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*RESEARCH ARTICLE - BIOLOGY*

# Detection of virulence factors of bacterial isolates associated with Acute Myeloid Leukemia (AML) and Acute Lymphocytic Leukemia (ALL) patients

**Zainab S. Helo[1], Eman N. Naji[1], Mazin A. Shubber[2]**

[1]Department of Biology, College of Science, Mustansiriyah University Baghdad, Iraq

[2] Hematology and Transplantation Center Medical City Complex

[*] Corresponding author E-mail: emannatiq@uomustansiriyah.edu.iq

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**Abstract**

Samples including blood, urine, pus, skin and throat swab) were taken from 200 leukemia patients who attended to Hematology and transplantation center in the medical city complex. The bacterial isolates were identified by phenotypic examination, biochemical tests, as well as VITEK-2 assay. From 176 positive culture samples, 195 isolates of gram-positive and gram-negative categories, no significant correlation was observed between the type of bacteria and their sources gram-positive isolates were more prevalent than gram-negative, *Staphylococcus epidermidis* was the prevalent bacteria isolated from different sources samples of leukemia patients followed by *Staphylococcus aureus* and *klebsiella and E.coli* among gram-negative. In conclusion, the bacteria isolated in our study demonstrated significant resistance to commonly used antibiotics, with most classified as MDR and others as PDR. Interestingly, Synergistic effects were detected among certain antibiotics against bacteria isolated from various sources. Most isolates hadn't hemolytic activity and the rest showed beta hemolysis except Micrococcus luteus and *Enterobacter cloacae* showed alpha hemolysis. Most isolates from numerous bacterial groups showed strong and moderate biofilm production.



***Keywords:*** : Acute leukemia**,** gram-positive, gram-negative bacteria, biofilm formation, hemolysis, antibiotic resistance

## 1. Introduction

Acute leukemia describes a collection of extremely aggressive blood illnesses that are defined by the uncontrolled proliferation of immature precursor cells in the bone marrow. This infiltration results in a significant decrease in platelet count, red blood cell count, and white blood cell count, ultimately leading to death within a few weeks if the condition is not treated. There are three primary categories of acute leukemia: acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). People diagnosed with cancer have an increased susceptibility to infections. In numerous instances, it is not possible to determine the source of infection. Therefore, it is necessary to employ empirical therapy in these patients [1]. The selection of treatment is based on established protocols, taking into account relevant microbiological information from cancer patients at the local, regional, and national levels [2]A large epidemiological investigation found that patients with haematological cancers had an eight-fold greater prevalence of bacterial infections than patients with solid tumours [3] Patients with leukemia are typically prone to

infectious or hemorrhagic and often life-threatening complications [4] The use of suitable empirical antibiotic therapy in conjunction with a thorough knowledge of frequently encountered microorganisms and drug sensitivity patterns forms the basis of infection control [5].

Prior studies have indicated that Gram-negative bacteria (GNB) were the primary pathogens responsible for bloodstream infections (BSIs) in cancer patients as a whole. Over the past two decades, there has been a noticeable change in this pattern, with a gradual rise in the occurrence of Gram-positive bacteria (GPB) as the cause of bloodstream infections (BSIs)[6].According to systematic investigations, invasive bacterial diseases in cancer patients are primarily caused by gram-positive organisms. Serious infections in cancer patients are caused by a wide variety of gram-positive bacteria, with staphylococci, streptococci, and enterococci accounting for the majority of disease burden [6]. Urinary tract infections (UTIs) are among the most prevalent illnesses among cancer patients due to their prolonged immunosuppression, complex cancer treatment, and catheterization [7])Antimicrobial resistance and bacterial bloodstream infections can lead to treatment failure and protracted infections in cancer patients[8]Blood cultures mostly detect bacteria. Despite advancements in pathogen identification and antibiotic susceptibility testing techniques, the current approaches for diagnosing bacterial bloodstream infections involve on biochemical and phenotypic assays on pure bacterial cultures or isolated colonies to identify the pathogens [9].According to recent research, 82% of patients in intensive care units (ICUs) and 51% of patients in normal wards are already taking antibiotics within four hours when blood samples were collected for culturing.[10] this present study aims to evaluate the common types of bacterial infections and their antibiotic susceptibility spectrum in blood cancer patients undergoing chemotherapy and virulence factors of bacteria including hemolysis and biofilm formation

## 2. Subjects and methods

### 2.1 Subjects

This study included 200 patients who attended HTC in Medical City between October 2023 to January 2024, their ages ranged from (14-81) years.

#### 2.1.1 Inclusion Criteria

All cases of chronic /acute leukaemia include acute lymphatic leukaemia (ALL), and acute myeloid leukaemia (AML) with and without fever the current study included four groups of 50 patients for each one as listed below:

G1= Acute Lymphatic Leukemia without fever (ALL W.O.F)
G2= Acute Lymphatic Leukemia with fever (ALL W.F)
G3= Acute Myeloid Leukemia without fever (AML W.O.F)
G4= Acute Myeloid Leukemia with fever (AML W.F)

#### 2.1.2 Exclusion Criteria

Patients less than 14 years, patients infected with viral diseases bone marrow transplant patients and other types of leukemia.

### 2.2 Samples Collection

Ten milliliter's of venous blood were withdrawn from each individual by venipuncture under an aseptic technique by syringe. The blood samples were divided into three parts; 5 millilitres for blood culture, 2.5 millilitres used to separate the serum which was used for procalcitonin and C-Reactive

Protein tests and the third part of the blood sample was dispensed in a sterile ethylene diamine tetra acidic acid (EDTA) tube as anticoagulant for further study. Blood, urine, sputum and swab samples were taken from different sites, including skin and throat. Amie's transport medium swabs and cups were used to collect swab samples from other patients' sites and urine and sputum samples.

Bacteria were isolated and identified by using standard bacteriological techniques [11]. Species were identified according to the morphological features on culture media, microscopic examination, and biochemical tests [12]) VITEK-2 was used as a confirmed test for the automated identification of isolates.

### 2.3. Antibiotics Susceptibility Test by Disk Diffusion Method

In vitro, an antibiotic susceptibility test was applied for all tested isolates divided into there groups in the present study by using the Kirby-Bauer method relied on measuring the diameter of the inhibition zone and comparing it with the Clinical and Laboratory Standards Institute ([13]as susceptible (S) and resistant (R), towards (13) antimicrobial agents that categorized into ten classes: IMI=Imipenem, AM=Ampicillin, AMC=Amoxicillin/ Clavulanic acid, CIP= Ciprofloxacin, TGC=Tigecycline, FOX=Cefoxitin, DA=Clindamycin, E=Erythromycin, MTZ=Metronidazole, VA=Vancomycin, CFM=Cefixime, CRO=Ceftriaxone, SXT=Trimethoprim/Sulphamethoxazole.

### 2.4. The biofilm formation detection

The biofilm development test was conducted using the Congo red plate assay, which utilized the Congo Red Agar (CRA) medium. The medium was prepared by dissolving sucrose (36 g/L), Brain Hart Infusion (BHI) broth (30 g/L), and agar-agar (18 g/L) in 900 ml of distilled water. A solution of Congo red dye was made by dissolving 0.8 grams of the dye in 100 milliliters of water and then filtering. After subjecting the agar to autoclaving and allowing it to cool to a temperature of 55°C, dye is introduced. The prepared mixture was poured and utilized to identify microorganisms that produce biofilm. A solitary colony of each strain was streaked onto agar plates and cultured at a temperature of 37°C for 24 hours. The presence of black colonies suggested positive outcomes. White or pink colonies indicated isolates that do not produce biofilm.[14].

**2.5. Statistical analysis**: To assess the significance level or P-value, among the different factors considered in the current study, percentages and chi-square were computed. The Fisher test with 95% confidence interval was utilized to determine variations in drug resistance levels. One-way analysis of variance (ANOVA) tests was employed to compare different groups. Results were presented as mean ± standard deviation (SD). The LSD test was used to identify significant differences among the tested means, with letters (A, B and C) denoting levels of significance, starting from (A) indicating high significance and decreasing accordingly. If letters were similar, it indicates no significant differences among the tested means. Values of $p \geq 0.05$ were deemed statistically nonsignificant, while $p \leq 0.05$ was considered significant in contingency table analyses. Statistical analyses were conducted using SPSS (V20).

## 3. Results and Discussion

Out of a total of 200 acute leukemia (AL) patients, 100 patients were diagnosed with (ALL) and 100 were diagnosed with (AML), each group is divided into two groups, one with fever (50) and the other without fever (50). The study includes 610 samples from different sources of leukemia patients (blood, skin, sputum, throat and urine) 137 total positive cultures out of 195 as total isolates. The statistical

analysis has revealed significant differences ($P < 0.05$) in age between ALL and AML patients with and without fever as listed in table 1.

However, there were highly significant differences (P value 0.02) in the aged group of ALL and AML (p value 0.01). Age distribution by disease groups has revealed that mean age was 40.48±3.2 years in ALL patients without fever; 36.8±4.2 years with fever; 57±2.6 in AML patients without fever; 43±1.8 in p=AML patients with fever.

Table 1: Characteristics of the sample included in the study and group distribution

| Characteristics of patients | ALL Total NO.100 | | AML Total NO.100 | | Total |
|---|---|---|---|---|---|
| | Without fever (ALL W.O.F) 50=G1 | With fever (ALL W.F) 50=G2 | Without fever (AML W.O.F) 50=G3 | With fever (AML W.F) 50=G4 | |
| Age/year | | | | | 200 patients |
| Mean ± SD | 40.48±3.2 | 36.8±4.2 | 57±2.6 | 43±1.8 | |
| Median | 41 | 31.5 | 55.6 | 42.3 | |
| Min | 15 | 14 | 17 | 15 | |
| Max | 74 | 72 | 47 | 81 | |
| P value | 0.02 sig | | 0.01 sig | | |
| Sources of Specimen collection(No) | Without fever (ALL W.O.F) 50=G1 | With fever (ALL W.F) 50=G2 | Without fever (AML W.O.F) 50=G3 | With fever (AML W.F) 50=G4 | 610 Specimen |
| Blood (22) | 0(0.0%) | 3(1.7%) | 0(0.0%) | 5(2.8%) | 8 (8)* |
| Skin swabs (200) | 28(15.9%) | 13( 7.3%) | 29(16.4%) | 9(5.1%) | 79(82) |
| Sputum (88) | 2(1.1%) | 8(4.5%) | 3(1.7%) | 6(3.4%) | 19(24) |
| Throat swabs (200 | 4(2.2%) | 13(7.3%) | 4(2.2%) | 11(6.25%) | 32(38) |
| Urine (110) | 11(6.25%) | 6(3.4%) | 14(7.9%) | 7(3.9%) | 38(43) |
| Total of +ve cls (pure or mixed) | 45(25.5%) | 43(24.4%) | 50(28.4%) | 38(21.5%) | 176(195) |
| Total of - ve cls | 436 | | | | |
| P value | 0.001 sig | | 0.001 sig | | |
| Acute lymphocytic leukemia (ALL)/ Acute myeloid leukemia (AML)/* isolates number | | | | | |

Table 2 shows that there were (195) isolates distributed into gram-positive and gram-negative isolates, high prevalence of gram-positive isolates was observed, and the total number of positive were 137(70.2%) where the highest number was found in ALL groups without fever (23.1%); and then with fever (17.3%) ;(15.4%) in AML patients with fever and the lowest number was found in AML patients without fever (14.4%). The total number of Gram-negative isolates 58(29.8%) where the highest (11.3%) in AML patients without fever ;( 7.8%) in ALL patients without fever ;( 5.6%) in AML patients with fever and the lowest number was (5.1%) in ALL patients with fever.

Table 2: Diversity and distribution of Bacterial isolates in all tested groups

| Diversity of Bacterial isolates | ALL Total NO.100 | | AML Total NO.100 | | Total |
|---|---|---|---|---|---|
| | Without fever (ALL W.O.F) 50=G1 | With fever (ALL W.F) 50=G2 | Without fever (AML W.O.F) 50=G3 | With fever (AML W.F) 50=G4 | |
| Gram-positive g/spp/isolates no | 6/7/45 (23.1%) | 5/6/34 (17.3%) | 2/2/28 (14.4%) | 2/4/30 (15.4%) | 137(%70.2) |
| Gram Negative g/spp/isolates no | 3/6/15 (7.8%) | 6/4/10 (5.1%) | 6/7/22 (11.3%) | 6/7/11 (5.6%) | 58(%29.8) |
| Total *g/spp/isolates no | 9/13/60 (30.9%) | 11/10/44 (22.4%) | 8/10/50 (25.7%) | 8/11/41 (21%) | 195(%100) |
| *genus/species/isolates number / Acute lymphocytic leukemia (ALL)/Acute myeloid leukemia (AML)/G1= Acute Lymphatic Leukemia without fever (ALL W.O.F)/G2= Acute Lymphatic Leukemia with fever (ALL W.F)/G3= Acute Myeloid Leukemia without fever (AML W.O.F)/G4= Acute Myeloid Leukemia with fever (AML W.F). | | | | | |

Results of table 3 revealed There were highly diversity in antibiotic sensitivity pattern in gram positive tested isolate for example in staphylococcus epidermidis represented the highest number of infections ; 45 out of 58 resistant to Ampicillin, 43 resistant to Amoxicillin/ Clavulanic acid,41 resistant to Cefixime,38 to Erythromycin,36 to Ciprofloxacin,33 to Trimethoprim/Sulphamethoxazole,31 to Ceftriaxone, 29 to Vancomycin ,28 to Metronidazole and Cefoxitin, 26 to Clindamycin,6 to Imipenem and only 2 to Tigecycline. The antimicrobial susceptibility of *Staph. aureus* showed that most isolates (38 out of47) were resistant to Amoxicillin/ Clavulanic acid and Trimethoprim/Sulphamethoxazole with highly resistant levels toward Cefixime (36 out of 47) and 33 isolates resistant to Metronidazole and Ampicillin with only 3 isolate resistant to Tigecycline, while other isolates resisted the range of (2-8) antimicrobial agents.The study also observed that all *Streptococcus thoraltensis* isolate sensitive to Tigecycline,7 out of 9 resist to Amoxicillin/ Clavulanic acid,6 to Ceftriaxone,5 isolate resist to Ampicillin, Cefixime and Erythromycin,4 to Trimethoprim/Sulphamethoxazole, Metronidazole ,3 to Clindamycin , Vancomycin and Cefoxitin while 2 isolate to Ciprofloxacin and Imipenem.Most of *Micrococcus luteus* isolate 5 out of 7 resist to Amoxicillin/ Clavulanic acid,4 to Ampicillin and Cefixime ,3 to Erythromycin, 2 to Cefoxitin , Clindamycin and Trimethoprim/Sulphamethoxazole while only 1 isolate to Ceftriaxone , Ciprofloxacin ,Imipenem, Metronidazole, Vancomycin. Beside that all isolate were sensitive to Tigecycline

*Kocuria kristinae* isolate were 3 resist to Amoxicillin/ Clavulanic acid id , 1 resistant to range of antimicrobial agent used in the study , Vancomycin while all sensitive to Imipenem , Metronidazole , Tigecycline ,Trimethoprim/Sulphamethoxazole. All four *Staphylococcus hominis* isolates were resist to Amoxicillin/ Clavulanic acid while 2 resisted to Vancomycin, Clindamycin, and one isolate resist to Cefoxitin, Ceftriaxone , Cefixime, Ciprofloxacin and Imipenem while all isolate sensitive to Ampicillin , Erythromycin , Metronidazole , Tigecycline and Trimethoprim/Sulphamethoxazole.

One out of 5 *Lactococcus gravieae* resist to a range of antimicrobial agent (6-7) while 2 Amoxicillin/ Clavulanic acid, Cefoxitin, Cefixime ,and Ceftriaxone all sensitive to Tigecycline. Finally All three isolates of *Granulicatella elegans* in this group sensitive to rang of antimicrobial agent (7-8) while 2 resist to Ciprofloxacin , Clindamycin and 1 isolate was resistant to Amoxicillin/ Clavulanic acid, Vancomycin.

Table 3: Antibiotic resistance pattern of Gram-positive bacterial isolates

| Bacterial species | NO. | *AMC 20/10 mg | AM 25 mg | FOX 30 mg | CRO 10 mg | CFM 5 mg | CIP 10 mg | DA 10 mg | E 15 mg | IMI 10 mg | MTZ 5 mg | TGC 15 mg | SXT 1.25/23.75 mg | VA 30 mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus epidermidis 58* | **58** | 43 | 45 | 28 | 31 | 41 | 36 | 26 | 38 | 6 | 28 | 2 | 33 | 29 |
| Blood | **2** | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 |
| Skin swabs | **25** | 18 | 21 | 9 | 10 | 20 | 22 | 11 | 22 | 1 | 5 | 0 | 6 | 6 |
| Sputum | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Throat swabs | **15** | 11 | 14 | 9 | 4 | 5 | 3 | 2 | 6 | 2 | 9 | 0 | 9 | 11 |
| Urine | **16** | 13 | 8 | 8 | 15 | 14 | 9 | 11 | 8 | 2 | 12 | 1 | 16 | 10 |
| *Staphylococcus aureus* | **47** | **38** | **33** | **21** | **27** | **36** | **29** | **19** | **22** | **9** | **33** | **3** | **38** | **34** |
| Blood | **1** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | **1** |
| Skin swabs | **9** | 6 | 3 | 3 | 5 | 5 | 5 | 4 | 3 | 1 | 5 | 0 | 6 | **5** |
| Sputum | **3** | 2 | 1 | 2 | 1 | 0 | 2 | 1 | 1 | 1 | 2 | 1 | 2 | **1** |
| Throat swabs | **7** | 5 | 6 | 3 | 5 | 4 | 4 | 5 | 4 | 1 | 3 | 1 | 3 | **5** |
| Urine | **27** | 24 | 22 | 12 | 15 | 26 | 17 | 8 | 13 | 5 | 22 | 1 | 26 | **22** |
| *Streptococcus thoraltensis* | **9** | 7 | 5 | 3 | 6 | 5 | 2 | 3 | 5 | 2 | 4 | 0 | 4 | **3** |
| *Micrococcus luteus* | **7** | 5 | 4 | 2 | 1 | 4 | 1 | 2 | 3 | 1 | 1 | 0 | 2 | **1** |
| *Lactococcus gravieae* | **5** | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | **1** |
| *Kocuria kristinae* | **4** | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | **1** |
| *Staphylococcus hominis* | **4** | **4** | **0** | **1** | **1** | **1** | **1** | **2** | **0** | **1** | **0** | **0** | **0** | **2** |
| *Granulicatella elegans* | **3** | 1 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | **1** |
| Total OF +ve isolates | **137** | **103** | **89** | **58** | **69** | **90** | **73** | **56** | **70** | **21** | **67** | **5** | **78** | **72** |
| % From 137 +ve isolates | | 75.2 | 64.96 | 42.3 | 50.4 | 65.9 | 53.3 | 40.9 | 51.1 | 15.3 | 48.9 | 3.6 | 56.9 | 52.6 |
| % From 195 Total isolates | | 52.8 | 46.4 | 29.7 | 35.4 | 46.2 | 37.4 | 28.7 | 35.9 | 10.8 | 34.4 | 2.3 | 40 | 36.9 |

* IMI=Imipenem, AM=Ampicillin, AMC=Amoxicillin/ Clavulanic acid , CIP= Ciprofloxacin, TGC=Tigecycline, FOX=Cefoxiti DA=Clindamycin, E=Erythromycin, MTZ=Metronidazole, VA=Vancomycin, CFM=Cefixime, CRO=Ceftriaxon SXT=Trimethoprim/Sulphamethoxazole.

Of all species in this group 75.2 %showed resistance to Amoxicillin/ Clavulanic acid followed by 65.9 %, 64.96%, 56.9%, 53.3%, and 52.6 % of species resistant to Cefixime, Ampicillin, Trimethoprim/Sulphamethoxazole, Ciprofloxacin , Vancomycin respectively. Followed by 51.1 % showed resist toward Erythromycin ; 50.4% toward Ceftriaxone; 48.9% toward Metronidazole; 42.3% toward Cefoxitin ; 40.9% toward Clindamycin; 15.3% toward Imipenem and only 3.6% toward Tigecycline.

Antibiotic resistance may be a sign of potential problems choosing an antibiotic course of treatment for GN management. This group included 58 isolates comprising *Klebsiella pneumoniae* as the prevalent species followed by *Escherichia coli*, *Enterobacter cloacae, Pseudomonas aeruginosa*, *Acinetobacter*

*baumannii, Acromobacter xylosis* , *Burkholderia cepacia* and *Aeromonas hydrophilia* The results of the current study in this group as listed in table 4 showed that

Oneout of 17 *Klebsiella pneumoniae* isolate isolated from blood resist to all antibiotic used in current study expect Tigecycline. while 5 isolate from sputum with highly resistance to Ceftriaxone (4) ;3 isolate were resist to Ampicillin , Amoxicillin/Cavulanic acid, Cefixime, Erythromycin, Metronidazole, Trimethoprim/Sulphamethoxazole, Vancomycin;2 toward Clindamycin and Ciprofloxacin ; 1 toward Imipenem and Cefoxitin .Besides that, all isolate sensitive toward Tigecycline. furthermore ,Isolate from urine sample with highly resistance to Amoxicillin/Cavulanic acid, Ciprofloxacin, Ampicillin (9,8,7 out of 11) respectively .

The antimicrobial susceptibility of *Escherichia coli* showed that 8 out of 10 isolate resist to Amoxicillin/Clavulanic acidand Ampicillin, whereas other isolates resisted the range of (2-5) antimicrobial agents and only one resist to Metronidazole was isolated from urine. In another hand all isolate sensitive toward Imipenem and Tigecycline. Furthermore, 7 out 8 Enterobacter cloacae isolate were resist to Amoxicillin/Clavulanic acidand 6 toward Ampicillin , Ciprofloxacin 5 to Erythromycin ,4 TO Cefixime while 3 toward Clindamycin, Ceftriaxone, Cefoxitin;2 toward Trimethoprim/Sulphamethoxazole ;1 toward Metronidazole , Vancomycin .In addition, it was found that all 8 isolates were sensitive to Imipenem and Tigecycline.

On the other hand, *P. aeruginosa* were resistant to Ampicillin , Amoxicillin/Clavulanic acid, Cefixime of the Present study with high resist 6 out 0f 7 toward Ceftriaxone, Ciprofloxacin, Clindamycin ,Erythromycin and 5 to Trimethoprim/Sulphamethoxazole while 4 to Cefoxitin , Imipenem and Metronidazole;2 to Vancomycin all isolate were sensitive to Tigecycline.

All five *Acinetobacter baumannii* isolate showed resist toward Ampicillin , Amoxicillin/Cavulanic acid, Cefoxitin , Ceftriaxone, Clindamycin with high resist 4 toward Cefixime, Ciprofloxacin while( 3,3,3,2,2) toward( Erythromycin , Metronidazole , Vancomycin, Imipenem , Trimethoprim/Sulphamethoxazole receptively and only one resist to Tigecycline.

All *Acromobacter xylosis* isolate were sensitive to Imipenem, Tigecycline, Trimethoprim/Sulphamethoxazole while two were resist toward Ampicillin , Clindamycin, Erythromycin, Ciprofloxacin and one toward Amoxicillin/Clavulanic acid, Ceftriaxone, Cefoxitin , Cefixime , Metronidazole , Vancomycin.

In addition, it was found that all *Burkholderia cepacia* isolates were resist to Ampicillin while 2 resist to Amoxicillin/Clavulanic acid, Erythromycin and 1 toward Cefoxitin , Clindamycin and Vancomycin. Besides that, all were sensitive to the other antibiotics that were applied.

Table 4: Antibiotic resistance pattern of Gram-negative bacterial isolates

| Bacterial species 58 | NO. | *AMC 20/10 mg | AM 25 mg | FOX 30 mg | CRO 10 mg | CFM 5 mg | CIP 10 mg | DA 10 mg | E 15 mg | IMI 10 mg | MTZ 5 mg | TGC 15 mg | SXT 1.25/23.7 25 mg | VA 30 mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | **17** | **13** | **11** | **4** | **6** | **9** | **11** | **5** | **8** | **2** | **7** | **1** | **9** | **7** |
| Blood | **1** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| Skin swabs | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sputum | **5** | 3 | 3 | 1 | 4 | 3 | 2 | 2 | 3 | 1 | 3 | 0 | 3 | 3 |
| Throat swabs | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urine | **11** | 9 | 7 | 2 | 1 | 5 | 8 | 2 | 4 | 0 | 3 | 1 | 5 | 3 |
| *Escherichia coli* | **10** | **8** | **8** | **3** | **3** | **4** | **5** | **2** | **5** | **0** | **1** | **0** | **3** | **4** |
| Blood | **2** | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 4 | 0 | 0 | 0 | 1 | 1 |
| Skin swabs | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sputum | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Throat swabs | **1** | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Urine | **7** | 6 | 5 | 2 | 2 | 3 | 4 | 1 | 4 | 0 | 1 | 0 | 2 | 2 |
| *Enterobacter cloacae* | **8** | **7** | **6** | **3** | **3** | **4** | **6** | **3** | **5** | **0** | **1** | **0** | **2** | **1** |
| *Pseudomonas aeruginosa* | **7** | **7** | **7** | **4** | **6** | **7** | **6** | **6** | **6** | **4** | **4** | **0** | **5** | **2** |
| *Acinetobacter baumannii* | **5** | **5** | **5** | **5** | **5** | **4** | **4** | **5** | **3** | **2** | **3** | **1** | **2** | **3** |
| *Acromobacter xylosis* | **5** | **1** | **2** | **1** | **1** | **1** | **2** | **2** | **2** | **0** | **1** | **0** | **0** | **1** |
| *Burkholderia cepacia* | **4** | **2** | **4** | **1** | **0** | **0** | **0** | **1** | **2** | **0** | **0** | **0** | **0** | **1** |
| *Aeromonas hydrophilia* | **2** | **2** | **1** | **1** | **1** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **1** |
| Total OF -ve isolates | 58 | 45 | 44 | 22 | 25 | 29 | 34 | 24 | 31 | 8 | 17 | 2 | 21 | 20 |
| %From 58 -ve isolates | | **77.5** | **75.6** | **37.9** | **43.1** | **50** | **58.6** | **41.4** | **54.4** | **13.8** | **29.3** | **3.5** | **36.2** | **34.5** |
| %From 195 Total isolates | | **23.1** | **22.7** | **11.3** | **12.8** | **14.9** | **17.4** | **12.3** | **15.9** | **4.1** | **8.7** | **1.02** | **10.8** | **10.3** |

*IMI=Imipenem, AM=Ampicillin, AMC=Amoxicillin/Cavulanic acid, CIP= Ciprofloxacin, TGC=Tigecycline, FOX=Cefoxitin, DA=Clindamycin, E=Erythromycin, MTZ=Metronidazole, VA=Vancomycin, CFM=Cefixime, CRO=Ceftriaxone, SXT=Trimethoprim/Sulphamethoxazole.

Furthermore, the two isolates of *Aeromonas hydrophilia* were resist to Amoxicillin/Clavulanic acidand only one resist to Ampicillin, Cefoxitin , Ceftriaxone and Vancomycin. while they were sensitive to the other antibiotics that were applied.

Out of the total rate of species, 77.5% and 75.6% showed the highest resistance against Amoxicillin/Clavulanic acidand Ampicillin respectively . followed by 58.6%,54.4%, 50%,43.1%,41.4%,37.9%,36.2%,34.5%,29.3% toward Ciprofloxacin, Erythromycin, Cefixime, Ceftriaxone , Clindamycin, Cefoxitin , Trimethoprim/Sulphamethoxazole, Vancomycin, Metronidazole while resist against Imipenem , Tigecycline it were the least 13.8% and 3.5 respectively

Table 5 display results of biofilm formation that revealed that strong biofilm producers were abundant by gram positive isolates, in the first was Staphylococcus epidermidis 22(11.5%) strong followed by Staphylococcus aureus 12 (6.15%) strong , Staphylococcus hominis was 3(1.53%) strong,moderately biofilm producer were *Staphylococcus epidermidis* 19(9.7%) moderate; *Staphylococcus aureus* were mostly moderate 16 (8.2%); Streptococcus thoraltensis was 4 (2.05%)moderate and Micrococcus luteus 2(1.02%) moderate, Lactococcus gravieae 2(1.02%) moderate .further more ,weakly biofilm producers were *Staphylococcus aureus* (6.15%)12 out of 47 **(**24.1%) ,5(2.56%) of *Staphylococcus epidermidis* ,4(2.05%) *Micrococcus luteus* ,and 2(1.02%) *streptococcus thoraltensis.* 1(0.5%) *Kocuria kristinae* while In gram negative Klebsiella pneumonia was 7(3.5%) weak ;5 (2.56%) strong 2(1.02%) moderate and 3(1.53%) non , Escherichia coli was 5 (2.56%) strong 4(2.05%) weak and 1(0.5%) non, Enterobacter cloacae was 6 (3.07%)weak and 2(1.02%) non, Pseudomonas aeruginosa was 5 (2.56%) strong and 2(1.02%) moderate, Acinetobacter baumannii was 4 (2.05%) strong and 1(0.5%) weak, *Acromobacter xylosis* was 5(2.56%) and all weak , Burkholderia cepacian was 1(0.5%) weak and 3 (1.53%) non , *Aeromonas* hydrophilia was 2(1.02%) weak in biofilm formation.

Table 5: Results of biofilm formation profile in all tested isolates

| Bacterial isolate 195 | Biofilm formation profile | | | | |
|---|---|---|---|---|---|
| | Non | Weak | Moderate | Strong | Total No. (%) |
| *Staphylococcus epidermidis* | 12(6.15%) | 5(2.56%) | 19(9.7%) | 22(11.2%) | 58 (29.7%) |
| *Staphylococcus aureus* | 7(3.5%) | 12(6.15%) | 16(8.2%) | 12(6.15%) | 47(24.1%) |
| *Streptococcus thoraltensis* | 3(1.53%) | 2(1.02%) | 4(2.05%) | 1(0.5%) | 9(4.6%) |
| *Micrococcus luteus* | 1(0.5%) | 4(2.05%) | 2(1.02%) | 0(0.0%) | 7(3.5%) |
| *Lactococcus gravieae* | 3(1.53%) | 0(0.0%) | 2(1.02%) | 0(0.0%) | 5(2.56%) |
| *Kocuria kristinae* | 3(1.53%) | 1(0.5%) | 0(0.0%) | 0(0.0%) | 4(2.05%) |
| *Staphylococcus hominis* | 1(0.5%) | 0(0.0%) | 0(0.0%) | 3(1.53%) | 4(2.05%) |
| *Granulicatella elegans* | 2(1.02%) | 0(0.0%) | 1(0.5%) | 0(0.0%) | 3(1.53%) |
| Total OF +ve isolates | 32(16.4%) | 24(12.3%) | 44(22.56%) | 37(18.9%) | 137(70.7%) |
| *Klebsiella pneumonia* | 3(1.53%) | 7 (3.5%) | 2(1.02%) | 5(2.56%) | 17(8.7%) |
| *Escherichia coli* | 1(0.5%) | 4(2.05%) | 0(0.0%) | 5(2.56%) | 10(5.1%) |
| *Enterobacter cloacae* | 2(1.02%) | 6(3.07%) | 0(0.0%) | 0(0.0%) | 8(4.1%) |

| | | | | | |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 0(0.0%) | 0(0.0%) | 2(1.02%) | 5(2.56%) | 7(3.5%) |
| *Acinetobacter baumannii* | 0(0.0%) | 1(0.5%) | 0(0.0%) | 4(2.05%) | 5(2.56%) |
| *Acromobacter xylosis* | 0(0.0%) | 5(2.56%) | (0.0%)0 | 0(0.0%) | 5(2.56%) |
| *Burkholderia cepacia* | 3(1.53%) | 1(0.5%) | 0(0.0%) | 0(0.0%) | 4(2.05%) |
| *Aeromonas hydrophilia* | 0(0.0%) | 2(1.02%) | 0(0.0%) | 0(0.0%) | 2(1.02%) |
| ve -Total OF isolates | 9(4.6%) | 26(13.3%) | 4(2.05%) | 19(9.7%) | 58(29.7%) |
| *TOTAL +VE AND –VE* | 41(21%) | 50(25.6%) | 48(24.6%) | 56(28.7%) | (100)195 |

The hemolytic activity of the bacterial isolate is displayed in **table 6**, fig.1 Within beta hemolysis *Staphylococcus aureus* was the highest percentage among gram-positive isolates 16.9% followed by *Staphylococcus epidermidis* and *Streptococcus thoraltensis* with 9% out of 137 the total number of isolate .*Staphylococcus hominis , Kocuria Kristinae* Granulicatella elegans ,*Micrococcus luteus* with(4,1,1,2) percentages respectively .while gram negative isolates Klebsiella pneumoniae, Escherichia coli were the two highest percentage (17,9)respectively Followed by *Pseudomonas aeruginosa*, Acinetobacter baumannii .On the other hand, the alpha hemolysis activity on blood agar with one percentage only for both gram-positive and gram negative display by *Micrococcus luteus* and Enterobacter cloacae.

Table 6: Hemolysis profile results of bacterial species isolated for all tested isolate**s**

| Bacterial isolate 195 | Hemolysis profile | | | |
|---|---|---|---|---|
| | beta hemolysis | α hemolysis | no hemolysis | Total No. (%) |
| *Staphylococcus epidermidis* | 9(4.6) | 0(%0.0) | 49(25.1) | 58 (29.7%) |
| *Staphylococcus aureus* | 33(16.9) | 0(%0.0) | 14(%0.5) | 47(24.1%) |
| *Streptococcus thoraltensis* | 9(4.6) | 0(%0.0) | 0(%0.0) | 9(4.6%) |
| *Micrococcus luteus* | 2(1.02%) | 1(%0.5) | 4(2.05%) | 7(3.5%) |
| *Lactococcus gravieae* | 0(%0.0) | 0(%0.0) | 5(2.56%) | 5(2.56%) |
| *Kocuria kristinae* | 1(%0.5) | 0(%0.0) | 3(1.53%) | 4(2.05%) |
| *Staphylococcus hominis* | 4(2.05%) | 0(%0.0) | 0(%0.0) | 4(2.05%) |
| *Granulicatella* | 1(%0.5) | 0(%0.0) | 2(1.02%) | 3(1.53%) |

| | | | | |
|---|---|---|---|---|
| *elegans* | | | | |
| Total OF +ve isolates | 77(39.5) | 1(%0.5) | 59(30.3) | 137(70.3%) |
| *Klebsiella pneumonia* | 17(%0.5) | 0(%0.0) | 0(%0.0) | 17(8.7%) |
| *Escherichia coli* | 9(4.6) | 0(%0.0) | 1(%0.5) | 10(5.1%) |
| *Enterobacter cloacae* | 2(1.02%) | 1(%0.5) | 5(2.56%) | 8(4.1%) |
| *Pseudomonas aeruginosa* | 7(3.6) | 0(%0.0) | 0(%0.0) | 7(3.5%) |
| *Acinetobacter baumannii* | 5(2.56%) | 0(%0.0) | 0(%0.0) | 5(2.56%) |
| *Acromobacter xylosis* | 0(%0.0) | 0(%0.0) | 5(2.56%) | 5(2.56%) |
| *Burkholderia cepacia* | 0(%0.0) | 0(%0.0) | 4(2.05%) | 4(2.05%) |
| *Aeromonas hydrophilia* | 0(%0.0) | 0(%0.0) | 2(1.02%) | 2(1.02%) |
| ve -Total OF isolates | 17(8.7) | 1(%0.5) | 40(20.5) | 58(29.7%) |
| *TOTAL +VE AND –VE* | 94(48.2) | 2(1.02%) | 99(50.78) | 195(100) |

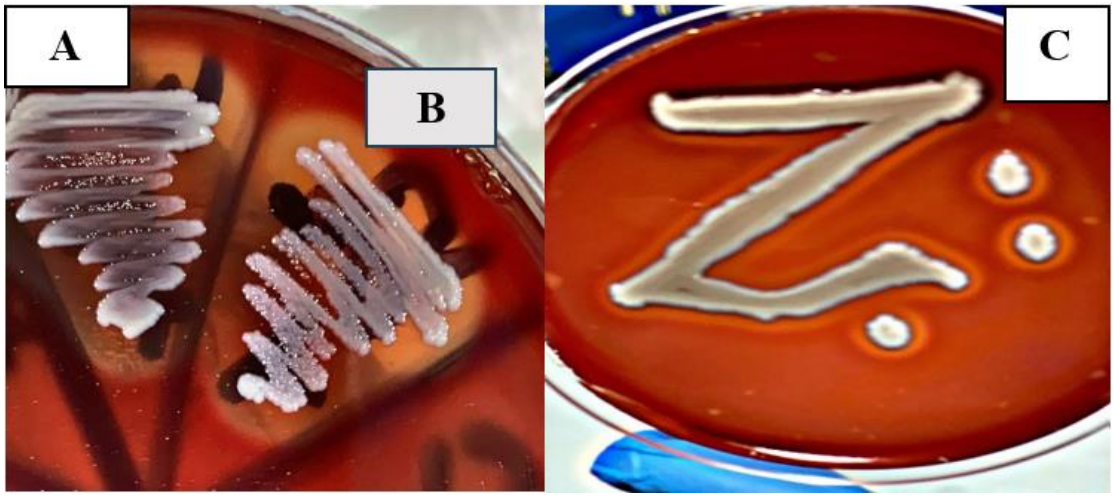


Fig.1. displays the hemolysis activity. Hemolysis on blood agar A) *Streptococcus sanguinis* B) *Staphylococcus aureus* C) *Serratia marcescens* -beta hemolysis

The study found that the average age of ALL patients without fever was 40.48±3.2 years, while the average age of ALL patients with fever was 36.8±4.2 years. In AML patients without fever, the average age was 57±2.6 years, and in p=AML patients with fever, the average age was 43±1.8 years[15]reported that Neutropenic acute leukemia patients, with a mean age of 13.26 ± 11.67 years and an age range from 1 to 46 years old, are being compared to non-neutropenic acute leukemia patients, with a mean age of 20.55 ± 19.061 years and age range from 3 to 64 years old.

[16]observed that the mean age was 34.3 ± 14.17, with a median age of 30 years. Out of the total number of patients, 37 (62.71%) were diagnosed with Acute Myeloid Leukemia (AML), while 22 patients (37.29%) were diagnosed with Acute Lymphoblastic Leukemia (ALL).

Age and acute leukemia have a high correlation but in distinct manners. Although peaks can occur in childhood, Acute Myeloid Leukemia (AML) is more prevalent among the elderly population. Therefore, the median age for AML diagnosis is 67 years old, while the median age for ALL diagnoses is 14 years old.[17]

The table also revealed the source of specimens and number of isolate in the current study table 1, Isolates commonly were from skin83, urine 43, throat 83, sputum 24, and blood 8. total of positive cultures (pure and mixed) was 176(195) out of 600.[18] reported Microorganisms were more commonly found in the bloodstream of the HIIC group (29 vs. 22), although this difference was not statistically significant ($p = 0.066$). Sputum, urine, throat swabs, and ET secretion showed no significant differences between the groups.

The current study observed a high prevalence of gram-positive isolates. these results agreed with[14]Reported that Gram-positive bacteria (GPB) were the most abundant microorganisms than gram-negative bacteria (GNB); it represents about 2:1 while in non-neutropenic acute leukemia patients growth only GPB were present. This is in contrast to the findings of [2]reveals that the most predominant causative organisms among cancer patients are Gram-negative bacteria.

Among gram-positive isolates *S. epidermidis* was the commonest isolate which in contrast to a study conducted by [8] found S. aures most prevalent isolate.in our study, S. aures represented the second most prominent. *Staphylococcus aureus* was the primary cause of sepsis in cancer patients. Treating S. aureus infections is challenging since the bacteria have a high level of adaptability to resist numerous medications, and there is currently no vaccination available.[19]

In our study resistance to vancomycin was found this line with the study of[20] reports of vancomycin resistance, once the preferred treatment for people with MRSA, have been documented. The results of our analysis revealed a 15.5% prevalence of vancomycin resistance in S. aureus isolates, all of which were also methicillin-resistant.

Among gram negative isolates klebsiella pneumonia and E.coli most prevalent types this line with a study of [21]found that most frequently isolated organisms were *Klebsiella pneumoniae* and *Escherichia coli* .

In the current study, *E.coli* highly resistance to ciprofloxacin agree with[22] reported E. Coli isolates from patients with HMs showed strong resistance to ciprofloxacin (84.40%). Additionally, we found *E.coli* highly sensitive to imipenem and this line[23]study.

Analysis of antibiotic resistance revealed high resistance to AMC and AM among gram-positive and negative isolates. An extensive examination and statistical analysis have shown a significant prevalence of extended beta-lactamase-generating Enterobacteriaceae colonization in patients with solid or hematological malignancies. The occurrence of this phenomenon raises the likelihood of bacteremia with the same pathogen and establishes significant reservoirs for horizontal transmission among oncological patients who are hospitalized.[24]

Carbapenem resistance, namely to imipenem, is a significant issue in antibiotic resistance due to the limited availability of other treatment options. The resistance of Gram-negative (GN) bacteria to carbapenems may be attributed to various processes that result in varying levels of resistance to this class of antibiotics. The expression of these mechanisms, either alone or in combination, determines the extent of resistance. The resistance may arise from structural alterations that impact the expression of certain components of the membrane, such as the efflux pump. [25]

Globally, the prevalence of Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) has lately grown There has been much discussion over the years on the relationship between the mortality of Bacterial Secondary Infections (BSI) caused by MDR-PA and incorrect empirical antibiotic therapy, with varying degrees of agreement. The differences between the studies show how complex this illness is and how important antibiotic therapy is in addition to host characteristics, the infection source, and infection site clearance. To the best of our knowledge, however, there haven't been any prior reports of specific research on the effects of improper antibiotic therapy on BSI caused by MDR-PA in the AL population. [26]

The study revealed that strong biofilm producers were abundant by gram positive isolates This finding supports the theory that biofilms are rarely linked to acute infections but are a major cause of persistent and recurrent infections. When a Strong Biofilm producer is present, it is an independent risk factor for the majority of invasive infections and end-organ diseases, even though it is less common than Weak Biofilm producers. This finding indicates that these bacteria's capacity to form biofilms enables them to travel and colonize distant organs and tissues once they enter the bloodstream, which can result in local metastatic infections. Septic arthritis, pneumonia, and infectious endocarditis are common instances of illnesses brought on by bacteria that form biofilms and spread to distant locations[27]The variations in the biochemical makeup of the biofilm and the resulting structure of the biofilm matrix among different bacterial species may account for the diversity in biofilm generation found among them.[28]In contrast to our Study [29]that shown that the relative abundance of weak biofilm producers (WBPs) and strong biofilm producers (SBPs) was substantially higher (P, 0.0001). Gram-negative bacteria were mostly responsible for the existence of SBPs. SBPs were specifically for CoNS, *P. aeruginosa*, K. pneumoniae, and E. coli. In 20.8% of incidents, catheter-related bloodstream infections (CRBSIs) were found. With a 65% frequency of isolation,Gram-positive cocci constituted the majority of the bacteria in these cases, whereas Gram-negative bacteria were detected in 35% of cases . The diversity of biofilm-producing isolates can be impacted by factors such as temperature and seasons, the presence of nutrition and oxygen gradients, antibiotic resistance, and quorum sensing [30].

The current study revealed a high prevalence of *S. epidermidis* non- biofilm-producing isolates. most isolate of *S. aureus* were beta hemolysis. The pathogenicity of S. aureus is mostly ascribed to the existence of virulence factors. For instance, S. aureus can cause the lysis of erythrocytes by generating several hemolysins, including α-, β-, and δ-hemolysins.[31].All Acinetobacter baumannii isolates were beta hemolysis in contrast to[32] study found that *A. baumanii* isolates from hospital samples in Baghdad lacked hemolysin activity; yet, many other variables could make it a pathogenic bacteria and cause nosocomial infections.

Hemolysins have traditionally been regarded as virulence factors, despite limited or absent direct experimental evidence supporting this idea. The majority of hemolysins Induce the breakdown of red blood cells by creating openings of different sizes in the cell membrane. Several hemolysins have the ability to target and damage various types of mammalian cells, likely using a similar process [33]. The ability of bacteria to survive in humans relies on their capacity to rapidly adapt to various environmental factors, including temperature, pH, osmolality, incubation duration, oxygen tension, nutrient availability, and iron reduction. Notably, the enhancement of hemolysis virulence factors is significantly influenced by the latter [34].

## 4. Conclusions

The current research findings confirm the extensive variety of microorganisms found in leukemia patients. This diversity encompasses both gram-positive and gram-negative bacteria from various bacterial

groups and isolation sources. The bacteria isolated in the current study demonstrated significant resistance to commonly used antibiotics, with most classified as MDR and others as PDR. Synergistic effects were detected among certain antibiotics against bacteria isolated from various sources. Most isolates hadn't hemolytic activity and the rest showed beta hemolysis expet *Micrococcus luteus* and *Enterobacter cloacae* showed alpha hemolysis. Most isolates from numerous bacterial groups showed strong and moderate biofilm production

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