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# Spectrum of Bacterial Infection and Antibiotic Susceptibility Profile among Clinical Samples of Febrile Pediatric Cancer Patients under Chemotherapy

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***Abstract---*** *Bacterial infection is one of the most frequent complications in malignant patients. Among them, those caused by multidrug-resistant (MDR) bacteria that increase mortality and morbidity mainly because of limited therapeutic options. The study material comprised of 155 febrile episodes occurring in 101 children aged less than 18 years with various malignancies from May 2018–April 2019 at at Basrah children's specialty hospital. All the episodes were worked up in detail including physical examination, history and relevant investigations. Bacterial isolated from different sites were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents Total of 61 bacteria were cultured in 155 febrile episodes. Gram-negative bacteria were more prevalent 53(86.9%) as a cause of infection in febrile pediatric cancer patients at our institution.* Escherichia coli *showed the highest isolation rate 64.1% (34/53) followed by* Klebsiellapneumonia *13.2%(7/53) and Proteus mirabilis 11.3% (6/53). The rate of MDR in Gram negative isolates were 90.5% (48/53). Gram positive consist 8(13.1%) with prevalence of Staph aureus 37.5% (3/8) and the rate of MDR 75%(6/8). The antibiotic susceptibility results showed meropenem, imipenem and amikacin the most effective antibiotics for Gram negative bacteria. Overall, urinary tract infections were the most common sites of infection (23.2%). The results of the analysis of the sequencing 16SrDNA of selected 43 isolates showed compatible 100% to genus and species level with results of VITEK 2. Ten isolates were registered as a new strain in Gene Bank. Gram-negative bacteria with high MDR were more prevalent cause of infection in febrile pediatric cancer patients. Escherichia coli being the most common pathogen. The changing pattern of infectious agents in cancer patients with significant distribution of MDR suggests the need for further studies to give physicians a more recent view of bacterial isolates and antibiotic resistance pattern for appropriate therapeutic approaches.*

***Keywords---*** *Febrile Pediatric Cancer, Chemotherapy, Antibiotic Resistance, E. Coli, PCR.*

## I. INTRODUCTION

In cancer patients, Infection is a significant problem due to both direct and indirect effect on a patient's immune system. There are many causes increase the susceptibility of immunosuppressed cancer patients to infection, such as the neutropenia during aggressive therapy, disruption of skin and damage of epithelial surfaces by cytotoxic agents, altered gut flora because of frequent antibiotic administration1,2. Although the advances in antimicrobial prophylaxis and chemotherapy have decreased the disease severity and improved the survival rate but infectious

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complications are still the major causes of morbidity and mortality in hematological and oncological patients3. It is estimated about 60 % of hematological malignancies patients and 50% of patients with solid tumors die from infectious complications4.

Infections in malignant patients cause reduction of effectiveness of anti-neoplastic treatments because of dose reductions and delays in drug taking. In addition the treatment of infections need high economic costs because of requires of hospital admission and antimicrobial drugs5 Fever may be the only manifestation of serious infection in the immunocompromised patient6.

Bacterial infection is one of the most frequent complications in malignant patients. Among them, those caused by multidrug-resistant (MDR) bacteria increase mortality and morbidity mainly because of limited therapeutic options7.

Currently the initial selection of an antibiotic regimen is depend on the types of organisms that cause infection in each institution, their susceptibility to antibiotics and the individual characteristics of each patient1. Although national guidelines are available for the management of febrile neutropenic children, local microbiological epidemiology is more important when deciding the empiric antibiotic regimen for the individual patient8.

Therefore, the current study aimed to determine the spectrum and antibiotic resistance pattern of bacterial isolated from febrile cancer patients that suspected having infection. This information may help clinicians to select effective empirical therapies and provide good epidemiological profiles to compare our situation with others.

## II. MATERIAL AND METHODS

### *Patients and Methods*

This study carried out at Basrah children's specialty hospital from May 2018-April 2019. Study population included 155 febrile episodes with various malignancies undergoing treatment who were <18 years of age.

The inclusion criteria of this study include all pediatric cancer patients undergoing chemotherapy that had presented with fever with or without neutropenia who suspected to have infection. The cases excluded from the study include the children who received treatment with antibiotics within the previous 72 hours or had fever due to a non-infectious cause, such as drug infusion, blood transfusion, and others.

All patients underwent complete physical examination, a detailed history and relevant radiological, hematological and microbiological investigations. Two sets of blood cultures and urine culture from each patient. All possible sources of infection were investigated and taken swabs from the suspected site of infections.

### *Ethical Approval*

The study protocol was approved by the ethics committee of Basrah Health Department and Basrah children's specialty hospital. Informed consent was taken from the parents of children before collecting the samples for the study.

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***Collection of Clinical Specimens and Processing***

***Blood:*** Under strict aseptic measures (disinfecting with 70% alcohol and 2% tincture of iodine), two sets of 1-3 ml of blood were collected for each patient. Immediately after collection, the blood was added to BacT/ALERT® PF Plus(Color- coded yellow ) bottles and incubated in a Bactec incubator BacT/ALERT 3D (Biomeriux France) for 7 days at 37°C. The positive culture bottles were mixed well then the specimen taken from the broth by using sterile syringe and plated on various media included : blood agar and macConkey agar were incubated aerobically, chocolate agar incubated in 5-10% $CO_2$ and blood agar incubated anaerobically. All the plates were incubated at 35-37ºC for 24 hours.

***Urine:*** Urine specimens were collected in sterile urine container. Once received the urine sample, it mixed well in the container and inoculated by using a calibrated loop (0.01/mL) on blood and MacConkey agar plates were labeled for each patient and incubated under $O_2$ at 35- 37ºC for 24 hours. Significant bacteriuria was defined as colony count $\geq 10^5$ CFU/mL urine.

***Swabs:*** included throat, wound, ear, skin and vaginal swabs were collected by specialist physician before start of antibiotic therapy by using amies transport media swabs. Swabs were streaked (inoculated) directly on blood agar and MacConkey agar plates (for aerobically incubation), chocolate agar plate (in 5-10% $CO_2$ ), blood agar plate (for anaerobically incubation). The inoculated culture plates were incubated at 35- 37ºC for 24- 48 hours.

***Intravenous Catheter:*** Catheter tip was cultured by the semi quantitative, which performed by rolling the external surface of a catheter tip back and forth on the surface of blood agar plate 3-4 times and then the plate was incubated for 24 hours in 5-10% $CO_2$ at 37ºC after which the number of colony forming unit (CFU) were quantified. The break point for detection of catheter tip colonization was> 15 CUF.

***Identification of Bacterial Isolates***

All plates were examined after 24-48 hours. If any of the media indicate a significant growth of a suspected pathogen, the microorganism was identified by colony morphology, Gram stained characteristics and biochemical test (such as catalase test, Coagulase test, Oxidase test).

The identification of bacterial isolates were confirmed by VITEK 2 compact auto analyzer system (Biomerieux, France). This technique was conducted in Microbiology Laboratory of Basrah children's specialty hospital.

***Antimicrobial Susceptibility Testing***

Antibiotic susceptibility testing of bacterial isolates was performed by Kirby Bauer disc diffusion method using Muller Hinton agar9. Pure colonies were transferred to 5 mL sterile normal saline solution and mixed well to obtain a homogenous solution of bacterial cells. The turbidity of the mixture adjusted by compared with 0.5 McFarland standard. A sterile cotton swab was immersed in to the bacterial suspension, then the cotton swab rotated numerous time steady on inside wall of the tube above the fluid level to remove extra inoculum from the swab, after that the immersion swab was streaking on surface ofMuller Hinton agar plate. The inoculated plates were left at room temperature for 3-5 minutes to dry. The antibiotic discs then placed on the top of cultural Muller Hinton agar with a sterile forceps, at a distance of about 20 mm from each other and 15 mm from the edge of the plate and discs were

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pressed gently to ensure complete touching base with the surface of agar. The plates were incubated at 37ºC for 18-24 hour. The results were read by measuring the diameter of inhibition zone around the disc according to the clinical and laboratory standard institute (CLSI) guideline10.

The antibiotics used were purchased from (Mast diagnostic, Oxoid Ltd., UK) and they are:ampicillin (10µg), ampicillin- sulbactam (10/10µg), piperacillin(100µg), piperacillin-tazobactam (100/10µg), amoxicillin/clavulanic acid (20/10µg), ticarcillin- clavanic acid(75/10µg), cefoxitin(30µg), cefotriaxone (30µg), ceftazidime (30µg), cefotaxime (30µg), cefixime (5µg), aztreonam(30µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), tobramycin (10µg), amikacin (30µg), azithromycin(15µg), ciprofloxacin (5µg), ofloxacin (5µg), levofloxacin (5µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), penicillin (10µg), vancoomycin (30µg), teicoplanin (30µg), erythromycin (15µg), clarithromycin (15µg), clindamycin (2µg), linezolid(30µg) and tetracycline (30µg).

According to CLIS guideline10recommendation, some antimicrobial susceptibility for some isolates performed automatically using AST VITEK 2 kit because it depends on the minimum inhibitory concentration rather than disc diffusion method such as Oxacillin.

***Confirmation of the Identified Bacterial Isolates by 16SrRNAgenes Sequencing***

***Genomic DNA Preparation***

The DNA of 43 bacterial isolates were extracted according to the procedure of Presto™ Mini g DNA bacteria kit ( Geneaid, Taiwan ) after refreshing the pure colony in Brain heart infusion broth (Afco, Jordan. ) for 24 h. at 37°C.

***Amplification of 16SrDNA***

16SrDNA was amplified by Polymerase Chain Reaction (PCR) according to11 using the following universal primers: forward primer: 27F (5´AGAGTTTGATCCTGGCTCAG-3´) and reverse primer:1492R (5´-GGTTACCTTGTTACGACTT-3´) (Alpha DNA, Canada).

Total PCR reaction mixture (50µl) contains 25µl of Go Taq Green master mix (Promega, USA), 18µl of nuclease free water, 3µl of DNA template and 2µl from each primer. The amplification conditions for PCR were as follows: initial denaturation at 92ºC for 2min followed by 35 cycles each consisted of denaturation at 94ºC for 30 sec, annealing at 54ºC for 45sec and extension at 72°C for 1.5 min, final extension at 72°C for 5 min usingthermal cycler (Biometra, Germany).

About 5µl of the PCR ampliconwere electrophoresed for 1 hrusing 2% agarose gel prepared in 1x TBE containing 0.2µl of ethidium bromide. The band product (1500bp) was indicative to 16SrDNA gene amplicon. The gel was photographed using Gel photo documentation system (

***16SrDNA Sequencing***

About 20µl of PCR product of 16SrRNAgene with the forward primer (17pmol) for each sample, were sent for sequencing (Macrogen, Korea). The sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. New sequenceswere submitted to the NCB1-GeneBank to obtain accession numbers.

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***Statistical Analysis***

Analyses were performed using SPSS™ software, version 25.0. The results are presented as descriptive statistics in terms of relative frequency.

## III. RESULTS

***Bacterial Profiles and Site of Isolation***

According to staining reactions, culture characteristics, biochemical tests and confirmation by VITEK2 system, there were 61 bacterial isolates detected by cultured different clinical samples including blood, urine, swabs from the site of suspected infection and intravenous catheter (Figure1). In general the result showed 86%(53/61) Gram negative isolatesand 13.1%(8/61)Gram positive bacteria isolates. Among the Gram negative isolates, E. coli was the most predominant (64.1%) followed by Klebsiellapneumoniae (13.2%) and Proteusmirabilis (11.3%). In Gram positive bacteria, S.aureuswas the most predominant 37.5% (3/8).

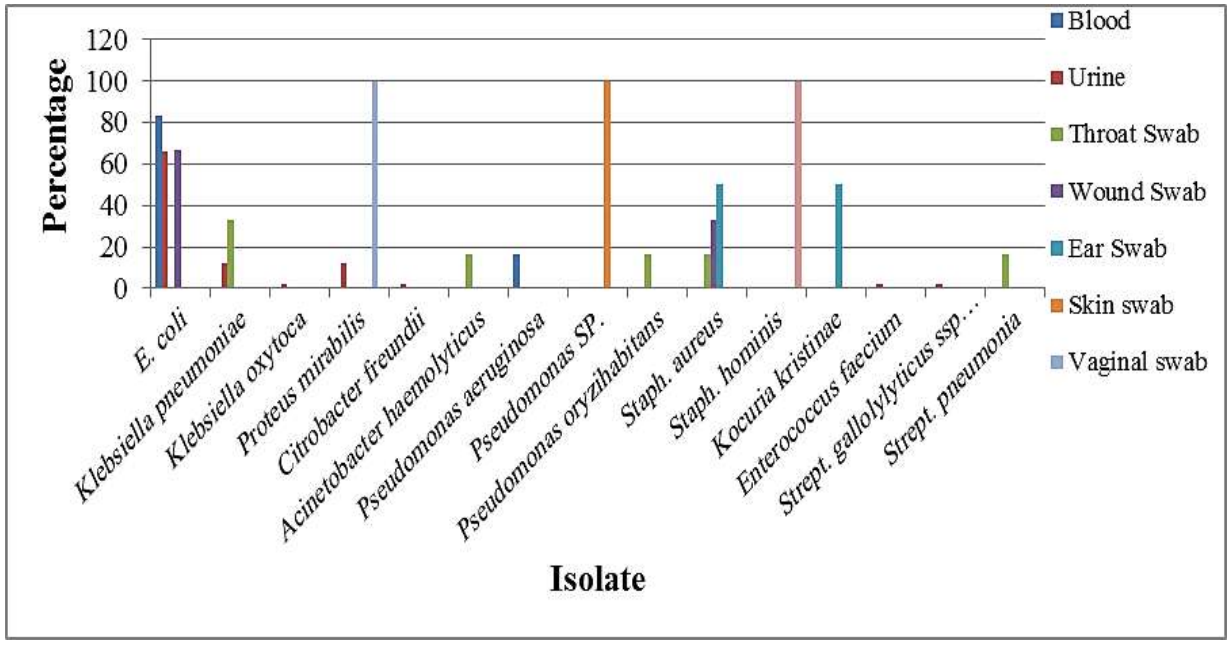


Figure 1: Percentage of Bacterial Isolates in Different Clinical Specimens

***Blood Culture***

Blood culture was performed for all the febrile cases (155), bloodstream infection occurred in 3.9% (6/155) of cases. Six bacterial isolates of*E. coli* 83.3%(5/6) were predominating.

***Urine Culture***

Urine cultures also carried out for all the patients included in this study. Urinary tract infection detectedin 23.2% (36/155). The total bacterial isolates from the positive urine culture were 41 isolates and *E. coli*waspredominating.

***Swabs Culture***

There were 11 swabs collected from different sites, all of these swabs gave positive results.Five throat swabs

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were collected from the patients with tonsillitis that clinically examined, there were six different bacterial isolates identified**.** Three wound swabs were collected from three patients and three bacterial isolates were identified. One ear swab was collected from one patient and two bacterial isolates were identified. One skin swab was taken from febrile patient suffered from skin infections and one bacterial isolate was identified. Finally one vaginal swab was taken from febrile case with vaginitis and one bacterial isolate was identified.

***Intravenous Catheter***

One intravenous catheter collected and gave positive culture with one bacterial isolate.

***Antimicrobial Susceptibility***

Antimicrobial sensitivity patterns of different Gram negative isolates (N= 53) against the twenty two antimicrobial agents is shown in(table1).

High resistance rates were observed among total Gram negative bacteria to most antibiotics tested including β-lactam and non β-lactam. On other hand low resistance rates were detected for imipenem (15.1%), meropenem (18.9%) and amikacin (15.1%). It should be noted that the use of tazobactam (β-lactamase inhibitor) enhanced the activity of piperacillin.

Table 1: Antibiotic Sensitivity among Gram Negative Bacteria

| Antibiotic | Number of isolates that antibiotic tested | Sensitive N(%) | Intermediate N(%) | Resistance N(%) |
|---|---|---|---|---|
| Ampicillin | 49 | | | 49(100) |
| Ampicillin- sulbactam | 49 | 6(14.6) | 2(4.0) | 41(83.6) |
| Piperacillin | 53 | 9 (17.0) | 3(5.7) | 41(77.3) |
| Piperacillin-Tazobactam | 53 | 26(29.1) | 5(9.4) | 22 (41.5) |
| Amoxicillin- clavanic acid | 49 | 10(20.4) | 4(8.2) | 35(71.4) |
| Ticarcillin- clavanic acid | 53 | 3(5.7) | | 50(94.3) |
| Cefoxitin | 52 | 0(100) | | 52(100) |
| Cefotriaxone | 50 | 2(4.0) | | 48(96.0) |
| Ceftazidime | 53 | 5(9.4) | 2(3.8) | 46(86.8) |
| Cefotaxime | 50 | 3(6.0) | | 47(94.0) |
| Cefixime | 53 | 32(60.4) | | 21(39.6) |
| Aztreonam | 52 | 9(17.4) | 2(3.8) | 41(78.8) |
| Imipenem | 53 | 45(84.9) | | 8(15.1) |
| Meropenem | 53 | 43(81.1) | | 10(18.9) |
| Gentamicin | 53 | 28(52.8) | 1(1.9) | 24(45.3) |
| Tobramycin | 53 | 27(50.9) | 3(5.7) | 23(43.4) |
| Amikacin | 53 | 45(84.9) | | 8(15.1) |
| Azithromycin | 49 | 18(36.7) | | 31(63.3) |
| Ciprofloxacin | 53 | 20(37.7) | 2(3.8) | 31(58.5) |
| Ofloxacin | 52 | 23(44.3) | 3(5.7) | 26(50.0) |
| Levofloxacin | 53 | 34(64.2) | | 19(35.8) |
| Trimethoprim-sulfamethoxazole | 53 | 19(35.8) | | 34(64.2) |

*E. coli* as the most frequent isolated bacteria among Gram negative isolates from all clinical specimens, showed the same pattern of total Gram negative bacteria (table 2).

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Table 2: Antibiotic Sensitivity of the Isolated E.coli

| Antibiotic | Sensitive N(%) | Intermediate N(%) | Resistance N(%) |
|---|---|---|---|
| Ampicillin | | | 34(100) |
| Ampicillin- sulbactam | 4(11.8) | 2(5.9) | 28(82.3) |
| Piperacillin | 5(14.7) | 1(2.9) | 28(82.4) |
| Piperacillin-Tazobactam | 13(38.2) | 3(8.8) | 18(52.9) |
| Amoxicillin- clavanic acid | 7(20.6) | 3(8.8) | 24(70.6) |
| Ticarcillin- clavanic acid | | | 34(100) |
| Cefoxitin | | | 34(100) |
| Cefotriaxone | 1(2.9) | | 33(97.1) |
| Ceftazidime | 2(5.9) | | 32(94.1) |
| Cefotaxime | 2(5.9) | | 32(94.1) |
| Cefixime | 18(52.9) | | 16(47.1) |
| Aztreonam | 2(5.9) | 1(2.9) | 31(91.2) |
| Imipenem | 28(82.4) | | 6(17.6) |
| Meropenem | 28(82.4) | | 6(17.6) |
| Gentamicin | 19(55.9) | | 15(44.1) |
| Tobramycin | 18(52.9) | | 16(47.1) |
| Amikacin | 30(88.2) | | 4(11.8) |
| Azithromycin | 14(41.2) | | 20(58.8) |
| Ciprofloxacin | 10(29.4) | | 24(70.6) |
| Ofloxacin | 11(32.4) | 3(8.8) | 20(58.8) |
| Levofloxacin | 20(58.8) | | 14(41.2) |
| Trimethoprim-sulfamethoxazole | 14(41.2) | | 20(58.8) |

Antibiotic sensitivity patterns of the 8Gram-positive isolates from cancer patients (n=8) against the fifteen antibiotics, showed high resistance to some antibiotics (table3).

Table 3: Antibiotic Sensitivity of Gram Positive Isolates

| Antibiotic | Number of isolates | Sensitive N(%) | Intermediate N(%) | ResistantN(%) |
|---|---|---|---|---|
| Penicillin | 7 | | | 7(100.0) |
| Oxacillin | 5 | 2(40.0) | | 3(60.0) |
| Vancoomycin | 8 | 6(75.0) | | 2(25.0) |
| Teicoplanin | 6 | 4(66.6) | | 2(33.3) |
| Azithromycin | 7 | 4(57.2) | | 3(42.8) |
| Erythromycin | 8 | 3(37.5) | | 5(62.5) |
| Clarithromycin | 6 | 4(66.6) | | 2(33.3) |
| Clindamycin | 7 | 5(71.4) | | 2(28.6) |
| Linezolid | 8 | 6(75.0) | | 2(25.0) |
| Tetracycline | 8 | 3(37.5) | | 5(62.5) |
| Gentamicin | 5 | 1(20.0) | | 4(80.0) |
| Ciprofloxacin | 6 | 5(83.3) | | 1(16.7) |
| Ofloxacin | 7 | 5(71.4) | 1(14.3) | 1(14.3) |
| Levofloxacin | 8 | 6(75.0) | | 2(25.0) |
| Trimethoprimsulfamethoxazole | 6 | 5(83.3) | | 1(16.7) |

Multiple drug resistant (MDR) was defined as a bacterial isolate, that resistant to one or more antibiotics in three or more categories of antimicrobial agents[12]. In the current study, the totally MDR bacteria among the Gram negative isolates were 90.6% (48/53).Table (4)shows the percentage of MDR for all Gram negative isolates. Among the Gram positive bacteria, MDR isolates were 75.0% (6 /8).Table (5)shows the percentage of MDR for all Gram positive isolates.

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Table 4: Multi Drug Resistance of Gram Negative Isolates

| Isolate | Multi Drug Resistant | | Non Multi Drug Resistant | | Total | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| *E. coli* | 33 | 68.8 | 1 | 20.0 | 34 | 64.2 |
| *Klebsiellapneumoniae* | 7 | 14.6 | | | 7 | 13.2 |
| *Klebsiellaoxytoca* | 1 | 2.1 | | | 1 | 1.9 |
| *Proteus mirabilis* | 5 | 10.4 | 1 | 20.0 | 6 | 11.3 |
| *Pseudomonas aeruginosa* | 1 | 2.1 | | | 1 | 1.9 |
| *Pseudomonas oryzihabitans* | | | 1 | 20.0 | 1 | 1.9 |
| *Pseudomonas sp.* | | | 1 | 20.0 | 1 | 1.9 |
| *Citrobacterfreundii* | 1 | 2.1 | | | 1 | 1.9 |
| *Acinetobacterhaemolyticus* | | | 1 | 20.0 | 1 | 1.9 |
| Total | 48 | 100 | 5 | 100 | 53 | 100 |

Table 5: Multi Drug Resistance of Gram Positive Isolates

| Isolate | Multi Drug Resistance | | Non Multi Drug Resistance | | Total | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| *Staph aureus* | 3 | 50.0 | | | 3 | 37.5 |
| *Staphylococcus hominis* | | | 1 | 50.0 | 1 | 12.5 |
| *Kocuriakristinae* | | | 1 | 50.0 | 1 | 12.5 |
| *Streptococcus pneumonia* | 1 | 16.7 | | | 1 | 12.5 |
| *Enterococcus faecium* | 1 | 16.7 | | | 1 | 12.5 |
| *Streptococcus gallolylyticusssppasteurianus* | 1 | 16.7 | | | 1 | 12.5 |
| Total | 6 | 100 | 2 | 100 | 8 | 100 |

***Molecular Identification of Bacterial Isolates Based on 16srrn Agene Sequencing***

Figure (2)Shows the results of agarose gel electrophoresis for the 1500bp amplicon.

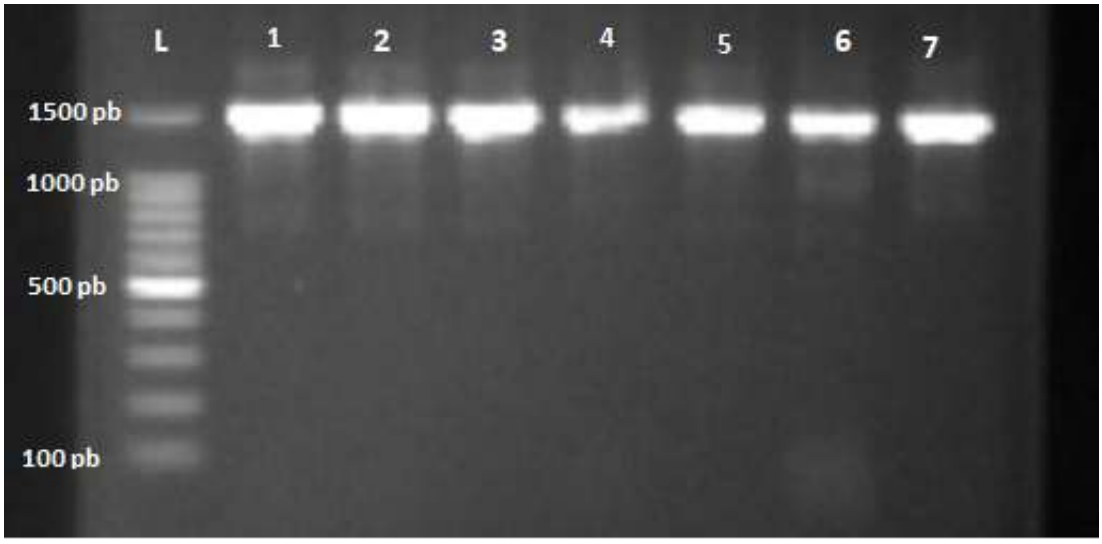


Figure 2: Agarose gel electrophoresis of the amplified PCR product of 16SrDNA. Lane L: DNA marker (100pb), lanes 1-7: 16SrDNA amplified bands

The sequencing results of the tested isolates gave identical (100%) at genus and species level with the identification by VITEK 2.

Ten bacterial isolates were registered as new strains at the Gen Bank which gave 99% similarity with their relative species (table6).

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Table 6: Sequencing Results of New Strains

| Code | Source | Nearest neighbor (Accession No.) | Identity % | Accession No. | Type of Mutation |
|---|---|---|---|---|---|
| BD6 | Urine | *Escherichia sp.* (KR190344.1) | 99% | **MN148397.1** | Frame shift(insertion G) at the position 1057 bp |
| BD14 | Urine | *Proteus mirabilis* (LC385636.1) | 99% | **MN148436.1** | Frame shift(insertion G) at the position 1054 bp |
| BD15 | Urine | *Proteus mirabilis* (MF576130.1) | 99% | **MN148437.1** | Frame shift(insertion G) at the position 1051bp |
| BD17 | Blood | *Escherichia coli* (CP023820.1) | 99% | **MN148509.1** | Frame shift(insertion G) at the position 1056 bp |
| BD18 | Urine | *Klebsiellaoxytoca* (MF429543.1) | 99% | **MN148510.1** | Frame shift(insertion G) at the position 1050 bp |
| BD19 | Throat swab | *Klebsiella pneumonia* (KU942506.1) | 99% | **MN148511.1** | Frame shift(insertion G) at the position 1050 bp |
| BD24 | Vaginal swab | *Proteus mirabilis* (CP012674.1) | 99% | **MN148513.1** | Frame shift(insertion G) at the position 1119 bp |
| BD25 | Urine | *Klebsiella sp.* (AB558497.1) | 99% | **MN148512.1** | Frame shiftmutation (insertion C, G) at the position 1034 bp, 1075 bp respectively and transversion mutation (C>G) at the position 1066 bp |
| BD 32 | Blood | *Pseudomonas aeruginosa*(KF598858.1) | 99% | **MN148514.1** | Four transversion mutations, these are: C, T, Aand A instead of G, G, G and G at the position 203 pb, 350 pb, 405 pb and 633 pb, respectively |
| BD 37 | Skin swab | *Pseudomonas sp.* (MH158303.1) | 99% | **MN148515.1** | Transversion mutation (G>A) at the position 550bp |

## IV. DISCUSSION

Children with cancer vulnerable to infection due to defect in host defense mechanisms. This may be related to disease itself, to intensive treatment with immunosuppressive/myelo suppressive drugs (mucositis, neutropenia, lymphopenia,) and frequent use of the central line. In this context, infection is an important complication which may be life threatening unless dealt with, appropriately and timely[13].The knowledge of likely pathogens and local bacterial spectrum is very important for effective treatment[14].

***Types of Microbiological Document Infections in Febrile Patients***

Current prospective study on the clinical relevance of the blood, urine and other site (throat, wound,ear, skin, vaginal and intravenous catheter ) as a source of bacterial infection and cause fever of included patients.

Bloodstream infections remain a major cause of morbidity and mortality in cancer patients[15]. Different rate of the positive blood culture were reported in previous studies, about 2.6%[16], 6.9%[17], 9.4%[13], 38%[18], 36.3%[19].

In a study done in Barcelona, Spain from 2006 to 2010, reveled overall bacteraemia was 5.6 episodes/1000 hospital stays in patients on quinolone[20]. With the use of prophylactic antibiotics and antifungal in neutropenic pediatric cancer patients in Basra Children Hospital, the prevalence of bacteraemia was low.

Urinary tract infection is one of the major causes of morbidity in cancer patients[21]. Previous studies of UTI reported 8.5% [22], 18.4% [13] and 65.6%[23]. Urinary tract infections may present with nonspecific symptoms and

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without any definitive clinical signs other than fever. Therefore the diagnosis of UTI may be missed unless urine cultures are routinely obtained[17].

Infectious Diseases Society of America (IDSA) guidelines state that sending urine cultures is indicated if signs or symptoms of UTI exist; a urinary catheter is in place, or the urinalysisis abnormal[24]. Moreover, IDSA states that routine culture of urine yields clinically irrelevant information. [25]recorded on the absence of pyuria in neutropenic pediatric oncology patients with UTI. Positive urine culture in children with cancer may not be correlated with urinalysis abnormalities, particularly in patients with neutropenia[26].

A report by17 performed urine and blood culture for 58 febrile episodes, and they found the frequency of UTI was 8.6% (5/58) in comparison for bacteremia 6.9% (4/58) (none of whom had a UTI), they concluded the UTI is as common as bacteremia in their study of pediatric cancer patients with fever and neutropenia.

In present study the other sites of infection includes (throat, wound, ear, skin, vaginal and intravenous catheter) is fewer than previous studies that may be due to limitation time of sample collection in addition some patients were excluded because they were under antibiotics treatment.

***Pattern of Infection***

Identification and determination of antimicrobial susceptibility of bacterial pathogens can help the clinician in selecting the appropriate antimicrobial agent to treat his patients27.

A predominance of infections due to Gram-negative organisms. These findings are similar to those reported in the literature28, 29, 13, 14, 30. In contrary, others studies had reported predominance of Gram-positive bacteria 18, 19, 31. While similar rate in gram positive and negative, in febrile neutropenic patients had been reported32. The epidemiology of bacterial infections among cancer patients showing a change in the trend from Gram positive to Gram-negative bacteria and vice versa remains controversial31. The higher rates of gram-positive pathogens in some studies may be due to some factors; For example, the use of prophylactic antibiotics such as fluoroquinolones, oral and intestinal mucosal damaging as a result of chemotherapy and the frequent use of central venous catheters33.

These differences in results even in the same country require frequent monitoring of local epidemiology understanding of bacterial infection among cancer patients and further studies are necessary to give a more recent view of the microorganisms to physicians.

E. coli was the most common among Gram negative bacteria identified among the clinical specimens. It should be noted that most of Gram negative bacteria strains examined were from urine specimens. This may explain the predominance of E. coli amongst our Gram negative bacteria isolates. Similar findings were reported23,27. Add to that, E. coli is a normal member of gastro-intestinal flora and a common cause of both community and hospital acquired urinary tract infection. The immune compromised cancer patients are easily infected by the bacteria, due to the fact that infection of cancer patients by this bacterium is inevitable31.

Among gram positive, Staph. Aure us was the most frequent that agrees with34. However at the same study of different clinical samples, they found that Pseudomonas aeruginos a the most frequent gram-negative bacteria. The difference between the results might be due to differences in the pattern of antibiotic medications, health and

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hygiene issues, underlying diseases in those countries, sample size, the source of infections and geographical distribution.

***Antimicrobial Sensitivity***

The results agree with what was reported that Infections due to Gram negative bacteria with high resistance rates to β-lactam and non- β-lactam drugs are common in cancer patients27,35,36.

In the current study showed highly resistance of gram negative bacteria to cephalosporin group, which is consistent with a study on leukemic patients at teaching laboratories of medical city of Baghdad37 and it is raising world wide41,42Other similar study performed by1 that reported high resistance of E. coli and Klebsiella species to cefotaxime and ceftazidime. The high resistance in Enterobacteriaceae may be attributed to the β-lactamase activity38, 39 and the resistance of cefotaxime, Aztreonamand ceftazidime are potential markers for the presence of Extended-Spectrum β lactamases (ESβL)40 that are resistant to flouroquinolones and amino glycosides thus making it difficult to treat43.

In this study, Gram negative isolates exhibited high resistance to Aztreonamthat is consistent with a study on pediatric cancer patients at South Egypt Cancer Institute44.

High resistance to Ciprofloxacin has been detected among Gram negative bacteria which is also comparable with the results reported by1 that found resistance in E. coli, Klebsiella and Enterobacter species to ciprofloxacin. At the same study, they showed that Acinetobacter species, Enterobacter species, E. coli and Klebsiella were relatively more susceptible to newer quinolones than ciprofloxacin, that is consistant with the findings of this study. The high resistance of others β-lactam and β-lactam/ β-lactamase inhibitor agents were found in this study. Same results were observed by1,13,31,40,45.

The Current study reported high resistance to aminoglycosides, similar finding of13,40.

The highest efficient antibiotics for treatment of Gram negative bacteria were Amikacin and Imipenem followed by Meropenem. Similar finding reported by44 for Amikacin and Imipenem.

Gram positive bacteria also exhibited high resistance to some antibiotics, however more data needed to well studying the sensitivity of antibiotic groups.

The rate of infections related to MDR Gram negative bacteria in patients with cancer is increasing globally46. In this study, the estimated rate of MDR Gram negative bacterial isolates was remarkable and agrees with a similar finding of resent study from neighboring Iran reported high rate of MDR isolates (91.5%) from cancer patients23.However, our estimated MDR rates were significantly higher compared to those that were previously reported for E. coli (29%) from cancer patients27. Add to that, Basrah Governorate have reported that E. coli isolates from hospital diarrheal cases were (100%)47.Despite the low incidence of gram positive isolates, there were 75% showed MDR. It is higher than those were previously reported (32.8%) isolated from the blood stream18.Despite the comparable antibiotic resistance and susceptibility results of our study with other studies, there was variation from others due to different in geographical area and policy of using antibiotic.

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***Molecular Identification***

Broad-range 16srDNA PCR is becoming increasingly available and is generally rapid and easy to carry out in a laboratory with relevant expertise in molecular microbiology and an understanding of bioinformatics48,49.

In this study, the bacterial isolates were identified phenotypically by conventional biochemical tests and then confirmed by VITEK 2 system and finally the most common confirmed by Molecular Characterization that using16SrRNA gene sequencing. VITEK 2 is automated identifications systems used in routine work of most clinical microbiology laboratory. All the bacterial isolates of this study were successfully identified and confirmed by molecular method that gave 100% identity atgenus and species level.

Ten bacterial isolates gave 99%similarity that were recorded as a new strains because the accumulative results from a many studies suggested a range of about a 0.5% to 1% difference (99.5 to 99% similarity) is often used for classification50.

## V. CONCLUSION

Gram-negative bacteria with high MDR were more prevalent cause of infection in febrile pediatric cancer patients where E. coli being the most common pathogen. The changing pattern of infectious agents in cancer patients with significant distribution of MDR suggests the need for further studies to give physicians a more recent view of bacterial isolates and antibiotic resistance pattern for appropriate therapeutic approaches.

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## ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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