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Yes, or No? Is there an effect of leukemia drugs on bacteria isolated from different sites of ALL and AML patients?

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ABSTRACT

Objective: The current research aims to evaluate the prevalence of causative bacterial species in acute myeloid and lymphoblastic leukemia patients, as well as the effect of certain chemotherapeutic agents on the growth of common bacterial pathogens isolated from various sites in these patients. Additionally, it investigates the relationship between elevated minimum inhibitory concentrations (MICs) of chemotherapeutic drugs and antimicrobial resistance.

Methods: This study included 200 patients who attended HTC in Medical City during a period between October 2023 to January 2024, ranging in age from 14 to 81 years. Blood, urine, sputum and swab samples were collected from various sites, including skin and throat swabs. The isolates were identified through phenotypic examination and biochemical tests, and confirmed by VITEK-2 assay. Antibiotic susceptibility pattern and the ability to form biofilms were studied for all bacterial isolates, followed by MIC testing for chemotherapeutic drugs including Cytarabine and Decitabine.

Results: Statistical analysis indicated significant differences ($p \leq 0.05$) in age between ALL and AML patients with and without fever notable discrepancies were observed in the age groups of both ALL and AML patients. While highly significant differences were indicated a distribution of 195 isolates into gram-positive and gram-negative categories, no significant correlation was observed between the type of bacteria and their sources .Synergistic effects were detected among certain antibiotics against bacteria isolated from various sources. Comparing groups revealed significantly higher differences in PCT concentration associated with both ALL and AML with fever when considering bacterial group .Also significant distinction was recorded among the analyzed groups of gram-positive and gram-negative bacterial isolates in ALL and AML patients concerning the mean concentration of CRP. No inhibitory effects were noticed across all minimum inhibitory concentration (MIC) values for both chemotherapy agents Cytarabine and Decitabine towards various multidrug-resistant bacterial isolates obtained from different sources.

Keywords: *Acute leukemia, gram-positive , gram-negative bacteria, Cytarabine , Decitabine, procalcitonin, C reactive protein*

1. INTRODUCTION

Acute leukemia refers to a form of blood cancer characterized via the transformation of immature progenitor cells in the bone marrow into clonal cells. develop into clonal cells. This infiltration leads to severe thrombocytopenia, anemia, and leukopenia if left untreated, which can prove fatal within a few weeks .[1](Hansen *et al*.,2020). There are various subtypes of the diseases, each requiring a distinct diagnosis and treatment approach. Chemotherapy is commonly employed to alleviate the condition, while hematopoietic stem cell transplants or alternative chemotherapy regimens are options for patients [2](Aljamali & Naser,2021). Acute myeloid leukemia affects the bone marrow's hematopoietic stem cells, resulting from genetic alterations in blood cell progenitors that lead to an excessive production of malignant clonal myeloid stem cells. This can be triggered by factors such as prior chemotherapy or exposure to specific chemicals [3](Grimwade *et al* ..,2016;).

Acute lymphoblastic leukemia (ALL) is a cancer characterized by the abnormal proliferation of immature lymphoid progenitors, that fail to mature. Different types of lymphoid cells can initiate the diseases, leading to T-cells or B-cells leukemia, or sometimes a combination of both [4](Cortes *et al.*,1995). Diagnosis typically involves morphological examination of bone marrow and blood samples [5](Fey,2007).

Several reports indicate that patients with blood cancer often contract bacterial infections, largely due to prolonged neutropenia following treatment. A large-scale epidemiological study revealed that individuals with hematological cancers had an eight-fold higher incidence of bacterial infections compared to those with solid tumors. Furthermore, a recent study found that hematological malignance exhibited higher levels of *Pseudomonas aeruginosa* bloodstream infections compared to solid tumors [6](Yusuf *et al.*,2023)

Infection plays a crucial role in the morbidity and mortality of individuals with hematological malignancies (HM). Febrile neutropenia (FN), the most significant treatment-related complication in HM patients, heightens susceptibility to infections[7,8] (Aslaner *et al*.,2023 & Shabeeb *et al*.,2023). Aggressive therapeutic interventions such as chemotherapy and stem-cell transplantation, aimed at managing these conditions, elevate the risk of infection and induce neutropenia [9](Mjali *et al*.,2021).

Recent meta-analyses suggest that procalcitonin (PCT) may offer better discrimination between infection and other sources of inflammation in critically ill patients compared to C-reactive protein (CRP)[10,11] (Yang *et al*.,2019 & Qasim *et al*.,2023)

Anticancer medications hold significant promise, as bacterial infections and tumor development share several characteristics, such as rapid replication rates, tendency to metastasize, high resistance to the immune system, and potential for treatment resistance. Indeed, many anticancer drugs exhibit notable antimicrobial properties[12] (Cruz-Muñiz *et al*.,2017) .

The hypomethylating agent Decitabine inhibits DNA methyltransferase, potentially exerting direct cytotoxic effects or influencing apoptosis and cellular differentiation. Recommended for treating de novo and secondary myelodysplastic syndrome (MDS) across all French-American-British subtypes, as well as intermediate-1, intermediate -2, and high-risk international prognostic Scoring System groups, Decitabine shows promise.[13] (Kantarjian *et al*.,2012)

This study aims to evaluate the bacterial distribution rates in infections among leukemia patients, along with identifying the common bacterial species associated with such infections. Additionally, it seeks to investigate the impact of two drugs, Cytarabine and Decitabine, widely used in leukemia treatment among Iraqi patients, on the growth of common bacterial pathogens isolated from various sites in these patients. Furthermore, it aims to explore potential relationships between elevated minimum inhibitory concentrations (MICs) of chemotherapeutic drugs and antimicrobial resistance.

2. SUBJECTS AND METHODS

Subjects

This research involved 200 individuals who visited the Hematology and transplantation center /Medical City Complex from October 2023 to January 2024, with ages spanning from 14 to 81 years old.

Inclusion Criteria

All tested cases of chronic or acute leukemia encompass both acute lymphatic leukemia (ALL) and acute myeloid leukemia (AML), with or without fever. As per the current study, four groups were examined, each comprising 50 patients, as outlined 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)

Exclusion Criteria

Patients aged 14, patients with viral infections, recipients of bone marrow transplants, and other forms of leukemia.

Samples Collection

Ten milliliters of venous blood were collected from each person through venipuncture using a sterile syringe and aseptic technique. The blood samples were then divided into three portions: 5 milliliters for blood culture, 2.5 milliliters for separating serum, which was subsequently utilized for procalcitonin and C-reactive Protein tests, and the remaining portion dispensed into sterile ethylene diamine tetra acetic acid (EDTA) tubes as an anticoagulant for further analysis. Additionally, samples including blood, urine, sputum, and swabs from various sites such as the skin and throat were collected. Amies transport medium swabs and cups were employed to gather swab samples from different patient sites, as well as urine.

Isolation and Identification of bacteria

Bacterial isolation and identification were conducted following established bacteriological methods[14] (Murray *et al.*,2020). Species were distinguished based on morphological characteristics observed in culture media, microscopic analysis, and biochemical tests [15](Brooks *et al.*, 2013). VITEK-2 was employed as a confirmatory test for the automated identification of isolates.

Antibiotics Susceptibility Test by Disk Diffusion Method

In vitro, all tested isolates in this study were subjected to an antibiotic susceptibility test, categorized into three groups, utilizing the Kirby-Bauer method. This method relies on measuring the diameter of the inhibition zone and comparing it with the guidelines provided by the Clinical and Laboratory Standers Institute[16] (CLSI, 2021), which classify isolates as susceptible (S) or resistant (R) to the antimicrobial agents. These agents are divided into ten classes: Carbapenem (IMI), Glycylcycline (TGC), Glycopeptide (VA), Nitroimidazole (MTZ), Fluoroquinolone (CIP), Lincosamide (DA), Cephalosporins (CFM), (CRO), and (FOX), Penicillin (AM) and (AMC), Sulfonamide (SXT), and Macrolides (E).

Measurement of PCT and CRP: Serum levels of PCT and CRP were assessed utilizing the AFIAS-6 system. Normal PCT levels ranged from 0.0 to 0.1 ng/ml, while normal CRP levels were below 10 mg/L. Results were obtained within 2 hours of the test being requested.

MIC OF Chemotherapeutic drug: Powders including cytarabine and dacagen were purchased from Getwell pharmaceuticals. Mueller-Hinton agar plates containing the drugs were prepared in a series of dilutions ranging from1.0 - 0.5 to 1.0 - 512. Additionally, drug-free control plates were prepared. The impact of the drugs was assessed through agar dilution, following the guidelines provided by CLSI (2021). Following an incubation period of 18 h at 35°C, plates were examined for growth and colony size, which were then compared to the growth observed in the drug-free control.

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

Out of a total of 200 patients diagnosed with acute leukemia (AL), 100 were identified with acute lymphoblastic leukemia (ALL) and the other 100 with acute myeloid leukemia (AML). Each group was further divided into two subgroups: one with fever (50 patients) and the other without fever (50 patients). The current study comprised 610 samples collected from various sources in leukemia patients (blood, skin, sputum, throat, and urine), resulting in 137 positive cultures out of a total of 95 isolates. Statistical analysis indicated significant differences ($p \leq 0.05$) in age between ALL and AML patients with and without fever. Furthermore, highly significant differences were observed in the age groups of ALL (P value 0.02) and AML (P value 0.01) patients. Analysis of age distribution via diseases groups revealed mean ages of 40.48 ± 3.2 years in ALL patients without fever, 36.8 ± 4.2 years in those with fever, 57 ± 2.6 years in AML patients without fever, and 43 ± 1.8 years in AML patients with fever.

Nevertheless, notable discrepancies were observed in the age groups of both ALL and AML patients, with highly significant differences evident (P value 0.02) for ALL and AML (P value 0.01) as well. Examination of age distribution among disease groups indicated mean ages of 40.48 ± 3.2 years for ALL patients without fever, 36.8 ±4.2 years for those with fever, 57 ± 2.6 years for AML patients without fever, and 43 ± 1.8 years for AML patients with fever.

The findings presented in table 2 indicated a distribution of 195 isolates into gram-positive and gram-negative categories, with a notable predominance of gram-positive isolates observed. Among the total positive cases (137,70.2%), the highest prevalence was found in ALL patients without fever (23.1%), followed by those with fever (17.3%), AML patients with fever (15.4%), and the lowest occurrence in AML patients without fever (14.4%). Gram-negative isolates accounted for 58 cases (29.8%), with the highest proportion observed in AML patients without fever (7.8%), AML patients with fever (5.6%), and the lowest occurrence in ALL patients with fever (5.1%).

Characteristics of patients	ALL Total NO.100		AML Total NO.100		
	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	Total
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)					610 Specimen

Table (1): Characteristics of the sample included in the study and groups distribution

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

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)

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

Table 3 displays the distribution and origins of bacterial isolates. No significant correlation was observed between the type of bacteria and their sources. *Staphylococcus epidermidis* constituted the highest number of isolates, accounting for 58 (29.7%), with 12.8% originating from skin samples, 8.2% from urine samples, and 7.69% from throat swabs. *Staphylococcus aureus* accounted for 47(24.1%) isolates, with 13.8% from urine, 4.6% from skin swabs, 3.5% from throat swabs, 1.5% from sputum, and 0.5% from blood samples. Other bacteria such as *Streptococcus thoraltensis*, Micrococcus *luteus, Lactococcus gravieae, Kocuria kristinae, Staphylococcus hominis*, and *Granulicatella elegan* were also identified, with percentages ranging from 1.53% to 4.6%, predominantly from sputum samples. Among gram-negative isolates, *Klebsiella pneumoniae, Escherichia coli,* and *Enterobacter cloacae* were the most prevalent, with *Klebsiella pneumoniae* being the predominant species (8.7%), mostly from urine samples, followed by *Escherichia coli* (5.1%) primarily from urine samples, and *Enterobacter cloacae* (4.1%). Other gram-negative bacteria such as *Pseudomonas aeruginosa, Acinetobacter* baumanni, Acromobacter xyloses, *Burkholderia cepacian* and Aeromonas *hydrophilia* were also identified, with lower percentages ranging from 1.02% to 3.5%.

Table 3: Distributions of bacterial isolates in all tested groups in combinations with sample sources

Sample source Bacterial isolate	Blood N(%)	Skin swabs N(%)	Sputum N(%)	Throat swabs N(%)	Urine N(%)	Total No. (%)
Staphylococcus epidermidis	(1.02%)2	(12.82%)25	(0.0%)0	(7.69%)15	(8.2%)16	58 (29.7%)
Staphylococcus aureus	(0.5%)1	(4.6%)9	(1.5%)3	(3.5%)7	(13.8%)27	(24.1%)47
Streptococcus thoraltensis	(0.0%)0	2(1.02%)	(2.56%)5	(0.5%)1	0(0.0%)	(4.6%)9
Micrococcus luteus	(0.0%)0	(1.02%)2	(1.02%)2	(0.5%)1	(0.0%)0	(3.5%)7
Lactococcus gravieae	(0.0%)0	(0.5%)1	(0.0%)0	(0.0%)0	(0.0%)0	(2.56%)5
Kocuria kristinae	(0.0%)0	(1.02%)2	(0.0%)0	(1.02%)2	(0.0%)0	(2.05%)4
Staphylococcus hominis	(0.0%)0	(0.5%)1	(1.02%)2	(0.5%)1	(0.0%)0	(2.05%)4
Granulicatella elegans	(0.0%)0	(0.5%)1	(0.5%)1	(0.5%)1	(0.0%)0	(1.53%)3
Total OF +ve isolates	(1.53%)3	43(22.05%)	(6.6%)13	28(14.35%)	(22.05%)43	(70.2%)137
Klebsiella pneumoniae	(0.5%)1	(0.0%)0	(2.56%)5	(0.0%)0	(6.15%)12	(8.7%)17
Escherichia coli	(1.02%)2	0(0.0%)	(0.0%)0	(0.5%)1	(3.5%)7	(5.1%)10
Enterobacter cloacae	(0.0%)0	(0.0%)0	(0.0%)0	(1.53%)3	(2.56%)5	8(4.1%)
Pseudomonas aeruginosa	(0.0%)0	(0.0%)0	(2.05%)4	(0.5%)1	(3.07%)6	(3.5%)7
Acinetobacter baumannii	(1.02%)2	(0.0%)0	(0.0%)0	(1.02%)2	(1.53%)3	5(2.56%)
Acromobacter xylosis	(0.0%)0	(0.0%)0	(0.5%)1	(0.5%)1	(1.53%)3	(2.56%)5
Burkholderia cepacia	(0.0%)0	(0.0%)0	(0.0%)0	(1.02%)2	(1.02%)2	(2.05%)4
Aeromonas hydrophilia	(0.0%)0	(0.0%)0	(0.5%)1	(0.0%)0	(0.5%)1	(1.02%)2
Total OF -ve isolates	(2.56%)5	(0.0%)0	11(5.64%)	(5.1%)10	(20%)39	(29.7%)58
TOTAL +VE AND -VE	(4.1%)8	(22.05%)43	(12.3%)24	(16.4%)38	(42.05%)82	195
Bad prism statistic is 27.853 The *p*-value is 0.001The result is significant at $p < .05$.						

A total of 195 bacterial isolates were identified, comprising 137 gram-positive and 58 gram-negative bacteria. Figure 1 illustrates a comparative analysis of antibiotic susceptibility patterns between gram-negative and gram-positive bacterial isolates. Gram-positive isolates exhibited a resistance rate of 52.8% to Amoxicillin/Clavulanic acid, while gram-negative isolates showed a resistance rate of 23.1%. Similarly, resistance rates for Ampicillin were 46.4% for gram-positive and 22.7% for gram negative bacteria. Other antibiotics also showed varying resistance rates between gram-positive and gram-negative isolates: Cefoxitin (29.7% vs 11.3%), Ceftriaxone (35.4% vs 12.8%), Cefixime (46.2% vs 14.9%), Ciprofloxacin (37.4% vs 17.4%), Clindamycin (28.7% vs 12.3%), Erythromycin (35.9% vs 15.9%), Imipenem (10.8% vs 4.1%), Metronidazole (34.4% vs 8.7%), Tigecycline (2.3% vs 1.02%), Trimethoprim/Sulfamethoxazole (40% vs 10.8), and vancomycin (36.9% vs 10.3). Moreover, the table indicates high sensitivity rates for both gram-positive and gram-negative isolates, notably in Tigecycline (96.41% and 85.13% receptively) and Imipenem, followed by varying sensitivity rates for other antibiotics. The current study revealed significant differences in resistance among most antibiotics tested, except for Ceftriaxone, Erythomycin, Trimethoprim/Sulfamethoxazole, and Vancomycin, where no significant difference was observed. Notably, there was a variation in resistance levels among isolates, especially among the same species isolated from similar source samples in different groups.

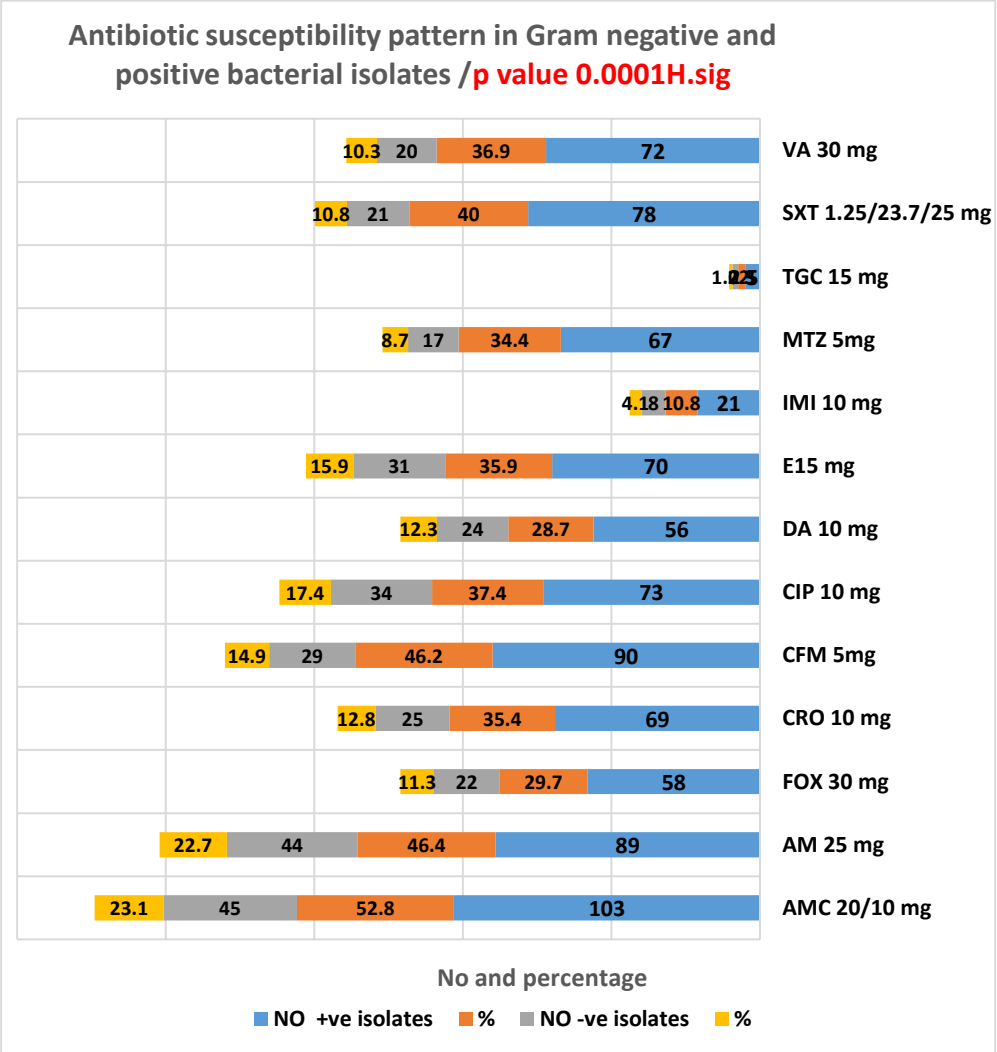

Figure 1: Antibiotic susceptibility pattern between Gram negative and positive bacterial isolates

The concurrent use of specific antibiotics can yield a more potent therapeutic outcome compared to the individual effect of each drug. This combined approach not only aids in reducing resistance but also offers broader coverage against bacterial invasion. Moreover, certain combinations may shorten the duration of therapy[17] (Edware *et al.*,2023). In the current study, synergistic effects were observed among certain antibiotics against bacteria isolated from various sources, as depicted in Figure 2.

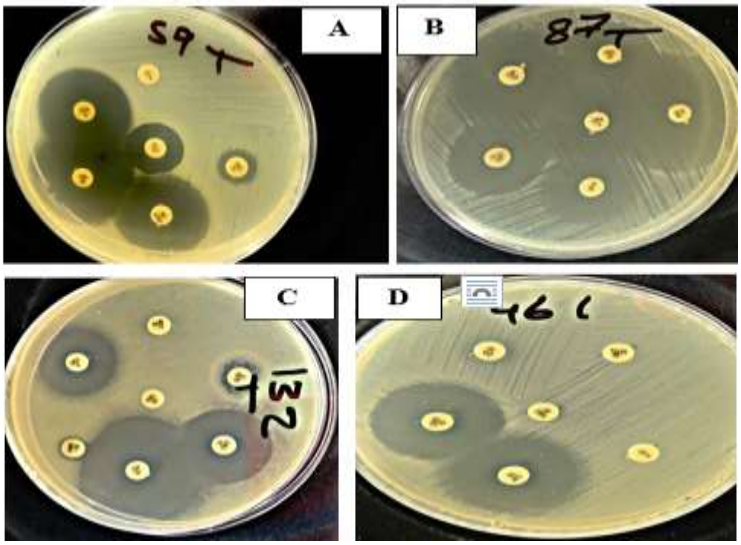

Figure 2 illustrates the phenomenon of antibiotic synergism on (Muller Hinton agar. A). *Streptococcus thoraltensis* isolated from a throat swab exhibiting synergy between (CIP, SXT, and FOX.B). *Staphylococcus aureus* isolated from a throat swab demonstrating synergy between (FOX, FOX and IPM, FOX and CRO, and FOX and CRO.C). *Pseudomonas aeruginosa* isolated from blood showing synergism between (CRO and CIP.D). *Klebsiella* isolated from a urine sample displaying synergy between CIP and CRO.

Comparing groups revealed significantly higher differences in PCT concentration associated with both ALL and AML when considering bacterial groups, whether gram-positive or negative isolates. These differences were highly significant according to the statistical analyses outlined in table 5. Specifically, highly significant differences were observed in gram-positive (0.44 ± 0.13) and gram-negative (0.65 ± 0.1) patients without fever (G1, ALL) with a p-value of 0.05. Similarly, highly significant variations were noted between gram-positive (0.78 ± 0.09) and gram-negative (0.98 ± 0.12) patients with fever (G2, ALL) with a p-value of 0.02. In the case of G3 AML, significantly differences were observed between patients infected with gram-positive (0.37 ± 0.05) and gram-negative isolates (0.28 ± 0.09) with p-value of 0.05, while in G4 AML, no significant difference was found (p-value of 0.41), indicating that bacterial types had no effect on PCT levels in this group. Notably, highly significant differences were observed between patients with negative and positive cultures, irrespective of the isolate being gram-positive or negative. In terms of gram-positive isolate, no significant difference were found between G1 and G3 (p-value 0.09) or between G2 and G4 (p-value 0.07). However, in the case of gram-negative isolates, highly significant differences were observed between G1 and G3 (p-value 0.0001) as well as between G2 and G4 (p-value 0.03).

Table 4 Levels of Procalcitonin in both ALL and AML patients regarding presence or absence of fever

Tested factors (mean ±SE) Procalcitonin N.V (0.10-0.15 ng/ml)	***ALL Total NO.100**		**AML Total NO.100**	
	Without fever (ALL W.O.F) 50=G1	**With fever (ALL W.F) 50=G2**	**Without fever (AML W.O.F) 50=G3 (10 tested)**	**With fever (AML W.F) 50=G4** (10 tested)
patients with gram +ve isolates	**0.44±0.13**	0.78±0.09	0.37±0.05	**0.86±0.11**
P value	**0.01**		**0.01**	
patients with gram -ve isolates	0.65±0.1	0.98±0.12	0.28±0.09	**0.83±0.07**
P value	**0.04**		**0.002**	

P value G –ve vis G+ve	**0.05**	**0.02**	**0.05**	**0.41**
Patients with Negative cultures	0.18±0.05		**0.22±0.08**	
P value tested groups and neg patients	G1+ve=0.02 G1-ve=0.001	G1+ve=0.001 G1-ve=0.000	G1+ve=0.02 G1-ve=0.08	**G1+ve=0.001** **G1-ve=0.0001**
P value	**G1**	**G3**	**G2**	**G4**
patients with gram +ve isolates	**0.09**		**0.07**	
P value	**G1**	**G3**	**G2**	**G4**
patients with gram -ve isolates	0.0001		**0.03**	
*** Acute lymphocytic leukemia (ALL)/ Acute myeloid leukemia (AML)** **Only 10 patients from each group were examined/ Patients with** **Negative cultures for all sample sources. p<0.05 were considered significantly different**				

Similarly, as illustrated in Table 5, there was a markedly significant distinction observed among the analyzed groups of gram-positive and gram-negative bacterial isolates in ALL and AML patients concerning the mean concentration of CRP. Notably, highly significant disparities were evident between patients with negative cultures and those with positive cultures, irrespective of the gram-positive or gram-negative nature of the isolates. Furthermore, there were highly significant difference noted in gram-positive- infected patients between G1 and G3(p-value 0.004), as well as between G2 and G4 (p-value 0.001). Similarly, significant disparities were observed in gram-negative patients between G1 and G3 (p-value 0.002), and between G2 and G4 (p-value

0.001), in their respective tested groups.

Table 5 Levels of C reactive protein in both ALL and AML patients regarding presence or absence of fever

Tested factors (mean ±SE) **CRP=C-reactive protein N.V (3 - 10) milligram/L**	***ALL Total NO.100**		**AML Total NO.100**	
	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**
patients with gram +ve isolates	**10.6±5.4**	33.9±**7.1**	22.2±**6.2**	**58.5**±13.4
P value	**0.002**		**0.001**	
patients with gram -ve isolates	17.5±**3.8**	45.9±**8.4**	35.7±**11.8**	66.8±14.9
P value	**0.001**		**0.001**	
Patients with Negative cultures	8.9±**2.3**		**13.9**±2.8	

P value tested groups and neg patients	G1+ve=0.19 G1-ve=0.05	G1+ve=0.001 G1-ve=0.001	G1+ve=0.04 G1-ve=0.002	**G1+ve=0.0001** **G1-ve=0.0001**
P value	**G1**	**G3**	**G2**	**G4**
patients with gram +ve isolates	**0.004**		**0.001**	
P value	**G1**	**G3**	**G2**	**G4**
patients with gram -ve isolates	**0.002**		**0.001**	

*** Acute lymphocytic leukemia (ALL)/ Acute myeloid leukemia (AML)**

Only 10 patients from each group were examined/ Patients with

Negative cultures for all sample sources. p<0.05 were considered significantly different

This study marks the initial investigation into the impacts of specific chemotherapy drugs like Cytarabine and Decitabine on bacterial strains derived from leukemia patients. These strains include a variety of genera and species of drug-resistant pathogenic and opportunistic bacteria. There is an increasing inclination towards repurposing these medications to capitalize on their precise effects on bacteria. The current study revealed that no inhibitory effects were detected across all minimum inhibitory concentration (MIC) values for both chemotherapy agents when tested against divers multidrug-resistant bacteria sourced from different origins, as illustrated in Figure 3.

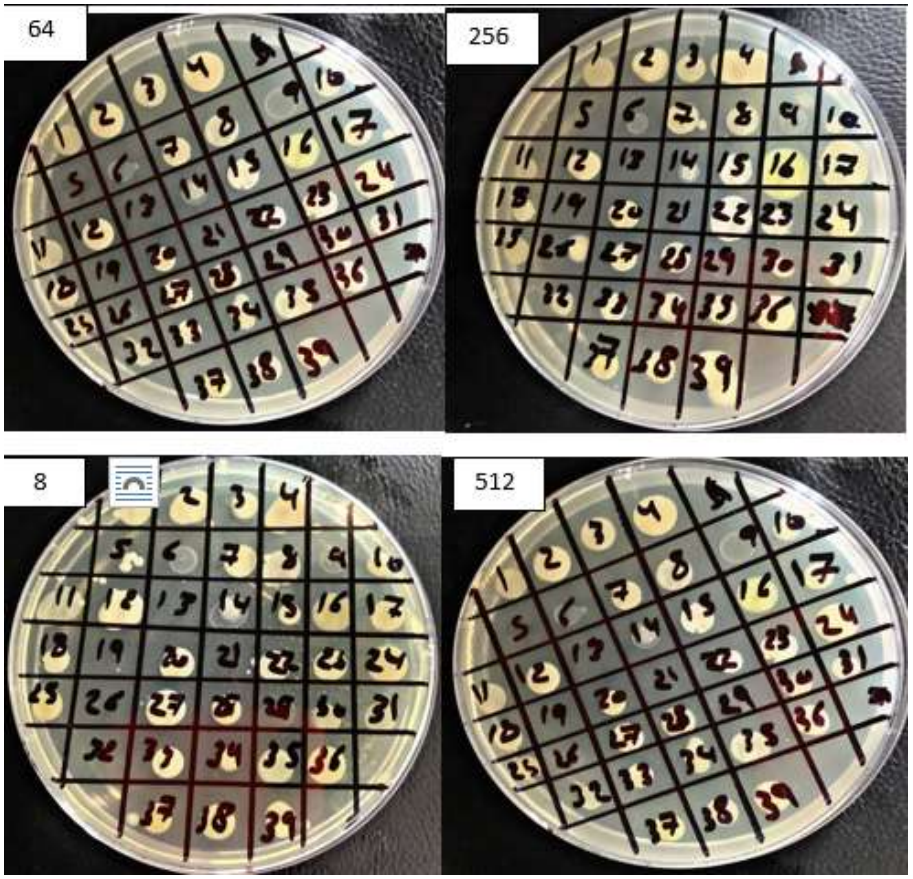

Figure 3 MIC of chemotherapy drugs ; Cytarabine and Decitabine on bacterial strains derived from leukemia patients.

4. DISCUSSION

This study findings revealed that the average age varied across different groups: 40.48 ± 3.2 years for ALL patients without fever, 36.8 ± 4.2 years for those with fever, 57 ± 2.6 for AML patients without fever, and 43 ± 1.8 for p = AML patients with fever. [9]Mjali *et al.,* (2021) observed a mean age of 34.3 ± 14.17, with a medium of 30 years. Of the patients, 36 (61.02%) were female and 23 (38.98%) were male. AML accounted for 37 patients (62.71%), while ALL comprised 22 patients (37.29%).

There exists a strong correlation between age and acute leukemia, albeit in different patterns. While ALL typically manifests in childhood, AML is more prevalent among older individuals. [18]Fiegls (2016).

Regarding age, the patient condition and the type of acute myeloid leukemia AML exhibit variation. It is widely acknowledged that elderly patients tend to have a poorer performance status and are more prone to comorbidities compared to their younger counterparts. As observed in this study. AML in older patients often involves a myelodysplastic phase preceding manifestation, unfavorable cytogenetics, multidrug resistance, and a less favorable response to chemotherapy compared to younger adults [19](Appelbaum *et al.*,2006).

The outcomes of the current study disclosed the specimen sources and isolates count. Commonly encountered specimens included skin (79), urine (38), throat (32), sputum (19), and blood (8). The total number of positive cultures (pure and mixed) amounted to 176 out of 600. [20]Majid *et al.,* (2024) noted that microorganisms were more frequently found in the bloodstream of the HIIC group (29 vs 22), although this discrepancy lacked statistical significance (p = 0.066). no notable difference was observed in sputum, urine, throat swabs, and ET secretion between the groups.

A notable prevalence was observed in the current study belongs to gram-positive isolates. These findings resonate with [21]Abedelnasser (2020), who highlighted a higher abundance of gram-positive bacteria (GPB) compared to gram-negative bacteria (GNB), with a ratio of approximately 2:1. Similarly, the results concur with [22]Abdul Hussein (2022), who evidenced that gram-positive bacteria were the predominant bacterial species in leukemia patients.

No significant correlation was observed between the type of bacteria and their sources. [23]Amanati (2021) documented that 262 (63.3%) and 152 (36.7%) gram-negative and gram-positive pathogenic organisms were respectively isolated from blood cultures. *Escherichia coli* emerged as the predominant gram-negative organism, followed by *Pseudomonas* spp and *klebsiella pneumoniae*. Among gram-positive bacteria, Coagulase -negative *Staphylococcus* (CoNS) were the most prevalent pathogens.

Moreover,[20] Majid *et al.,* (2024) noted a higher incidence of microorganisms in the bloodstream of the HIIC group compared to the LIIC group (29 vs 22), though this disparity lacked statistical significance (p = 0.066). No significant discrepancies were observed in sputum, urine, throat swabs, and ET secretion between the groups. Additionally, fever without a clear source was recorded in 7 participants from the HIIC group and 2 from the LIIC group, with no statistically significant difference (p = 0.725).

In the current study, resistance of antibiotic pathogens was examined for all blood cancer patients. Obtained results revealed a high resistance to AMC and AM in both gram-positive and gram -negative isolates. Through thorough examination and statistical analysis, we identified a significant prevalence of extended beta-lactamase producing Enterobacteriaceae colonization in patients with solid or hematological malignancies. This occurrence increases the likelihood of bacteremia with the same pathogen and establishes significant reservoirs for horizontal oncological patients[24,25] (Alevizakos et al.,2016; Edwar *et al* 2023). Results indicate an observed resistance to fluoroquinolones and cephalosporins in the isolates. The escalating prevalence of fluoroquinolone resistance and ESBL production by gram-negative bacteria may require focused attention, especially during hospital admission for specific patients groups. In such scenarios, employing a clinical risk score could be advantageous[26,27] (Tumbarello *et al.*,2011; Al-Kadmy *et al.*,2023) Quinolone prophylaxis did not result in an increase in the incidence of gram-positive bacteremia. Furthermore, there were no notable differences in the number of patients infected by quinolone-resistant organisms[28,29] (De Rosa *et al.*,2013; Awayid *et al.*,2017). Research demonstrated a 72% resistance to vancomycin in isolates of gram-positive bacteria. Cases of vancomycin resistance, previously the preferred treatment for MRSA patients, were reported by[30,27] Ashour *et al.,* (2017); Al-Kadmy *et al.,* (2023). In another study [31,32]Eleni Isidora *et al.,* (2021) & Edwar *et al.,* (2023) , results revealed that 14% of participants patients, had previously been colonized by multidrug resistant bacteria. This colonization could potentially elevate the risk of developing infections resistant to multiple drugs. According to our findings, both Glycylcycline and Carbapenem showed efficacy against multidrug resistant isolates and are thus recommended for treating associated infections in cancer patients. Despite reports indicating a growing trend of Carbapenem resistance among Enterobacteriaceae, the current study results underscore the effectiveness of these medications.

Additionally, because of the ongoing evolution of antimicrobial resistance in bacteria, it is crucial to regularly update data on antimicrobial susceptibility profiles. This ensures that pathogen-specific antimicrobial treatments remain effective and safe. Enforcing limitations on antimicrobial use could help reducing the development of resistant organism and improve the efficacy of treating nosocomial infections. It is also essential to establish and implement comprehensive infection control

measures in hospitals to lower the risk of nosocomial infections in cancer patients. When starting empirical antibiotic therapy, clinicians should examine the full spectrum of microorganisms, not just those associated with bloodstream infections (BSIs). The rate of infection in neutropenic acute leukemia patients is higher and involves more virulent and resistant bacteria compared to non-neutropenic acute leukemia patients. This fundamental understanding was linked to a higher mortality rate among all patients, as insufficient or inappropriate antimicrobial treatment is also associated with an increased hospital mortality rate. Current study results identified a notable proportion of multidrug resistant (MDR) isolates

Detecting fever early is essential for patients with hematologic conditions. This retrospective study aimed to assess how effectively PCT and CRP identify bacterial infections in these patients. PCT showed superior diagnostic accuracy for bacteremia compared to CRP. In this study, infection was defined as a culture-positive bacterial infection during febrile episodes. Patients with bacteremia had notably higher serum PCT levels than those without fever. PCT typically peaked 24 hours after fever onset and declined if the infection cleared, but not if fever persisted.

According to multivariant analysis, a high CRP cutoff (> 9.5 mg/dL) was a more reliable indicator of infections than PCT in patients undergoing stem cell transplantation (HSCT). Conversely, in non-HSCT patients, a positive PCT was a better indicator of infections than any CRP cutoff level.

One of the important goal of the current study is differentiate between various infection causes in febrile patients with hematologic diseases using serum PCT or CRP levels. Patients with infections had significantly elevated PCT levels compared to those without infections. The cutoff for serum PCT to distinguish between bacteremia and non-bacteremia infections ranged from 0.5 to 1.3 ng/ml in several studies, with sensitivity and specificity ranging from 44% to 88% and 61% to 88% respectively.

Although pathogenic organism culture remains the gold standard for identifying bacterial infections, delays in obtaining results could negatively impact neutropenic patients following acute leukemia chemotherapy. Hence, evaluating biomarkers like PCT may offer prompt evidence of bacteremia and aid in antibiotic management in this high-risk group. Utilizing PCT to assess bacteremia risk seems reasonable based on this study findings. Current study data align with previous studies by [33]Yan *et al.,* (2017), indicating differences in PCT levels between gram-positive and gram-negative infections and according to infection site, aiding in selecting appropriate antimicrobial therapy when bacterial culture results are unavailable or the infection site is uncertain. However, this contradicts a pervious study by [10]Yang *et al.,* (2019), which found no significant difference in PCT levels between gram-positive and gram-negative bacteremia.

Leukemia patients may experience infection, inflammation, and neuroendocrine system dysfunction even with a normal body temperature. Elevated CRP levels in some leukemia patients may indicate bacterial infection or inflammation caused by the diseases itself.

Increases in CRP, a marker of inflammatory activity, are associated with a higher mortality risk. Mortality risk also rises in patients recovering from neutropenia due to increased CRP levels, emphasizing the importance of monitoring CRP levels alongside neutrophil count. CRP was 100% sensitive but only 41% specific in predicting sepsis.

Drug discussion part

The results of current study observed promising outcomes regarding the impacts of chemotherapy medications, particularly decitabine and cytarabine, which have long been utilized in leukemia treatment. Hence, the dosage, administration procedures, and pharmacological aspects of these drugs are well established. The current study findings indicate that chemotherapy drugs lack antibacterial properties against bacterial isolates. We tested this effect on 39 isolates from various sources in leukemia patients, using different concentrations ranging from 0.5 to 512 of the mentioned chemical agents. In contrast to the study by [12][Cruz-Muniz *et al.,* (2017) which illustrates the notable antibacterial potential of MMC (mitomycin C) against A. *baumanni*, repurposing it for A. *baumanni* infections might present a viable option in clinical practice . Another study revealed that 5-FU (5-Fluorouracil) acts as a highly effective coating agent, surpassing even the positive control, in preventing bacterial colonization of central venous catheters. Clinical trials are presently underway to assess the effectiveness of gallium nitrate in treating cystic fibrosis patients infected with *Pseudomonas* aeruginosa[34] (Soo et al., 2017). [35]Montassier *et al.,* (2015) noted that chemotherapy was linked to decreased diversity of intestinal microbiota.

Conclusion: In summary, our research findings affirm 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. Notably, patients with acute infections, particularly those with elevated PCT levels and fever, Once again, PCT appears to be an effective indicator of the presence of acute bacterial infection exhibited significant differences. The bacteria isolated in our study demonstrated significant resistance to commonly used antibiotics, with most classified as MDR and others as PDR. Interestingly, these bacteria proved to be non-sensitive to the antileukemia drugs utilized in our study, suggesting no correlation between their antibiotic resistance and sensitivity to leukemia treatment. Some scholars argue that administering these drugs negatively impacts bacterial virulence, evident in inhibition zones on bacterial cultures. MIC techniques support this notion, revealing isolates across a spectrum of concentrations. Notably, patients with acute infections, particularly those with elevated PCT levels and fever, Once again, PCT appears to be an effective indicator of the presence

of acute bacterial infection exhibited significant differences.

Ethical approval: Mustansiriyah University oversaw and approved this study by their Ethics Committee.

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