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European Journal of Experimental Biology, 2014, 4(2):1-6

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Isolation, identification, and profile of antibiotic resistance of bacteria in childhood febrile neutropenia patients

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ABSTRACT

Cancer patients are particularly susceptible to nosocomial infections because of their compromised immune system, and because of the nature of treatment practices they experience. This study analyzed the distribution and the antimicrobial resistance of bacteria isolated from cancer patient during two time periods in the Basrah - Iraq. A total of 428 patients presenting with febrile neutropenia during years 2012 and 2013. During the study period 63.6% Gram-negative bacteria and 36.3% Gram-positive bacteria were isolated from cancer patients during year 2012 while this ratios were increased through 68.1% Gram- negative bacteria and 31.8% Gram- positive bacteria during year 2013. The most common Gram- negative bacteria were Escherichia coli and Pseudomonas aeruginosa while Gram- positive bacteria were Staphylococcs epidermidis and Staphylococcus aureus. Many of Gram- negstive and Gram- positive bacteria were high resistance for many of antibiotics and significant differences during the two study periods, especially against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis and Staphylococcus aureus.

Keywords: Identification, Bacteria, Antibiotic resistance, Febrile Neutropenia patients.

INTRODUCTION

Pediatric cancer is the second most common cause of death following accident in children and the most common cause of death from pediatric diseases [1]. Although the potent anticancer therapy increased survival rates or cure rates of pediatric cancer patients, the risk of infection became even higher due to profound and protracted neutropenia[2].

Leukemia is the most common childhood cancer, accounting for 25% to 35% of the incidence of all childhood cancer among most populations. The commonest type of childhood leukemia is acute lymphoblastic leukemia (All), which occurs in approximately 80% of leukemia cases, followed by acute myeloid leukemia (CML), and a few in other categories [3]. In other studies to assess the rates and trends of childhood leukemia in Basrah, Iraq, from 2004 to 2009, this results were 2.7% to 3.1% respectively [4].

Blood steam infection is one of the main causes of morbidity and mortality in patients who receive cancer treatment. Most of infections in such patients are related to the neutropenia, aplastic anemia, tissue necrosis, and hypogamma-globulinemia [5]. Normal microflora and semi-pathogen organisms can play a significant role in such acute medical conditions . It was found that 15-25% of bacteremia in cancer patients is polymicrobial. The polymicrobial infections are associated with higher mortality , sometimes more than 50%. Literature review indicates that the risk of anaerobic blood stream infection in malignancy is high[7]

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In many institutions in developed countries, more Gram- positive bacteria, mainly staphylococci, than Gram-negative bacteria are isolated from cancer patients [8]. Emergence of antimicrobial resistance among staphylococci (e.g. methicillin- resistance *S. aureus* (MRSA) and Gram- negative bacilli (e.g. *E. coli*) associated with infections in cancer patients is of particular concern in recent years [9].

Reports on the microbial spectrum and antimicrobial resistance profile of bacteria from cancer patients in the country of Iraq is lacking. The aim of this study was to determine the profile and susceptibility patterns of bacterial pathogens associated with infections in cancer patients in neutropenia patients during the two calendar years, we planned this study at the Basrah, Iraq.

MATERIALS AND METHODS

Four hundred and twenty eight patients with cancer who were receiving chemotherapy were included in the present study and suffering from febrile neutropenia. The patients consisted of children with acute and chronic leukaemia, lymphoma and solid tumors treated at the hospitals between 2012 and 2013 . In addition ,the patients were re-examing clinics in the city of Basrah .

Demographic data and clinical characteristics of the patients were collected in a questionnaire. The questionnaire was designed for the specific needs. According to the patients clinical status, the number of blood samples and the time of sample collection were determined by the physicians [10]. Organisms were isolated from blood, urine, stool, pus swab, wound swab and sputum specimens from the patients at the time infection occurred. All specimens were cultured on different media according using standard bacteriological procedures [11]. Isolated organisms were identified to the species level and tested for their susceptibility to avariety of antimicrobial agents by the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks MD, USA) according to the manufacture´s instructions. The PAMS uses combination panels for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria. These include the Phoenix NMIC/ ID panels intended for the *invitro* rapid ID and AST by minimal inhibitory concentration (MIC) of Gram-negative aerobic and facultative anaerobic bacteria from pure culture belonging to the family Enterobacteriaceae and non-Enterobacteriaceae. In addition, this system used to diagnosis of Gram-positive bacteria belong to the genera *Staphylococcus* , *Streptococcus* , *Enterococcus* , and other Gram-positive cocci and Gram-positive bacilli. PAMS provides AST results for antimicrobials as susceptible (S), intermediate susceptible (I) and resistant (R) and is interpreted according to CLSI criteria [12].

Statistical Analysis

The data in this study are shown as frequencies. The t-test was used for the group statistics of dependent and independent samples. In addition, the Chi-Square test was used to calculate the p-value. p-value of less than or equal to 0.05 was considered statistically significant. All statistical analysis were performed with use of SPSS Software, version 10 .

RESULTS

The study population comprised 428 patients presenting with febrile neutropenia during years 2012 and 2013. This characteristics are shown in table (1). The mean age of the patients was 5.5 and 6 years for children. Of 168 patients, 65.4% suffered from leukemia and remaining (34.5%) had other various forms of malignancies during year 2012 while year 2013 were 68% and 31.9% respectively. In addition, the bacterial cultures were 40.4% and 48% positive and polymicrobial growths were 18.4% and 11.1% during years 2012 and 2013 respectively.

Table (1): Characteristics of pediatric cancer patients with neutropenia during years 2012 and 2013

characteristics		Year 2012	Year 2013
Total patients (n=428)		(n=168)	(n=260)
Mean age		5.5 Years	6 Years
Sex	Males	97 (57.7 %)	158 (60.7 %)
	Females	71 (42.2 %)	102 (39.2 %)
Leukemia (ALL and AML)		110 (65.4 %)	177 (68 %)
Solid tumors and others*		58 (34.5 %)	83 (31.9 %)
Bacterial culture positive		68 (40.4 %)	125 (48 %)
Polymicrobial growth		31 (18.4 %)	29 (11.1 %)

** Others : hepatoblastoma , pineoblastoma*
All : acute lymphoblastic leukemia
AML : acute mycloid leukemia

The most common pathogens identified were gram-negative bacteria (63 out of 99 isolates, 63.6 %) and (105 out of 154 isolates, 68.1 %). *Escherichia coli* (52.3 % and 54.2 %) was the most common isolates, followed by *Pseudomonas aeruginosa* (28.5 % and 29.5 %) and *Klebsiella oxytoca* (1.5 % and 6.6 %) during year 2012 and 2013 respectively. Gram-positive bacteria (36 out of 99 isolates , 36.3 %)and (49 out of 154 isolates , 31.8 %) such as *Staphylococcus epidermidis* (22 isolates, 61.1 % and 34 isolates, 69.3 %), *Staphylococcus aureus* (8 isolates , 22.2 % and 10 isolates , 20.4 %) during year 2012 and 2013 respectively (Table 2, 3, 4 and Figure-1). No significant differences in bacterial spectrum were recorded between the two years.

Susceptibility of bacterial isolates from cancer patients to antimicrobial agents is shown in Table (5). Many of gram-negative and gram-positive were high resistance for many of antibiotics and significant differences during the two study periods (2012 and 2013).

Table (2): Gram-negative and Gram-positive organisms isolated from different clinical specimens of cancer patients during years 2012

Organism (Gram-negative)	No. (%)						
	Urine (n=38)	Blood (n=18)	Sputum (n=4)	Pus (n=12)	Wound (n=19)	Stool (n=8)	Total (n=99)
Escherichia coli	12(55.2%)	2(11.1%)	1(25%)	2(16.6%)	2(10.5%)	5(62.5%)	33(52.3%)
Pseudomonas aeruginosa	8(21%)	2(11.1%)	1(25%)	1(8.3%)	3(15.7%)	3(37.5%)	18(28.5%)
Klebsiella pneumoniae	0(0%)	0(0%)	1(25%)	0(0%)	0(0%)	(0%)	1(1.5%)
Klebsiella oxytoca	1(2.6%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(1.5%)
Enterobacter aerogenes	3(7.8%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	3(4.7%)
Proteus mirabilis	2(5.2%)	1(5.5%)	1(25%)	0(0%)	0(0%)	0(0%)	4(6.3%)
Citrobacter koseri	1(2.6%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(1.5%)
Acinetobacter baumannii	0(0%)	1(5.5%)	0(0%)	1(8.3%)	0(0%)	0(0%)	2(3.1%)
Organism (Gram-positive)							
Staphylococcus aureus	1(2.6%)	3(16.6%)	0(0%)	4(33.3%)	0(0%)	0(0%)	8(22.2%)
S. epidermidis	0(0%)	5(27.7%)	0(0%)	4(33.3%)	13(68.4%)	0(0%)	22(61.1%)
S. hominis	1(2.6%)	4(22.2%)	0(0%)	0(0%)	1(5.2%)	0(0%)	6(16.6%)

Table (3): Gram-negative and Gram-positive organisms isolated from different clinical specimens of cancer patients during (years 2013)

Organism (Gram-negative)	No. (%)						
	Urine (n=68)	Blood (n=22)	Sputum (n=8)	Pus (n=14)	Wound (n=31)	Stool (n=11)	Total (n=154)
Escherichia coli	41(60.2%)	4(18.1%)	1(12.5%)	2(14.2%)	4(12.9%)	5(45.4%)	57(54.2%)
Pseudomonas aeruginosa	15(22%)	3(13.6%)	1(12.5%)	1(7.1%)	6(19.3%)	5(45.4%)	31(29.5%)
Klebsiella pneumoniae	0(0%)	2(9%)	4(50%)	0(0%)	0(0%)	0(0%)	6(5.7%)
Klebsiella oxytoca	5(7.3%)	1(4.5%)	0(0%)	1(7.1%)	0(0%)	0(0%)	7(6.6%)
Enterobacter aerogenes	1(1.4%)	0(0%)	0(0%)	0(0%)	0(0%)	1(9%)	2(1.9%)
Proteus mirabilis	2(2.9%)	0(0%	0(0%)	0(0%)	0(0%)	0(0%)	2(1.9%)
Citrobacter koseri	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Acinetobacter baumannii	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Organism (Gram-positive)							
Staphylococcus aureus	1(1.4%)	3(13.6%)	0(0%)	5(35.7%)	1(3.2%)	0(0%)	10(20.4%)
S. epidermidis	2(2.9%)	7(31.8%)	2(25%)	5(35.7%)	18(58%)	0(0%)	34(69.3%)
S. hominis	1(1.4%)	2(9%)	0(0%)	0(0%)	2(6.4%)	0(0%)	5(10.2%)

Table (4): Gram-negative and Gram-positive organisms isolated during years 2012 and 2013

Organism	Year 2012 n= 99	Year 2013 n= 154
Gram-negative organisms	63(63.6%)	105(68.1%)
Escherichia coli	33(52.3%)	57(54.2%)
Pseudomonas aeruginosa	18(28.5%)	31(29.5%)
Klebsiella pneumoniae	1(1.5%)	6(5.7%)
Klebsiella oxytoca	1(1.5%)	7(6.6%)
Enterobacter aerogenes	3(4.7%)	2(1.9%)
Proteus mirabilis	4(6.3%)	2(1.9%)
Citrobacter koseri	1(1.5%)	0(0%)
Acinetobacter baumannii	2(3.1%)	0(0%)
Gram-positive organisms	**36(36.3%)**	**49(31.8%)**
Staphylococcus aureus	8(22.2%)	10(20.4%)
S. epidermidis	22(61.1%)	34(69.3%)
S. hominis	6(16.6%)	5(10.2%)

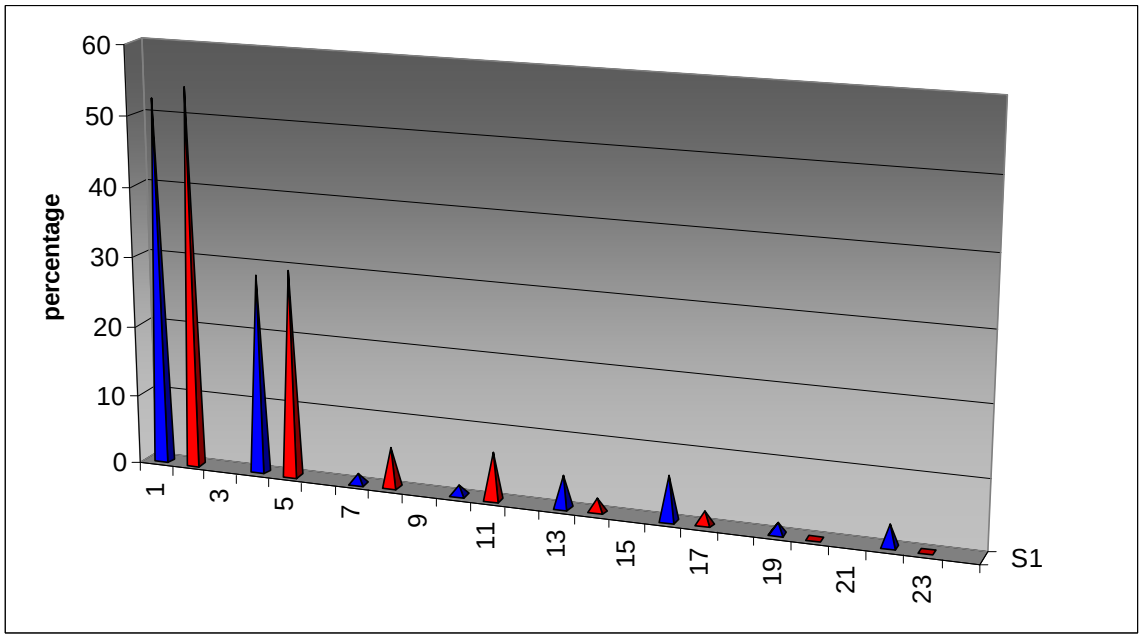

Figure (1): The percentages of Gram-negative and Gram-positive bacteria isolated during years 2012 and 2013

: year 2012,

:years 2013

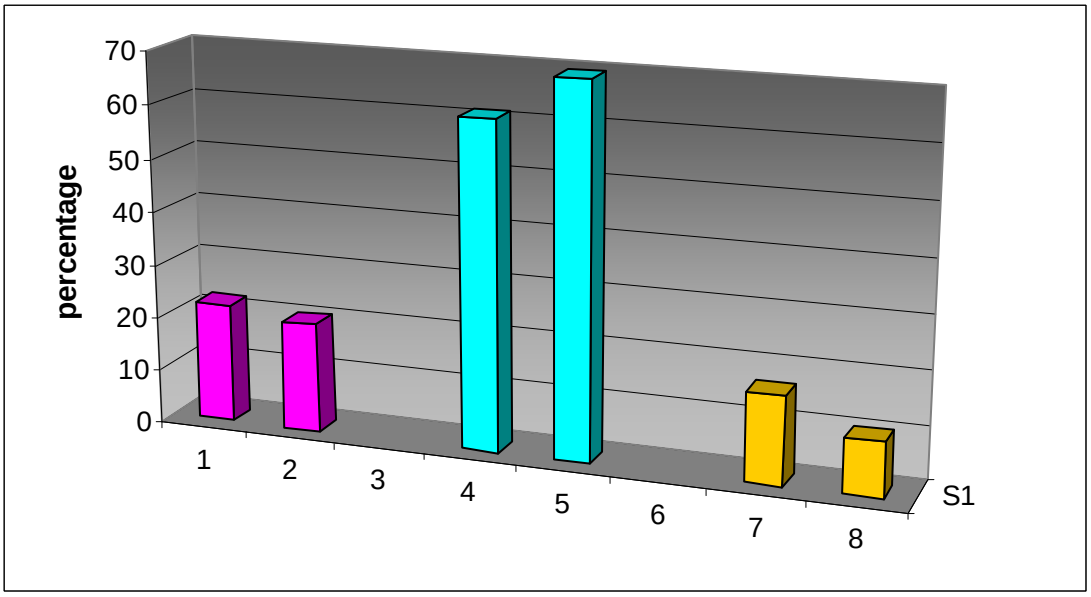

Table (5): The percentages to resistance patterns of antibiotics against common isolates pathogens during years 2012 and 2013

Antibiotic	No. Of Resistance %							
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Proteus mirabilis*	*Staphylococcus aureus*	*S. epidermidis*	*S. hominis*
Aztreonam	10 13.7	5 8.7	35 42	32 37	10 16.4	8.3 7.5	10 13.6	8 12
Erythromycin	50 53.3	30.5 25	25.4 20.8	15 10.5	13.9 11.2	80.5 89.2	50 60.8	44.4 40
Methicillin	80 80.4	77 78	76.5 78.5	80 82.2	80.4 80	40.5 36	40 33.3	46 48
Vancomycin	70 70.6	80 83	70.5 74	73 78	83 80	30 33.3	38 35	40 46
Ciprofloxacin	60 65.5	55 58	50 58.5	30.5 37.6	40.6 40	40.6 40.5	40 37.7	35 32
Amikacin	30 32	30 33.7	26.6 26	28.5 30	27 25	40 42	46 43.2	43.2 40
Impienem	30 25	37 40	20 22.2	18.5 16	20 23.3	36 40	27 27	30 31.6
Cephalothin	20 22.4	30 26	32 33.8	26 25	33 30.5	28 29	24 20	27 27
Cefoxitin	30 33	36 40	33 37.1	40 40	40.5 36.6	50 52	60.5 63.8	55 50
Gentamicin	37 40.6	32 32	33 40	40.8 50	32.6 39	62 62	64 67.7	56 58.2

Note: the upper number in 2012 while the lower number in 2013

DISCUSSION

To our knowledge, this is the first evaluation of the antimicrobial susceptibility profiles of Gram- negative and Gram- positive bacteria isolated from cancer patients in Basrah.

Infection in one of the important causes of high morbidity and mortality encountered during the treatment of pediatric cancer patients [13]. As newly developed more potent chemotherapeutic agents are widely used and the use of broad- spectrum antibiotics are increased, the trends of infections in immunocompromised pediatric cancer patients are changing [14]. In the 1970´s Gram- negative bacteria were thought to be the major pathogens of bacteremia in pediatric cancer patients. But during 1980´s , Gram- positive bacteria were recognized to be the major pathogens of bacteremia in pediatric cancer patients [15].

This study demonstrates that Gram- negative bacteria are still the predominant pathogens causing bacteremia in febrile neutropenia patients. Our study is similar to what has been reported in other studies. *Escherichia coli* was the most frequently isolated Gram- negative bacteria and *Staphylococcus epidermidis* was the most commonly isolated Gram- positive bacteria [16,17].

Identification and determination of antimicrobial susceptibility of bacterial pathogens can aid the clinician in selecting the appropriate antimicrobial agents to treat his patients . Infections due to Gram- negative bacteria with high resistance rates to β-lactam and non-β-lactam drugs are common in cancer patients [18,19]. Similarly, our Gram- negative bacteria isolates showed high resistance rates to different classes of antimicrobials, particulary among *E. coli* and *Pseudomonas aeruginosa* isolates with multidrug resistant . However, high susceptibility (< 4% resistance) rates were observed among the examined Gram- negative bacteria isolates to Aztreonam and Cephalothin [19]. In Iran , reported multidrug resistance in 37% and 33% of *E. coli* and *Klebsiella* spp. from cancer patients.

CONCLUSION

The present study shows the spectrum and antibiotic susceptibility patterens of pathogens in children febrile neutropenic patients at our cities . Continous surveillance of the spectrum of locally prevalent pathogens and their susceptibility patterns is essential for formulation of therapeutic regions for chemotherapy induced febrile neutropenic patients .

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