle, taking into account the sterilization of the skin with iodine as well as alcohol

to prevent contamination of the sample with the bacteria present on the skin, the blood samples were collected at 10-12 ml from adults and 5-7 ml from children, Two ml of blood sample in tube containing sodium citrate used to evaluate the PCV, WBC tests, while blood sample for clotting time was taken directly from the main blood sample according to[5, 6].

Blood culture

The blood samples collected were used to isolation and diagnosis the bacterial species caused the Septicemia infection after incubation on optimal media according [7, 8,9]. The isolates ability to antibiotic resistant were tested according the Kirby and Bauer [10] **.**

Results and Discussion

Tables 1 and 2 Show that16 bacterial growth, while 84 samples were negative growth from total 100 samples, 15 isolates appear as aerobic bacteria growth and one isolate of anaerobic bacteria *Clostridium perfringens*. The results showed that 11 isolates were gram positive bacteria, 10 of its where Staphylococcus species. While the results showed 5 samples was positive growth of the Gram negative bacteria (*Pseudomonas alcaligenese, Burkloderia cepacia, Klebsiella pneumonia, Stenotrophonas maltiphili, Escherichia coli*).

Table (1). Account and percentage of isolates.

Growth Results	Account	%
Growth	16	16
No growth	84	84
Total	100	100

This showed the high potential of these bacteria on the incidence of infection more than other bacterial genes. This is due to their ability to infect various injury events and habitant places in human body as well as its high resistance to antibiotics [8]. The samples were identified according to scientific references [7] These results are consistent with studies [5,9].

Table (2). Bacterial isolates accounts from blood samples of septicemia patients

Bacterial type	Account	%
Staphylococcus aureus	4	4
Staphylococcus epidermis	4	4
Staphylococcus spp	2	2
Pseudomonas alcaligenese	1	1
Burkloderia cepacian	1	1
Stenotrophonas maltiphilia	1	1
Klebsiella pneumonia	1	1
Escherichia –coli	1	1
Clostridium perfringens	1	1

Results of sensitivity tests against antibiotics in this test, various antibiotic commonly used in the sensitivity tests of bacterial isolates from septicemia the procedure was working according to [11].

Table(3). Sensitivity tests for *Staphylococcus spp* isolates against different antibiotics

Bacteria isolated	Antibiotics types												
	IPM	OX	VA	AK	CD	C	AZM	CFM	AMC	E	TE	AMP	CTR
S. aureus	S	S	S	R	R	R	R	R	S	R	R	R	S
	S	S	S	S	S	R	R	R	S	R	R	R	S
	S	R	S	S	R	S	R	R	R	R	R	R	S
	S	R	S	R	S	R	R	R	R	R	S	R	R
S.epidermis	S	S	S	S	S	S	R	R	S	R	S	S	S
	S	R	S	S	R	S	R	R	S	R	S	S	S
	S	R	S	S	S	S	R	R	S	R	S	R	R
	S	R	S	S	R	S	R	R	S	R	S	S	R
S. spp	S	R	S	R	S	S	R	R	S	R	S	R	S
	S	R	S	S	S	S	S	R	S	S	S	R	S

R=resistant, S=sensitive.IPM=imipenem, OX=oxacillin,VA=vancomycin, AK=amikacin, CD=clindamycin, C=chloramphenicol, AZM=azithromycin, CFM=cefixime, AMC=amoxicillin.clavulanic acid, E=erythromycin=tetracyclin, AMP=ampicillin=ceftriaxone

The results of antibiotic susceptibility tests, as shown in Table 3, all isolates of *Staphylococcus* sensitivity to imipenem. This antibiotic belongs to the carbapenem group containing the B-lactam ring, which inhibits the building of the bacterial peptidoglycan [12]. The results showed that all *Staphylococcus* isolates were sensitive to Vancomycin. This was due to the high estimate of this antagonist in inhibition of peptidoglycan synthesis by its association with d-alanyl-d-alanine peptide, On the inhibition of the enzyme transglucosylase, which enters the synthesis of the chain glycan composition of the peptidoglycan. Eight isolates of *Staphylococcus* showed their sensitivity to Amoxicillin-Clavulanic acid. Seven isolates showed sensitivity to Chloramphenicol while 3 were resistant to this antibiotic through the production of Chloramphenicol acetyl transferase. Results also seven isolates were sensitive to Ceftriaxone and Amikacin, while three other isolates were resistant to these antibiotics. The results showed that all *Staphylococcus* isolates were resistance to Cefixime antibiotic, because the inherent resistance against the bacterial cell and the loss of binding sites with PBPs, these results are consistent with both [13,14]. The results found that 9 isolates of *Staphylococcus* were resistance to both Erythromycin and Azithromycin, where resistance was controlled by plasmids by

substituting antibiotic contact sites with the S50 ribosome where the drug was added by drug efflux, these results are consistent with. [15]. Seven isolates of *Staphylococcus* showed resistance to Oxacillin and Ampicillin, while 3 isolates showed different susceptibility to this antibiotic. The resistance was due to the production of endopeptidase enzymes, which are encoded in the MecA-gen, which stimulates the erroneous and random use of penicillin's in animal breeding to the development of resistant strains that can reach hospitals and cause limited infection in space and time. [16] The results also indicate *Staphylococcus aureus* was one of the most resistant antibiotic-resistant bacteria in the study. Three isolates showed ability of this isolates to resistance for 8 types of the antibiotics, which reflects the seriousness, these results are consistent with many studies [14].

Table (4). Sensitivity tests for *gram negative* bacteria isolates against different antibiotics.

Bacteria isolate	Antibiotics									
Stenotrophomonas maltophilia	IPM	OX	ATM	AK	C	CEC	CTR	TE	CIP	TMP
	R	R	R	R	R	R	R	R	S	R
Pseudomonas alcaligenese	IPM	AZM	ATM	AK	CFM	CEC	AMP	GEN	CIP	TMP
	S	R	R	S	R	S	R	S	S	R
Klebsiella pneumonia	IPM	AZM	ATM	AK	NET	CEC	CTR	AMC	CIP	TMP
	S	R	R	S	R	S	S	R	R	R
Burkholderia cepacia	IPM	TX	CLT	AK	CFM	AZM	CTR	AMC	CIP	PI
	S	R	R	R	S	R	R	R	S	S
E coli	IPM	C	PI	AMC	CTR	NOR	CRO	GEN	CIP	TMP
	S	R	R	R	S	R	S	R	S	S

R=resistant,S=sensitive. TMP=trimethoprim, CIP=ciprofloxacin, CRO=Co-trimoxazole, PI=piperacillin GEN=gentamicin, CEC=cifotoaxim.olavul, NET=natamycin, TX=cefotoaxime, CLT=cephoalathin CEC=cifotoaxim.clavulanicacid, NOR= norfloxacin, ATM=aztreona

The results of sensitive against antibiotics for *Stenotrophomonas maltophilia* ability of these bacteria to resist all antibiotics used under study except Ciprofloxacin where it was appear sensitive, these results correspond to many studies [9,17,18].

The results in Table 4 found that *Pseudomonas alcaligenese* was sensitivity to (imipenem, amikacin, cefotaxime - clavulanic acid, gentamicin, ciprofloxacin). These antibiotics have mechanisms to resist antibiotics analysis-enzymes, which are produced by certain types of Gram-negative bacteria, represented by the production of B-lactamase enzymes penicillinase and cephalosporinase. These results correspond to [19]. These bacteria were resistant to azithromycin, aztreonam, cefixime, ampicillin, trimethoprim. These bacteria have high resistance against most antibiotics by controlling protein membrane permeability and possessing B-lactamase enzymes, and the occurrence of resistance mutations in the chromosome, which that squire multiple bacterial resistance against most antibiotics [20, 21].

The results in Table 4, showed that *Klebsiella pneumonia* isolate was sensitive to imipenem, amikacin, cefotaxime-clavulanic acid, ceftriaxone, in spite of these antibiotics were highly effective against many Gram-negative bacteria [12]. also showed this bacterial was resistance to azithromycin, aztreonam, natamycin, amoxicillin-clavulanic acid, ciprofloxacin, and trimethoprim. These appear that bacteria have multiple resistance mechanism against many antibiotic spectrum [20].

Table 8, showed that *Burkholderia cepacia* isolate was sensitive to 4 of the antibiotics used: imipenem, cefixime, ciprofloxacin, piperacillin. These bacteria have ability to secrete the destroyed penicillinase and cephalosporinase enzymes, It also showed anti-amikacin resistance, which prevented the entry of this antibiotic into the bacteria the resistant of azithromycin was also shown by substituting at the 50 S-ribosome binding sites and resistance to amoxicillin-clavulanic acid was shown by altering the PBPs on the cellular envelope Changes by plasmids and chromosomal mutations [22,23,24].

Table (5). Sensitivity tests for *Clostridium perfringens* against different antibiotics

Bacteria isolated	male	Female	Age years	Antibiotics									
				IPM	C	CD	AMC	E	VA	TE	BCT	LNM	CTR
Clostridium perfringens	1	0	54	S	S	R	S	R	R	R	R	R	S

R=resistant, S=sensitive. BCT= bacitracin, LNM=lincomycin.

The results in Table 9. were found the ability of the *Escherichia coli* to sensitive for imipenem, ceftriaxone, ciprofloxacin, trimethoprim, co-trimoxazole and this reflects the ability of these antibiotics to inhibit the Gram-negative bacteria. These results correspond with [25,26]. Also showed ability of this bacteria to resistance the chloramphenicol, piperacillin, amoxicillin-clavulanic acid, norfloxacin, gentamicin, and this reflects their ability to resist multiple antibiotic spectra. These results are consistent with [27,[28].

The results showed that *Clostridium perfringens* were sensitive to imipenem, chloramphenicol, amoxicillin-clavulanic acid, ceftriaxone and resistance to both clindamycin, erythromycin, vancomycin, tetracycline, lincomycin and bacitracin. These results are consistent with [29,30].

Physiological tests:

Table (6). Blood parameters septicemia samples.

Bacteria isolated	PCV %	WBCs counts ($\times 10^3/mm^3$)	Clotting time(mint.)
Staph aureus	36	10393	12.5
	31	7172	14.5
	33	13930	14.6
	36	13440	15.5
S. epidermis	35	12971	14.0
	39	12514	14.5
	39	10164	14.5
	22	10433	13,5
Staph spp	34	1300	13
	35	12100	14
Stenotrophomonas altophilia	35	10383	11.3
Pseudomonas lalueke	39	8400	13
Klebsiella pneumonia	32	8000	15.5
Burkholderia cepacia	31	7000	13.7
E.coli	38	12175	9.6
Clostridium perfringens	40	6800	13.5
Growth negative sample	43	6200	8

The results of some blood parameter for blood samples of patients infected with *Staphylococcus aureus, staphylococcus epidermidis, staphylococcus spp* are found in table (6) decrease in the PCV value of 22-39% compared with not infected peoples at 43%. The results indicate that best a lack of red blood cells due to the invasion of bacteria and is a result of the destruction of the membranes leads to the exit of the contents into the blood where these substances are nutrients to the bacteria such as iron, resulting hemolytic anemia. The results also indicated the increased the time of blood clotting in all samples of patients and become between 12.5 to 15.5 minutes compared with the people not infected at 8 minutes, which are the result of the loss and integration of proteins and factors that help to clotting blood because of its association with bacteria or toxins, which makes blood clotting abnormal. The results also showed increased of WBC and appear between 7172 to 13930 cell/mm^3 when compared with the people not infected at 6200 cell/mm^3 it is a defensive result of the body of the injured to get rid of the invasive bacteria through phagocytosis and affected by the immune system of the body [31,32,33]. These results show that there is a convergence of the causes of variance in WBC, PCV, and Clotting time in the Staphylococcus species, although species differ. The other gram negative bacteria (*Stenotrophomonas maltophilia, Pseudomonas laluek, Klebsiella pneumonia, Burkholderia cepacia, Escherichia-coli*) were similar with *Staphylococcus* bacteria in effect on PCV, WBCs and blood clotting time. These results are consistent with [34-39]. Blood tests for patient infected with an anaerobic bacteria *clostridium perfringens* showed a slight increase in PCV values of 40% compared with the other bacteria under study. PCV increased gradually after injury with a high incidence of 30-40% Due to liver damage and the occurrence of fusion in plasma size in the latter stages of the infection, the decomposition and hemorrhage of the infected tissues and lymphocytic poisoning occurs in the injury areas as well as an increase in the total number of WBC (eosinophil, neutrophil) occur during the first hours of bacterial infection and increase in PCV through the occurrence of Edema and hypo proteinemia [40].

Conclusions

The Conclusion of the study appear that. Most of the blood septicemia infected on some blood test were negative effects caused by *Staphylococcus spp* and some gram negative species such as *Stenotrophomonas maltophilia, Pseudomonas laluek, Klebsiella pneumonia, Burkholderia cepacia, Escherichia-coli* and anaerobic bacteria *clostridium perfringens*.

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دراسة بكتريولوجية وفسلجية لتجرثم الدم

محسن حمد ادهام[1] ، نسرين قادر كامل[2]

[1]قسم علوم الحياة ، كلية العلوم ، جامعة كركوك ، كركوك ، العراق
[2]كلية الطب ، جامعة كركوك ، كركوك ، العراق

الملخص

شملت الدراسة 100 عينة دم لأشخاص مشكوك بإصابتهم بتجرثم الدم Bacteremia من مختلف الأعمار ومن كلا الجنسين في مستشفى كركوك العام ومستشفى أزادي أظهرت النتائج نمو بكتيري ل 16 عينة دم بينما لم تظهر 86 عينة دم أي نمو بكتيري حيث سجلت البكتريا السالبة لصبغة كرام +G أحدى عشر عينة نامية عشرة منها كانت تابعة لجنس *Staphylococcus* بينما سجلت البكتريا اللاهوائية عينة نمو واحده فقط مثلتها *Clostridium perfringens* وسجلت البكتريا السالبة لصبغة كرام-G خمس عينات نامية تمثلت ببكتريا *Burkloderia cepacia, Pseudomonas alcaligenese, Stenotrophonas maltiphilia، Klebsiella pneumonia، Escherichia –coli* .

أظهرت اختبارات الحساسية للبكتريا المعزولة اتجاة المضادات الحياتية أن جميع عزلات *Staphylococcus* كانت حساسة لمضادات imipenem, vancomycin يليها المضاد amoxicillin-clavulanic acid حساس ل 8 عزلات تليها المضادات Ceftriaxone, Chloramphenicol و Amikacin حساسة ل 7 عزلات بينما أظهرت جميع عزلات *Staphylococcus* مقاومة للمضاد الحيوي cefixime يليها المضادين الحيويين erythromycin, azithromycin ، كما أظهرت الدراسة أن بكتريا *Staphylococcus aureus* كانت أشد أنواع البكتريا التابعة لهذا الجنس مقاومة للمضادات الحياتية، بينت الدراسة أن بكتريا *Stenotrophomonas mltophil* كانت أشد العزلات مقاومة للمضادات الحياتية المستخدمة قيد الدراسة حيث كانت حساسة فقط لمضاد ciprofloxin، بينما حلت كل من بكتريا *Burkloderia cepacia, Klebsiella pneumonia, Clostridium perfringens* بالمرتبة الثانية من حيث مقاومتها للمضادات الحيوية، من جهة أخرى أظهرت نتائج الاختبارات الفسلجيه انخفاض قيم ال PCV في اغلب عينات الدم للأشخاص المصابين بتجرثم الدم بينما سجلت النتائج قيم عالية لكل من Clotting time, WBC نتيجة للتأثيرات السمية للبكتريا الغازية على كريات الدم الحمراء وعلى المركبات الدخلة في تخثر الدم. من جهة أخرى تحفز هذه البكتريا وسمومها الجهاز المناعي على زيادة أعداد خلايا الدم البيض.