Bacteriological and Physiological Study of bacterial infection of the bloodstream

Muhsin H. Edham¹, Nasreen K. Kamel ²

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

Article Info

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

Keywords:
Bacteremia, physiological effects bacteria, Antibiotic sensitivity.

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, Burkloideria 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 maltophilia 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 that 16 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, Burkloeria cepacia, Klebsiella pneumonia, Stenotrophonas maltiphili, Escherichia coli).

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 1. Account and percentage of isolates.

<table>
<thead>
<tr>
<th>Growth Results</th>
<th>Account</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>No growth</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Antibiotics types</th>
<th>IPM</th>
<th>OX</th>
<th>VA</th>
<th>AK</th>
<th>CD</th>
<th>C</th>
<th>AZM</th>
<th>CFM</th>
<th>AMC</th>
<th>E</th>
<th>TE</th>
<th>AMP</th>
<th>CTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td></td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermis</td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. spp</td>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 transglycosylase, 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].

The results of sensitivity against antibiotics for *Stenotrophomonas maltophilia* ability of these bacteria to resist all antibiotics used in 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 alcigenese* 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, cefotaxim-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, nalatamycin, 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, pipercacillin. 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 (4). Sensitivity tests for *gram negative* bacteria isolates against different antibiotics.

<table>
<thead>
<tr>
<th>Bacteria isolate</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>IPM</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Pseudomonas alcigenese</td>
<td>IPM</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>IPM</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>IPM</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>E coli</td>
<td>IPM</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
</tbody>
</table>

R=resistant, S=sensitive. TMP=trimethoprim, CIP=ciprofloxacin, CRO=Co-trimoxazole, PI=pipercacillin. GEN=gentamicin, CEC=cefotaximolavul, NET=natamycin, TX=cefotaxime, CLT=cephoalathin.

The results of sensitivity against antibiotics for *Stenotrophomonas maltophilia* ability of these bacteria to resist all antibiotics used in 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 alcigenese* 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, nalatamycin, 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, pipercacillin. 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

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>Female</td>
</tr>
<tr>
<td>IPM</td>
<td>C</td>
</tr>
<tr>
<td>Clorstridium perfringens</td>
<td>1</td>
</tr>
</tbody>
</table>

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, pipercacillin, 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:**
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/mm³ when compared with the people not infected at 6200 cell/mm³ 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.

References
[34] Brooke, J. (2007). Mutation of a lipopolysaccharide synthesis gene results in increased
دراسة بكتريولوجية وفسلجية لتجرثم الدم

محسن حمد ادهام 1, نسرین قادر كامل 2

قسم علم الحيوان، كلية العلوم، جامعة كركوك، كركوك، العراق
كلية الطب، جامعة كركوك، كركوك، العراق

الملخص
شملت الدراسة 100 عينة دم من أشخاص مشكوك بإصابتهم بجرح 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 البكتيريا المصلحة Staphylococcus كرام G+ كأحد عشر عينة نامية عشرة منها كانت تابعة لجنس Staphylococcus كرام G+ وسجلت البكتيريا المصلحة Lصىة كرام G خمس عينات نامية تمتلئ بكتيريا Burkholderia cepacia, Escherichia –coli, Klebsiella pneumoniae, Pseudomonas alcaligenes, Stenotrophomas maltiphilia

أظهرت اختبارات الحساسية للمضادات الحيوية اتجاه المضادات الحيوية أن جميع عزلات Staphylococcus كانت حساسة لمضادات Ceftriaxone, amoxicillin-clavulanic acid, imipenem, vancomycin, cefixime، مقابلة للمضادات الحيوية، Staphylococcus ل 7 عزلات بينما لم تظهر جميع عزلات Amikacin و Chloramphenicol كأحد أنواع Staphylococcus aureus كأحد أنواع البكتيريا البكتيريا المضادات الحيوية، اتجاه المضادات الحيوية، حيث استخدمت 12 عينة باختبارات مضادات البكتيريا المضادات الحيوية المستخدمة في الدراسة، حيث كانت حساسة لتوسط معدل مضادات Clostridium perfringens، Klebsiella pneumoniae، Burkholderia cepacia

تؤثر الاختبارات الفسلجية اختلاف في احصائيات الدم للأشخاص المصابين بجرح الدم بينما سجلت النتائج قيمًا منخفضًا في تلك الدراسات، من جهة أخرى، أظهرت نتائج Clotting time, WBC على زيادةертвاض الدم في تلك الدراسات، من جهة أخرى، أظهرت نتائج Clotting time, WBC على زيادة تأثير فطريات الدم، من جهة أخرى، أظهرت نتائج Clotting time, WBC على زيادة تأثير فطريات الدم، من جهة أخرى، أظهرت نتائج Clotting time, WBC على زيادة تأثير فطريات الدم.