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Original Research Article

Isolation and Identification of Gram-Negative Bacteria Responsible for Bacteremia in Leukemia Patient and Detection of Procalcitonin Levels in Serum of Leukemic Patients

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Abstract: Gram negative bacteria (GNB) is the most common causative agent for morbidities and mortalities in leukemic patient, because of their receiving immunosuprresive chemotherapeutic agents, aggressive devices like catheters used for giving them those treatments and Hospitalization, in addition to GNB increasing resistance to many of antibiotics. The study include isolation of GNB from blood samples of leukemia patients and identification of GNB species isolated from those samples then testing its susceptibility to 15 antibiotics, moreover, calculating Procalcitonin (PCT) concentration in the samples as immunological marker to detect bacteremia in those patients. The results show presence of 9 GNB species in blood samples of leukemia patients including: *Enterobacter cloacae, E. sakazakii, Serratia marcescens, S. ficaria, S. liquifaciens, S. rubideae, S. odorefera, Klebsiella oxytoca, and Pseudomonas fluorescens*. The most common species was *E. cloacae* followed by *E. sakazakii.* The higher effective antibiotics were Ciprofloxacin, Gentamicin and Amikacin; also PCT concentrations were ranged between (0.1 and 8.23) $ng.ml^{-1}$. The study concluded that Gram negative bacteremia was common causative agent for infections in Leukemia Patients, Enterobacteriacae was the the most common GNB causing infection, and the Most effective antibiotic for it were Ciprofloxacin, Gentamicin, and Amikacin which can be used as prophylactic therapies for those infections**.**
Keywords: Gram Negative Bacteria, Bacteremia, Leukemia, Procalcitonin, Immunological marker, Antibiotic Resistant.

INTRODUCTION

Leukemia is a worldwide disease, it happened in all ages and both genders from Females and males [1]. It's one of the basic reasons for Pathogenicity and mortality in Iraq according to Cancer registration center in Iraq [2] since The world health organization report demonstrate that Leukemia is the second cause for mortalities in Females and the third in males in Iraq in 2014 [3].

Bacterial infections is one of the most important reasons for morbidity and mortality in Leukemia, [4] Including Blood stream infections particularly bacteremia which is the basic factor for life-threating infections in leukemic patients receiving chemotherapy, because of complications resulted from these infection; in spite of development in Antibiotics therapy and health care especially in Malignant diseases including leukemia which still one of the therapeutics' challenges as a result to immune system defect causing deficiency in immunity, also because of little using of prophylactics therapy [5-10]. In addition chemotherapy related neutropenia is a main cause of immune defects, because they receive either myelosuppressive or immunosupperessive drugs. Moreover, the early treated patients develop infections greater then untreated early. so that early diagnosis of those infections will be helpful largely to take decisions related to Antimicrobial drugs and hospitalization [11,12,5]. So that the expert physicians consider infections to be the possible causative factor of fever in any patient especially leukemic patients [13]. Because of the weak immune response in those patients, the traditional signs and symptoms being unclear and the high temperature considered to be the single diagnostic sign, so that the blood culture results take the axial diagnostic role for bacteremia [7,14,11]. So that it's considered to be the most relevant bacteria in blood particularly in adults in spite of Gram positive bacteremia is more predominant in children with association with leukemia [15]. and it responsible for most frequently infections with high mortality [16] although developments in antibiotics' therapy, it stay the basic reason for mortalities in hospitalized patients especially critically ill patients like leukemic patients [17].

Bacteremia is the presence of live bacteria in bloodstream which can translocate from commensally bacterial flora or from injection of some bacterial contaminated materials directly into circulation, which can be filtrated often from blood in minutes; so that the bacteremia considered to be silent and transient, but if the immune system destroyed or in weak performance; this bacteria will stay in the blood causing pathogenic symptoms, and the basic symptom is fever [6]. Most of gram negative bacteria causing these infections arise from intestinal flora and reach to blood stream by process called bacterial translocation which induce the intestine colonization and the increased growth of transferred bacteria because immunosuppression or by changes in intestinal mucosa , which all happened in leukemic patients [16].

The potential inducer for PCT production in the body were bacterial endotoxin and PCT concentrations stay high in immunocompromised patients in infections case also PCT in the serum stay very stable and its doesn't analyzed to hormonally active calcitonin. The pathophysiology rule of PCT was still under investigation and supposed to be one of acute phase proteins. Also according to the study, PCT good maker for infections incidence, moreover; its detect the intensity of infections also [18]. So many studies focused on the importance of Procalcitonon (PCT) in diagnosis of bacteremia, as Giani study show that PCT concentrations were high in bacteremic patients so that it was considered to be immunological marker for detecting the intensity of bacteremia [19,11,20]. AS PCT concentrations changes during infection were rapid; and its melcules were constant and easy detectable, its concentrations can raise to high values in the blood because of its releasing in the blood during bacterial infection [21,20,11]. The study aim to detect the incidence of Gram Negative Bacteremia in leukemia patients, its susceptibility to Antibiotics, the relation between Those infections and leukemia types, and detect PCT values and its relation to Bacteremia incidence.

MATERIALS AND METHODS

Sample Collection

The samples were collected from Leukemic patient with different types of leukemia (Including: AML, ALL, CLL and CML) , who were hospitalized in Baghdad teaching hospital in Medicine city, Baghdad and outpatients of National center of Blood diseases` treatment and research, Al-Mustansyria university. Whose suffering from one or more of clinical symptoms (Including: Fever, Accelerated heartbeats and dyspnea) which were important for predicting Bacteremia in those patients. And they receive chemotherapeutic agent like what mentioned in table (1).

Table 1: The chemotherapeutic agents used for treatment of leukemia patients in the study specimen.

Types of Leukemia	Chemotherapy Types of
Acute myeloid leukemia	Doxorubicin
	Cystosar
Acute lymphoblastic leukemia	Doxorubicin
	Cystosar
	Methotrexate, 6 –Mercaptopurine
Chronic myloid leukemia	Gleevec
	Tasigna
Chronic lymphoblastic leukemia	Endoxan
	Fludara

Isolation and Identification of Gram negative bacterial species

(2- 3) ml of leukemic patients' blood were taken in test tubes containing Brain-Heart Infusion medium for performing Blood culturing to insure the presence of bacteria in The blood samples and then prove bacteremia status; then it's left for 24 hours in the incubator at 37^{o}c to improve bacterial growth. Then it was transferred to MacConky agar medium and Blood agar medium to detect whether it was Gram positive or negative, as MacConky was a selective medium for Gram negative bacteria. The bacterial strains appeared in the positive blood cultures were purified on Nutrient agar medium to get pure strains eligible for characterization, Then the single bacterial strains were Identified by using (APi 20E, bioMérieux®) .

Calculating Procalctonin (PCT) Concentration for Detection of Bacteremia

3 ml of blood sample were taken in Gel tube to get serum by using Centrifuge at 14000 rounds for 10 minutes. After having serum, the serum was undergoing Procalcitonin's concentration calculation by using (ichromax^{TM} PCT) as an immunological diagnostic tool for Bacteremia.

Testing Antibiotic Susceptibility of Bacterial Species

After identification of bacterial species, the Antibiotic susceptibility test was performed according to Kirby-Bauer method, for testing its resistance to particular Antibiotics [Including: Trimethoprem (TMP), Imipenim (IPM), Ampicillin (IM), Erythromycin (E), Amikacin (IK), Ciprofloxacin (CIP), Gentamicin (CN), Cefotaxim (CTX), Tetracyclin (TE), Nitrofuranton (F),

Piperacillin (PRL), Chloramphnicol (C), Cefalothin (KF), Naldixic acid (NA) and Amoxicillin (AUG)] purchased from Bioanalyse® , by culturing bacteria on Muller-Hinton agar and using antibiotic's discs for 24 hours in 37° c in the incubator, then calculating the inhibition zone for all of them according to CLSI [22].

Statistical Analysis

The statistical analysis was performed by using chi- square test and UNIANOVA test (SPSS program, version 19).

RESULTS

Patients Sample

The study performed on 60 patients whose suffering from leukemia and their ages range from (16 – 90) year with a mean of ages (41± 1.24) year. The highest mean of leukemia incidence was happened in age group located between (36-45) years with percentage more than 28.3% like what appear in the table (2).

Table 2: Age and gender distribution in leukemic patients

Age of the patients/year	No. of the patients		Total	Percentage
	M	F		
15-25	6	3	9	15%
26-35	5	6	11	18%
36-45	12	5	17	28.3%
46-55	5	6	11	18.3%
56-65	5	4	9	15%
66-75	1	0	1	1.6%
76-85	1	0	1	1.6%
86-95	1	0	1	1.6%
Total	36	24	60	100%

In the study Acute myelogenous leukemia episodes were 23 episode (in percent 38.3%), and the acute lymphoblastic leukemia episodes were 8 episodes (with percent 13.3%) while the chronic myelogenous leukemia episodes were 22 (with percent 36.6%) and 7 episodes for chronic lymphoblastic leukemia. The incidence of gram negative bacteremia in the study was 33 episodes from the whole specimen (60 patients) which represent 53% of the patient in the specimen. while the higher incidence was in CML patients (with percent 40.6%), followed by AML (with percent 34.3%) then CLL (with percent 18.7%) and the least mean was in ALL patients (with percent 6.2%). Like what appear in table (3).

Table 3: The types of leukemia and its rates in patient's specimen and the incidence of positive blood cultures for each one

Types of leukemia	No. of cases	Percentage	Positive blood culture	Percentage
AML	23	38.3%	12	36.36%
ALL	8	13.3%	2	6.06%
CML	22	36.6%	13	39.39%
CLL	7	11.6%	6	18.18%
Total	60	100%	33	100%

*ALL: Acute lymphoblastic leukemia, AML: Acute myelogenous leukemia, CML: Chronic myelogenous leukemia, CLL: for Chronic lymphoblastic leukemia

The Bacterial Species which were isolated from Blood Samples of Leukemic Patients Suffering from Bacteremia:

Blood culture results of blood samples of leukemic patients showed 33 positive blood cultures from 60 blood sample of the patients. Table (4) shows the isolated bacterial species in the blood cultures. The positive blood cultures showed that *Enterobacter Cloacae* was the most common species, as the number of isolates which show positive results for this species (12 isolate with percent equal to 36.4%) followed by *Enterobacter Sakazakii* whose number of isolates equal to 7 isolate from the whole number of isolates (with percent equal to 21.2%). The other isolates contain *Serratia marcescens* (4 isolates with percent equal to 12.1%) and 3 isolates for each of *Serratia Ficaria* and *Pseudomonas flourescens* (with percent 9.0% for each of them), then its followed by *Serratia Liquefacien* and *Klebsiella oxytoca* and *Serratia odorifera* and *Klebsiella oxytoca* which represent 3.0%for each of them and produce just one isolate from the whole number of isolates. Like what appear in table (4).

Table 4: The bacterial species which were isolated from patients Specimen and the place of acquisition for each them and its percent from whole number of isolates

Bacterial species	No. of Isolates	Percent %	Place of Acquisition			
			Nosocomial		Community acquired	
			No.	Percent%	No.	Percent%
Enterobactor cloacae	12	36.4%	6	23.0%	5	71.4%
Enterobactor sakazakii	7	21.2%	7	26.9%	1	14.2%
Serratia odorifera	1	3.0%	1	3.8%	0	0%
Serraia marcescens	4	12.1%	4	7.7%	0	0%
Serratia rubidaea	1	3.0%	1	3.8%	0	0%
Serratia liquefaciens	1	3.0%	1	3.8%	0	0%
Klebsiella oxytoca	1	3.0%	1	3.8%	0	0%
Pseudomonas fluoresense	3	9.0%	2	7.6%	1	14.2%
Serratia ficaria	3	9.0%	3	11.5%	0	0%
Total	33	100.0%	26	100.0%	7	100.0%

In addition of that, the study results showed that nosocomial bacteremia episodes were highest from community acquired bacteremia with percent 78.78% and 21.21% for each of them sequentially. While the highest nosocomial bacterial isolates was *Enterobacter sakazakii* and *Enterobacter* cloacae with 26.9% and 23.0% sequentially, followed by *Serratia marcescens* and *Serratia ficaria* with percent 7.7%and 11.5% sequentially. While community acquired Bacteremia include only *Enterobacter cloacae* (with percent 71.42%) then *Enterobacter sakazakii* and *Pseudomonas fluorescens* (with percent equal to 14.28% for each of them) like in table (4).

Procalcitonin concentration measurement:

According to table (5). The PCT concentration as immunological marker of Bacteremia ranged from 0.10 $ng.ml^{-1}$ and 8.23 $ng.ml^{-1}$ with compare to cutoff value of PCT which calculated to be 0.5 $ng.ml^{-1}$ [23] and the highest mean was in *S. rubideae* also in CML patients.(table 6 and 7)

Table 6: The means of PCT concentration of the types of isolated bacteria from patient's specimen

BAC	N	Mean	Minimum	Maximum
Enterobacter cloacae	12	1.5242	.10	8.23
Enterobacter sakazakii	7	1.8229	.15	6.78
Klebsiella oxytoca	1	.1000	.10	.10
Pseudomonas fluorecens	3	.3467	.25	.52
Serratia ficaria	3	2.5467	.50	6.47
Serratia liquefaciens	1	.4500	.45	.45
Serratia marcescens	4	.3625	.10	.56
Serratia odorifera	1	.7800	.78	.78
Serratia rubidaea	1	4.7500	4.75	4.75
Total	33	1.4321	.10	8.23

Table 7: The means of PCT concentration of the types of leukemia patient's specimen.

Dis	N	Mean	Minimum	Maximum
ALL	2	.2750	.10	.45
AML	12	1.3650	.15	8.23
CLL	6	1.4850	.14	8.00
CML	13	1.6477	.10	6.78
Total	33	1.4321	.10	8.23

*ALL: Acute lymphoblastic leukemia, AML: Acute myelogenous leukemia, CML: Chronic myelogenous leukemia, CLL: for Chronic lymphoblastic leukemia

Antibiotics Susceptibility Test of The Isolated Bacterial Species:

The antibiotics susceptibility test of the isolated bacterial species show that Ciprofloxacin and Gentamicin were the most efficient antibiotics for treatment of bacteremia's episodes, as all bacterial isolates show susceptibility toward these antibiotics in percent 100% for each of them, followed by Amikacin in 99% percent.

While antibiotics susceptibility test of the isolated bacteria show results which demonstrated in table (8)

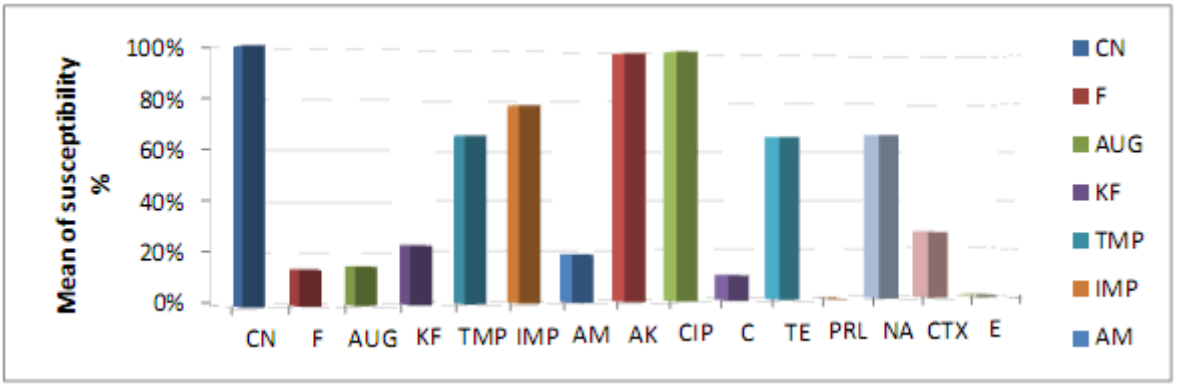

Fig-1: Antibiotics susceptibility means of bacterial species isolated form leukemic patients specimens

*[Including: Trimethoprem (TMP), Imipenim (IPM), Ampicillin (IM), Erythromycin (E), Amikacin (IK), Ciprofloxacin (CIP), Gentamicin (CN), Cefotaxim (CTX), Tetracyclin (TE), Nitrofuranton (F), Piperacillin (PRL), Chloramphnicol (C), Cefalothin (KF), Naldixic acid (NA) and Amoxicillin (AUG)]

Table 8: The results of antibiotics susceptibility test of the bacteria which isolated from leukemic patients' blood samples

No.	Type of Antibiotics	Pattern of Response	Enterobacter cloacae	Serratia ficaria	Serratia marcescens	Enterobacter sakazakii	Pseudomonas fluorescens	Serratia rubidaea	Serratia liquefaciens	Serratia odorifera	klebsilla oxytoca
1	CN	S	100%	100%	100%	100%	100%	100%	100%	100%	100%
		M	-	-	-	-	-	-	-	-	-
		R	-	-	-	-	-	-	-	-	-
2	F	S	18%	10%		-	-	100%	-	-	-
		M	9%	-		-	-	-	-	-	-
		R	72%	90%	100%	100%	100%	-	100%	100%	100%
3	AUG	S	9%	-	10%	14%	-	100%	-	-	-
		M	18%	10%		14%	-	-	--	-	100%
		R	72%	90%	90%	71%	100%	-	100%	100%	-
4	KF	S	-	-	-	10%	-	100%	100%	-	-
		M	-	-	-	-	-	-	-	-	-
		R	100%	100%	100%	90%	100%	-	-	100%	100%
5	TMP	S	90%	100%	100%	0%	100%	-	-	100%	100%
		M	10%	-	-	100%	-	100%	100%	-	-
		R	-	-	-	-	-	-	-	-	-
6	IMP	S	100%	100%	100%	100%	100%	-	-	100%	100%
		M	-	-	-	-	-	-	-	-	-
		R	-	-	-	-	-	100%	100%	-	-
7	AM	S	36%	-	90%	10%	33%	-	-	-	-
		M	-	-	-	-	-	-	-	-	-
		R	63%	100%	10%	90%	66%	100%	100%	100%	100%
8	AK	S	100%	100%	90%	100%	100%	100%	100%	100%	100%
		M	-	-	10%	-	-	-	-	-	-
		R	-	-	-	-	-	-	-	--	-
9	CIP	S	100%	100%	100%	100%	100%	100%	100%	100%	100%
		M	-	-	-	-	-	-	-	-	-
		R	-	-	-	-	-	-	-	-	-
1	C	S	100%	100%	100%	100%	100%	100%	100%	100%	100%
		M	-	-	-	-	-	-	-	-	-

		R	-	-	-	-	-	-	-	-	-
11	TE	S	63%	90%	90%	14%	33%	100%	100%	-	100%
		M	-	-	-	14%	33%	-	-	-	-
		R	36%	10%	10%	71%	33%	-	-	100%	-
12	PRL	S	-	-	-	-	-	-	-	-	-
		M	--	-	-	-	-	-	-	-	0%
		R	100%	100%	100%	100%	100%	100%	100%	100%	100%
13	NA	S	81%	100%	100%	90%	33%	100%	100%	-	-
		M	9%	-	-	-	-	--	-	100%	100%
		R	9%	-	-	10%	66%	--	0%	-	-
14	CTX	S	27%	-	10%	28%	0%	100%	100%	-	-
		M	54%	-	--	42%	33%	-	-	-	100%
		R	18%	100%	90%	28%	66%	-	-	100%	-
15	E	S	-	10%	-	-	-	-	-	-	-
		M	10%	-	-	28%	-	-	-	-	-
		R	90%	90%	100%	71%	100%	100%	100%	100%	100%

* Trimethoprem (TMP), Imipenim (IPM), Ampicillin (IM), Erythromycin (E), Amikacin (IK), Ciprofloxacin (CIP), Gentamicin (CN), Cefotaxim (CTX), Tetracyclin (TE), Nitrofuranton (F), Piperacillin (PRL), Chloramphnicol (C), Cefalothin (KF), Naldixic acid (NA) and Amoxicillin (AUG).

DISSCUSION

According to the results established from the study , leukemia has slightly more incidence in males than in females while males number in the study was 36 while females number was 24 patient from the whole number of specimen which was 60 patients. which agree with other researchers finding like Abdallah who found that male has slightly epidemiology with percent 53.5% in males and 46% in females, [24] also the study performed by Burns which mentioned that the incidence of leukemia was higher in males then in females [25]. Conversely to what mentioned by another researcher, that females epidemiology percent was larger than males with little difference [25]. While Iraqi cancer registry in Iraq in 2009 said that incidence rate in males higher than females, but its incidence in older age group higher than younger ones [2]. also in 2011, it's became in third rank in males and second ones in females [2] according to World Cancer Declaration, progress Report (2016); the mortalities in males higher than females [27].

In the study, the higher incidence was in age group (36-45) year with 28.3% followed by (26-35) year age group which slightly fewer with 18.3%. and the incidence of leukemia in ages lesser than 55 year was more than 79.3% which was higher than Abdallah study which mentioned that 61.9% of episodes located in age groups lesser than 49 year. [24] The ages mean in the study was (41± 1.24) while in Burns study its 52 and the ages range from 16-87 [25] which indicate that leukemia in the study has increased rate in younger groups and in adults other than elderly ones who were younger than 55 year .

The higher incidence in younger age groups be returned to the negative reflections of wars and events happened in Iraq starting from explosions which send so many contaminated chemicals in addition to increasing cancer incidence in Iraq especially leukemia after 1990 as a result to using depleted uranium in wars accomplished on Iraq, and apart of patients in the study were suffered from siege conditions in their cities as a result to the events happened in Iraq which expose them to starvation which is one of the risk factors for cancers like patients who were trapped in Fallujah city and were estimated in the study with 15 episode nearly, in addition to the nature of nutrition that people eat which contain a lot of synthesized food and genetically transferred food which increase the risk of cancer development particularly leukemia. Leukemia one of the most common cancers in children and Ranked first in 2006 [2]. because some types of leukemia take place largely in children like Acute myelogenous leukemia which was one of the common type of leukemia and particularly ALL which most common in children [28]. The study show that AL percent was more that 51% in patients specimen while it's more than 38% in chronic leukemia. Like what showed in table (3). The incidence of Bacteremia in leukemia patients.

33 patients from 60 leukemia patients who were suffering from fever show positive blood culture for gram negative bacteria which compose 55% of leukemic patients receiving chemotherapy. Because the gram negative blood stream infections were the most frequent infections with high mortality rates [16]. so many studies performed on the Bacteremia diffusion in cancer patients, and the rates of Infections in patients sample differ from 5.7% to 44% , while one of the studies mentioned that 18% of febrile Neutropenic patients show positive blood cultures [28]. Also yahya found that the incidence of Bacteremia in AL patients was 34% in Iraq [29]. which differ from what mentioned in the study and this can be explained according to the great time difference between the two studies that can explain the change the virulence and resistance of bacterial infections, children who suffering from leukemia and they develop FN were in increased risk for sever bacterial infections like Bacteremia and

the gram negative Bacteremia prevail traditionally because of fewer using of prophylactic therapy [10]. According to Kern, The number of GNB episodes were 5 from the whole number of episodes which mean presence of very little incidence GNB [30]. which conflict with the study because of high incidence of Bacteremia episodes in study's' specimen resulted from GNB. Also gram negative bacteria responsible for 14% of blood stream infections in AML patient Receiving extensive chemotherapy in a study performed in united states between 1995 and 2000 which also conflict with the results of other studies found that gram negative bacteria was the main cause of infections in those patients. This study returned its result to the possibility that prophylactic antibiotics inhibit GNB resulting in increasing of GPB and increase GNB infections [31].

The high incidence of gram negative bacteremia in the study can be explained according to many reasons including the defects in Immunological functions take place in leukemic patient which cause weakness in their ability to resist infections, and the other procedures which accomplished on those patients starting with diagnostics tests like bone marrow aspiration and aggressive devices included in chemotherapy presentation like a catheter as blood stream infections caused by GNB take place during these aggressive therapies using for cancer patients [28]. Also the high percent of bacteremia in leukemic patients specimen can be explained according to the chemotherapies used not only by its immunosuppressive effect and WBC decreasing especially Neutrophil but also by its side effects represented with mouth ulcers and lesions and sores happened in gastrointestinal tract also which will be a focus for infections and inflammations while bacteria translocate from these places to blood stream causing Bacteremia as a secondary infection. The patients in the study suffer from these ulcers which can prove these conclusions. As so many gram negative bacteria causing bacteremia arise from intestinal flora and reach to blood stream by bacterial translocation mechanism and this translocation induced by intestinal colonization and increasing growth of translocated bacteria by patients' immune-suppression or by changes happened in intestinal mucosa and all these changes take place in cancer patients [16].

In addition to previously mentioned effects, it's also induce mucositis which make patients more sensitive for infections caused by endogenous flora (opportunistic infections) as a result to the scares in epidermis and mucosal membranes [31] so that most of episodes were endogenous [32] in leukemic patients, using prophylactic antibiotics and intensive chemotherapies which cause mucositis and intravascular devise contribute largely in changing the type of microorganisms causing bacteremia. According to Collin, *Klebsiella* spp. and E. coli the most common bactereia (compose 51.5% from whole isolates) while Enterobacter spp. and *Serratia* spp. represent 17.8% and *pseudomonas aeruginosa* [33].

There's so little data take in frequency, position and infection causes before initiating chemotherapy, in early reports, appear that15-25% of patients were infected in diagnosis time. Before the availability of active therapeutic regimen, most of infections were thought to be due to the deficiency in immunoglobulins related to the disease process itself, including: hypogammaglobulinemia (which occur in early and developed stages of disease) deficiencies in inhibiting factors of neutrophil, neutropenia, morbid response of antibodies and deficiency in the CD4+ lymphocyte count. But the deficiencies related to traditional chemotherapy include: neutropenia, morbid functions of macrophage, lymphopenia and inhibition of antibodies productions [12]. In the study, the chemotherapeutic agents used correlate generally with those effects, omidvari proves doxorubicin effect which lead to Neutropenia and stomatitis which happened for intestine system. Also Batra find oral mucositis presence and granulocytipenia development and infections as most frequently effects in the treatment period with doxorubicin as a result to WBC count decreasing which make it hard to be fought and its include heavily infections and sometimes fatally ones including opportunistic infections which can be diagnosed by fever with 38 ^{o}c . Sweeting and mouth ulcers and another signs [34,35] Throat sore, and mouth sore happened after 5 days of each treatment with doxorubicin or may be after two weeks [34] because mouth and pharynx lining cells were Rapid growth cells, so that some chemotherapeutic agents like doxorubicin and methotrexate effect on these regions causing scares formation and drying of these regions. In addition to that, methotrexate also can lead to infection because of its effect on WBC count decreasing [34] and treatment with doxorubicin also lead to morbidities in effector functions of T cells and its proliferation morbidity. In addition to lymphopenia [36] which seem to what Gleevec cause which was one of protein tyrosine kinase inhibitors which effect on T cell in adaptive immune system, as high doses cause suppression of proliferation and activation of T cell presumably inhibition of protein Tyrosine kinase which lead to increase susceptibility to bacterial infections and increase lymphocyte apoptosis and defects in CD8 + T-cell (memory cells) functions [36].

Also, one of the side effect of 6-mercaptopurine was bone marrow suppression and infections also which follow severe neutropenia or immunosuppression that contribute in mortalities and hospitalization significantly. Brandalise found mortalities in leukemic patients receiving methotrexate and 6MPlinked to infections, moreover infections and throat sore were high in those patients, [37, 34] in addition to the above, Enodxan (Type of cyclophosphamide) lead to myelosuppression and

significant suppression of immune response and cause leukopenia and neutropenia leading to intensive and sometimes fatal bacterial infections like sepsis or septic shock also activation of potential infection and its must be stopped or its decreased dose in case of severe infections and can cause sores and bladder necrosis [34] Treatment with fludarabine (fludara) one type of purine analogues was high risk factor for side effects in GIT [12] while the most common side effect of Anthracyclines (doxorubicin) was myelosuppression and stomatitis, [38] Tasigna cause many common side effects including infections like upper respiratory tract infections and stomatitis and not common to develop sepsis abnormal hepatic functions and gastrointestinal ulcer perforation but acute pancreatitis and mouth ulceration were less common. Also its lead to myelosuppression and can cause neutropenia, septic shock and sore throat and low blood count and the latest effect was so common in case of Tasigna treatment [39].

By all what previously mentioned, There's explanation for the high percentage of bacterial isolates subsidiary to Enterobacteriaceae in the study like *Enterobacter cloacae*, *Enterobacter sakazakii* and *Serratia* spp. as most of bacteria in GIT were from Enterobacteriaceae which can linked to the side effects of chemotherapies as its can produce primary source of infections (secondary infections) also wisplinghoff found that most of secondary BSI resulted from intravenous catheter and from UTI followed by lower respiratory tract and GIT but there's no significant difference noticed between Neutropenia and those who didn't suffer from it about infection source [40]. most of BSI in immunocompromised patients arise from previous local infections compared with BSI in Neutropenia patient which arise largely from endogenous infections like GIT infections [40,25] The data taken from extensive studies in cancer research centers in USA and Europe show that Enterobacteriaceae represent 65-80% from registered gram negative infections [28] also Batzar found that *E. cloacae* was the most common bacteria in ICU patients from children [41] in addition to all of that Lio-ten foe isolate *Klebsiella oxytoca* and *E. cloacae* and *P. fluorescens* from pharynx, saliva and rectum from ICU patients [42] in the study. The highest percentage of positive isolates were in CML with percent higher than 40% from the whole number of specimens followed by AML with percent higher than 34%, Then CLL (18%) and the lesser percent was in ALL patients (6.25%) which differ from what noticed in Abdallah study and AL -Neemy [24,26] which found higher percent of Bacteremia in AML with 41% percent in Abdallah study [24] and mortality rates caused by infections in AML patients range from (5.5-13)% during treatment. [43] also it differ from Burns study who find ALL infections higher than AML ones [25]. according to study accomplished in Eduard Herriot hospital in France about the annual mean of Hospital acquired infections, this study showed that Bacteremia percent in AML was 12.9% and ALL (13.4%) and gram negative bacilli bacteremia was (4%) but cocci was 1% from whole episodes of bacteremia, this study also show increasing incidence of bacteremia and most of them were Endogenous [32] as *Enterobacter* from one of endogenous flora of human [44] in addition to that *E. cloacae* was opportunistic pathogen [54] also chemotherapeutics regimens for AML induce Neutropenia which stay for a long periods and expose them for frequent infections, Basically arise through the first cycle of chemotherapy [43] in spite of the fact that statistical analysis from the study show absence of significant difference in the relationship between bacteremia and leukemia patient, also between the types of bacterial isolates species and leukemic types which agree completely with what mentioned by Abdallah study [24] in spite of using appropriate number of patient in specimen which can give statistical results near from reality also Kern found that incidence of bacteremia in leukemic patients was 36% [30] so that its differ from the study that show higher incidence also Kern found that 4 patients from study specimen died, [30] while in the study 2 patient were died and they show positive blood cultures for *Enterobacter sakazakii* (PCT 4.60ng.ml^{-1}) and the other for *Serratia marcescens* (PCT 0.45ng.ml^{-1}) so that mortality rate in the study 6.25% while in Kern study, 4 patients died. [30] from the whole number of isolates, *Enterobacter cloacae* from the highest percent with 37.5% followed by *Enterobacter sakazakii* with 25% which form the most common species in the study while 12.5% from the isolates affiliate to *Serratia marcescens* and Both of *pseudomonas fluorecens* and *Serratia ficaria* was 9.37% and the least percent of isolates was for *Serratia rubidaea*, *Serratia liquefaciens*, *Klebsiella oxytoca* which represent 3% of the whole number of isolates, which agree with the isolated species causing bacteremia in Kern study whose include *Enterobacter* sp. In addition to *E. coli*. [30] It's important to notice Abdallah results whose study accomplished in the same environment of the study, and it's found that the most common species was *Klebsiella pneumonia* with 21.42% followed by *E. coli* and *Pseudomonas aeruginosa* with 14.28% then *Salmonella typhi* with 7.14% and the least percentage was for *proteus penneri* with 3.57% [24] all these results differ from what found in the study, which can be explained according to the time difference between the two studies and the possibility of prophylactic treatments difference and the resistance and virulence differences of bacteria which were in continuous progress. In his book, Faguet mentioned that the most common bacterial isolates were P.aeruginosa, Klebsiella sp. And E.coli [12] which agrees somewhat with study finding because of emergence of species affiliate to *Klebsiella* represented with *Klebsiella oxytoca*. inaddition to emergence of *Pseudomonas fluorescens*. As in one of studies which accomplished in Benha hospital, the incidence of bacteremia was 30.61% and from this percent, GNB

represent 63.9% and E. coli was the Hugo percent as its form 46.1% and *Klebsiella* form 30.8% followed by 15.4% of *Pseudomonas* then *Acinobacter* form 7.7% [54] Body and his collagenous consider FN and specific pathogens like *Pseudomonas aeruginosa* and *Serratia marcescens* to be challenge for cancer treatment. In this time, the mortalities followed *P. aeruginosa* Bacteremia form nearly 90% in spite of availability of laboratory active antibiotics [7]. in recent studies, BSI volume estimated in Neutropenia AML to be ranged between (34-38)% also in Madani study, who said that GN bacilli was 12.1%. Composed of *Klebsiella pneumonia* (3%) followed by *Enterobacter cloacae* (1.5%) [43]. The epidemiological observations on community acquired pneumonia (CAP) show increasing incidence of severe Bacteremia resulted from *Enterobacter* as one study show that En CAP incidence stay severe in number of cancer episodes including CML [55]. In India, parabgsh and his collagenous in 2010 found that the most common causative agents of infections in leukemic patients was *Pseudomonas* spp. with 30.37% percent. [28] while chency study GNB from 60% from infections in Neutropenic patients with 60% including *E. coli* (12%) and *Klebsiella pneumonia* (10%), [28] The most common Bacterial pathogen in Santolya study which composed pediatric cancer patients receiving chemotherapy and suffering from FN and Bacteremia was *E, coli* followed by *Klebsiella* sp and *Pseudomonas aeruginosa* then *Enterobacter aerugenes*. (221) but Wisplinghoff mentioned nearly same results to Santolya that E.coli, The most common isolates followed by *Klebsiella* sp. and *Pseudomonas* sp. Then *Enterobacter* sp [40]. These studies differ from the study with important point, which involve that E.coli the most common pathogen while in the study, there's not any isolate of *E. coli* and *Enterobacter* spp. Were the most common isolates which can be explained by the type of prophylactic antibiotic used which selected to target the most common pathogens like *E. coli* and the other types which appear with little percent in the study. Because prevention and treatment of infection critical part of leukemia treatment which can be accomplished with empirical treatment with antibiotics which cover the wide range of pathogens [8] also Abdollahi found that *E. coli* was the most common isolate from Blood cultures of leukemia patients with 34%. [28] because of increasing mortalities in leukemic patients resulted from *E. coli* and *P. aeruginosa* [40] *Serratia* cause 1.4% of nosocomial bacteremia because its contain chromosomally induced Amc β lactamases which give it the ability to develop rapid resistance to many of β lactam antibiotic and give it ability to cause Bacteremia and sever nosocomial infections. In Choi study which contains leukemic patients Receiving immunosuppressive agents and chemotherapeutic agents, *Serratia* bacteremia diagnosed with higher percentage in the first group while 96% of them returned to *S. marcescens* and 4% for *Serratia liquifaciens* [45]. in the study the higher percent of Bacteremia resulted from nosocomial infections with 81.25% while little percent resulted from community acquired Bacteremia. Because leukemia patients were in increased risk for nosocomial BSI because of disease severity which need intensive chemotherapy or stem cell or bone marrow transplantation which lead to severe infections and worsen the cases so that mortalities of Nosocomial bacteremia were ranged from 10-20% [25, 32].

Apostlopolou and his collagenous calculate Bacteremia incidence in 102 leukemic patients who were hospitalized for more than 48 hour and he find that 21.99/1000 patient perday suffering from Bacteremia especially women, in contrast, the age doesn't have any effect on Bacteremia expectance in spite of that its considered to be risk factor for severe Neutropenia. Also Garcia -Suarez found the same incidence of Bacteremia in leukemic patient from elderly and young who suffering from FN. there's so little data available on the effect of comorbidities of leukemia as risk factor for Bacteremia, [7] also *E. cloacae* considered to be the most common causative agent for Nosocomial infections as its cause sepsis and can be found in GIT, UT, RT, also its resistant to disinfectants and Antimicrobial agents from Enterobacteriaceae which can explain its increasing responsibility of Nosocomial Infections and its wide spreading [45].

PCT concentration measurement

Some studies suggest that PCT have secondary mediated rule in immunological pathogenicity in sepsis cases, and many studies focused on the diagnostic rule of PCT for diagnosis of Bacteremia and sepsis in patients who require intensive care, and one study found sensitivity between 65% to 97% and specfity between 48% to 94% for PCT in diagnosis of Bacteremia, Three studies found that PCT was the best immunological marker for sepsis, and the best marker for bacterial infections according to Chan study [19] also according to Leli and his Collageous study, [23] as patients suffering from severe sepsis have 10 times PCT concentrations with comparison to sepsis episodes. Several studies focused on the abilities of PCT for diagnosing infections ICU [19]. which help physicians to determinate the primary antibacterial therapy suitable for those patients while there's correlation between starting unsuitable therapy with resulted complications largely [23]. The PCT values in the study range between (0.1 to 8.23) $ng.ml^{-1}$ and Eleven sample from 33 sample in the study show PCT concentration higher than cut–off value which was 0.5 $ng.ml^{-1`}$ which show that they were infected with bacteremia, while the other values were less than 0.5 $ng.ml^{-1}$, so that it may indicate two possibilities; first one that there's no bacteremia in those patient and the other possibility that its values decreased as a result to disease itself. As Hepatocytes considered to be known sources of PCT so that any damages in the liver resulted from chemotherapy may be possible source for decreasing PCT levels in cancer patients [46,11] Like in Giamarellos-Baboulis study,

who mentioned that PCT concentrations in leukemic patients before chemotherapy in his study were in 0.16 mean while its 0.05 mean in Febrile neutropenic patients [47]. which suppose that in case of neutropenia, PCT values less than non-neutropenic patients. Which agree with what Schuttrumpf and his collagenous mentioned when they calculate PCT concentrations in plasma in for 111 patient with hematological malignancies conditions and they found that PCT concentrations higher significantly in patients with infections than in those without infections also its higher in patients with infections who didn't suffer from leukopenia from those who have leukopenia and PCT concentrations higher in Bacteremic patients than in local viral o fungal infections [48]. Engle and his colleagues found that PCT levels during fever incidence stay under 0.5 $ng.ml^{-1}$ also Hambach study found that PCT levels higher in Bacterial infections with mean equal to 2.3 $ng.ml^{-1}$. Also Pihusch and his collagenous found increasing in PCT levels after infections complications but only PCT can distinguish between infections and related complications to stem cells transplantation in cancer patients [48].

Also PCT levels in Chan study were equal or larger than 0.6 $ng.ml^{-1}$ in 69.5% of patient with infections and PCT mean was 5.30ng/ml for both infected and non-infected patients while its mean in non-infected patients only were 0.5 $ng.ml^{-1}$ and there's no relation between PCT concentration and the type of infected organism. Also PCT level was the best for predicting bacteremia incidence was 1 $ng.ml^{-1}$ with 63% specifity according to Chan study. PCT concentrations in healthy plasma were under 0.1 $ng.ml^{-1}$ [18] while in our study the cut-off value used according to ichromax™ PCT instructions was 0.5 $ng.ml^{-1}$ But Leli study found that cutoff value of PCT to distinguish between Enterobacteriaceae and non fermentive gram negative was 3.1 $ng.ml^{-1}$with 90% sensitivity [23] While Ugarit study indicate that PCT cutoff value was 0.6 $ng.ml^{-1}$ with 67.6% sensitivity [18] but So many studies mention the cutoff values of PCT to distinguish bacterial infections from other infections which range between 0.5 $ng.ml^{-1}$ to 1.3 $ng.ml^{-1}$ [48] PCT values ranged from 0.5 to 2 considered to be indicator for life threatened conditions [46] which agree with what used in the study. The PCT values larger than 2 $ng.ml^{-1}$ seem to be enough to distinguish between sever sepsis and local infections with 90.9% sensitivity approximately and 80.9% specifity [47].

The study results statistics indicate that there's no significant relationship between PCT values and the types of bacterial species, or with types of leukemia while SVALDI study found that PCT concentrations in AML patients were higher than 2 $ng.ml^{-1}$ with high percent in comparison with CML followed by ALL [46]. also one study found that PCT levels were less in children who suffer from neutropenia and bacterial infections from those without neutropenia [11] Hatzitilianon found high significant difference in PCT levels between bacterial infections and non-bacterial infections moreover its significantly lower in Neutropenia who have bacterial infections [11]. PCT concentration in gram negative bacteremia was higher than gram positive bacteremia significantly in positive blood cultures, [23] because GNB induce production of significantly higher PCT levels than GPB and the LPS composition of outer membrane was the responsible for that effect. [46] One study found that GN bacteremia had largest PCT values then gram positive bacteremia while another study didn't find any difference between them and this study didn't contain any sepsis episodes [47]. the higher PCT mean found in *S. rubideae* (4.75 $ng.ml^{-1}$) followed by *S. ficaria* then in *E. cloacae* and *E. sakazakii* (1.82 and 1.52) $ng.ml^{-1}$ sequentially and the least value was for *Klebseilla oxytoca* (0.1 $ng.ml^{-1}$) moreover Leli study found that PCT concentrations in BSI resulted from GNB including Enterobacteriaceae (*Enterobacter cloacae*, *E. coli*, *Klebsiella* sp. *Serratia marcescens*, *K. oxytoca* and *proteus mirabilis*) were 17 $ng.ml^{-1}$ which were higher significantly than non fermentive bacteria including *Pseudomonas aeruginosa* (3.5$ng.ml^{-1}$) and *Serratia marcescens* whose value was 14.9 $ng.ml^{-1}$and *Klebsiella oxytoca* (2.3 $ng.ml^{-1}$) and *Pseudomonas aeruginosa* (6.8 $ng.ml^{-1}$) [23]. and PCT concentrations in bacteremia patients resulted from GNB in the first day of fever were 12.37 $ng.ml^{-1}$ mean ranged from 0.09 to 143.98 and its high particularly in immunocompromised patients serum who suffer from sepsis. After incidence of fever, PCT levels say within normal limits for Both Neutropenic patients who suffer from cancers and Neutropenic patients without fever (The values means were 0.29 $ng.ml^{-1}$and 0.18 $ng.ml^{-1}$ sequentially while PCT values mean in the first day of bacteremia was 8.23 $ng.ml^{-1}$ also high significant difference noticed between the mean value of PCT in the first day of bacteremia and PCT main in the first day of local bacterial infections whose mean was 0.86 $ng.ml^{-1}$ [47]. In the study, there's no ability to detect whether PCT concentrations measured in the first day or any other day because it wasn't taken in mind during information's registration.

Antibiotic susceptibility test

Antibiotic administration in the beginning of disease symptoms may prevent its diffusion and completely decrease mortality rates and other complications resulted from cancer [28] while the first source of infection often stay unknown, so that choosing of first therapy of antibiotic often being empirically and targeted primarily toward GNB [43] because of noticed significant increasing in GN Bacilli resistance to antibiotics [28] and the changing in the spectrum of pathogens causing infections in last decades [43] many studies accomplished on main microbes which cause infections and its susceptibility to specific antibiotics in cancer patients, because of high rates of bacterial infections and difficulty to detect the patients who were more exposure to severe infections

risk. so that all neutropenic patients receive wide spectrum antibiotics until fever excluding and maintain integrity immune system this strategy was very successful for decreasing mortalities and secondary complications of infections. Instead of side effects risk of severe regimens, resistant bacterial strains, fungal infections and followed psychological and economic effects. Because isolated bacteria from clinical and ecological samples acquired increased resistant to conventional antibiotics and GNB represent the largest risk on public health. Not just by its rapid resistance development then positive bacteria but also by little availability of new and developed active antibiotics against it, and its increasing resistance resulted mainly by mobile genes on plasmids which rapidly transferred through bacterial groups [49]. According to results obtained in the study; the most efficient antibiotics were Ciproflxacin and Gentamicin with percent equal to 100% followed by Amikacin antibiotic (with 99%) so that these antibiotics can be used effectively, also in case of prophylactic antibiotic therapy. Since Rahman study concluded that fluoroqinolone therapy (like ciprofloxacin) effect on bacterial infections as its delay fever in FN patients resulted from chemotherapy [28] as ciprofloxacin used for recent years intensively to prevent bacterial infections in severe Neutropenia. In spite of it's decreased GNB infections but its cause increasing emergence of GPB with Resistance emergence for many GNB. Also its must be limited for patients with high risk of developing severe Neutropenia follow chemotherapy [12] in addition to that Mohammed study concluded that Amikacin the most efficient one [50] while Kumarasamy study showed Resistance of Enterobacteriacea to Imipenem and Amikacin in each of USA, China and India and sensitive with 8% to Ciprofloxacin and 3% for Gentamicin [49] while study results show 78% sensitivity to Imipenem but Patzer mentioned that Imipenem, Ciprofloxacin and Gentamicin the most effective antibiotic against GNB [41] as in *E. cloacae* Acr AB efflux pump mechanism play role in its pathogenicity and resistance [49] as some antibiotics considered to be good substrates to be excluded by efflux mechanism which provide internal resistance for these some antibiotics, as high resistance found in isolates express this mechanism including Ciprofloxacin [51] also Perez found that its resistance depend largely on efflux pump mechanism which interfere also with its pathogenicity [45]. Burns indicate that *Enterobacter* spp. Resistance were uncommon, because its slightly resistant to Gentamicin without any significant difference between Neutropenic patients and others [25] while wisplinghoff noticed that Imipenem was the most effective antibiotic toward *Enterobacter* isolates and 33% of them resistant to Piperecillin and so little percent of the isolates were resistant to CIP and Gentamicin [40]. In another study, most of *E. cloacae* was sensitive to Ciprofloxacin, TMP, aminoglycosides and piperacillin but also resistant to Ampicillin and Amoxicillin β lactmases was the most important mechanism responsible for β lactam Antibiotics resistance because its contain all the types of β lactamaces (Including class A penicillinaces, class β metallo-enzymes and class C cephalosporinases and class D oxacillinaces) and able for increasing reduction of Ampc β lactamaces also with removing of depression from chromosomal genes or by acquisition of Ampc genes known with Ampc plasmid mediated genes Which can be distinguished from chromosomal genes. Also increasing production of chromosomal cephalosporines with porines changes responsible for Imipenem resistance [44] also Paterson found that 37% of infections caused by *Enterobacter* in ICU include resistant strains to third generation cephalosporines. Because of its increasing production of Ampc β lactamases and some *E. cloacae* were Amp and ESBL β lactamaces producers which give it resistance toward cephalospoius while Quinolone resistance in Enterobacteriaceae always come from chromosomal mutations lead to changes in the enzymes. Hospital acquired *Enterobacter* sp. were Amp β lactamaces producers, so that any exposure to β lactam antibiotics can induce production of those enzymes also mutations can lead to its increasing and produce permanent resistance. And Quinolone resistance in Enterobacterceae. Result from changes or morbidities in DNA gyrase and topoisomerase enzymes which happen either by changes in porine expression or Efflux mechanisms and both of those mechanisms resulted from chromosomal mutations [52]. *Klebsiella oxytoca* show compeletely sensitivity toward Imipenem; while In one study, 50% of isolated *Klebsiella* spp. Show multi resistance toward antibiotics including Imipenem [28] also resistance of *Klebsiella* sp noticed toward Imipenem with 1% according to Burns study [25]. Abdollahi study agree with the study results in spite of some differences in Bacterial species isolates, that all isolated bacterial species from blood cultures of leukemia patients were sensitive toward CIP [28] but in spite of CIP using through Neutropenia, GN Bacteremia study high risk [43] also Abdollahi said that *Pseudomonas* and *Enterobacter* isolates didn't show any resistance toward Imipenem [28] which agree with study results, which indicate that *P. flourescens*, *E. cloacae* and *E .sakazakii* toward Imipenem with 100% [28] also Mohammed study show that *E. cloacae*, *E. sakazakii*, *K. oxytoca* and *S .marcescens* isolated from UTI were completely sensitive toward Amikacin, Gentamicin and Imipenem while its 100% resistant toward Ampicillin and *E.sakazakii* sensitive toward CIP, TMP and Piperacillin in spite of *E. cloacae* and *K. oxytoca* were resistant for them. [50] Collin demonstrated that (40-45)% of *Enterobacter* spp. And *Serratia* spp. were sensitive toward Piperacillin and *Kllebsiella* spp. completely sensitive toward Piperacillin and *Pseudomonas* sp. Were sensitive for many factors including Imipenem [33] also Wisplinghoff study noticed that 80% from *Klebsiella* spp. were sensitive toward 3rd generation cephalosporins, Imipenem, Aminoglycoside and

flouroquinolons while 98% of them and *Serratia* spp. were resistance toward Ampicillin and little percentage were resistant for Piperacillin, Imipenem, CIP and Gentamicin [40, 45] while in Choi study, most of isolates were resistant to Ampicillin, Piperacillin, Gentamicin and Coprofloxacin moreover its sensitive toward Imipenem which show the least resistance rates followed by Amikacin, so that Imipenem stay the important therapeutic choice but its excessive using lead to later resistance to IMP with 3rd generation cephalosporins recent study showed emergence of significant high resistance rate of *Serratia* bacteremia during treatment with wide spectrum cephalosporins while quinolone therapy didn't link to any resistance emergence so that, 3rd generation cephalosporins were described to be avoided as bacteremia treatment [45] also blood culture results from samples of ICU patient in Lockhart study showed species which include E. cloacae, *S. marcescens* and *K. oxytoca*, since *K. oxytoca* show the higher resistance toward β lactam antibiotics with decreasing in *E. cloacae* sensitivity toward CIP, at the same time, he indicated that Amikacin effective widely toward *Pseudomonas* sp. and Enterobacteriaceae [53]. Flouoquinolone work by interfering with Type II topoisomerases (DNA gyrase and Topoisomerase III), according to this mechanism, Bacteria work on developing resistance mechanisms which include mutations target Gyra / GYRB for DNA Gyrase and parC/parE for Topoisomerase III or decrease the access of the target its self by decreasing its permeability or by Efflux pump mechanism, and most effective antibiotics were Gentamyicin and Amikacin since isolates stay sensitive in spite of presence of gene responsible for Imipenem resistance [44] also most of studies mentioned that its using doesn't decrease the frequency of fever incidence and empirical antibacterial therapy and its should be limited for patients who have great possibility to produce sever neutropenia follow chemotherapy [12].

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