Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute

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Abstract: Blood stream infection (BSI) remains the major cause of morbidity and death in patients undergoing treatment for cancer. Approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation. Staphylococci are the most frequently isolated organisms from blood cultures of febrile neutropenic (FN) cancer patients.

Aims: This study aimed to define the main causative organisms of 139 episodes of bacteremia in 100 febrile neutropenic pediatric patients admitted to South Egypt Cancer Institute (SECI), pediatric oncology ward. Also to study the prevalence of biofilm forming capability of the coagulase-negative staphylococci (CONS) and *Staphylococcus aureus* (*S. aureus*) blood isolates (n=36, group A) and in 29 staphylococcal strains isolated from skin and nasal mucosa of healthy care workers (group B).

Methods: All Isolates were identified and tested for antibiotic susceptibility by MicroScan WalkAway System. The CONS and *S. aureus* isolates from blood cultures of pediatric patients were then tested for slime production using qualitative congo red agar plate test (CRA test), quantitative microtitre plate assay (MTP). The presence of *icaA* and *icaD* genes by polymerase chain reaction (PCR) was also determined.

Results: Among 139 episodes of fever and neutropenia recorded in 100 patients, bacteremia represented 54.7% in which Gram negative organisms constituted 52.6 % from the total episodes obtained and Gram positive staphylococcal isolates were 47.4%. *S. aureus* were 14 strains and CONS were 22 strains. Of the 14 *S. aureus*, 10 strains were *icaA* and *icaD* positive versus 8 strains were CRA test positive and also were MTP positive. Two strains of *S. aureus* were PCR positive for *ica* genes and slime negative on CRA and MTP. Of the 22 CONS, 12 (53%) were *ica* genes positive versus 11 strains (46%) were positive using CRA test and 9 strains were MTP positive. One strain of CONS was positive using MTP and PCR negative. Group B isolates were CRA, MTP and *ica* genes negative. Biofilm forming staphylococcal strains on CRA (15/19) and (16/22) with *ica* genes were resistant to imipenem, amoxicillin/clavulanic, cephlosporins, and oxacillin.

Conclusions: The present study shows a high percent of Gram negative bacteremia in pediatric oncology ward and the isolates expressing *ica* genes were exhibiting more resistance to broad spectrum antibiotics. This supports that biofilm adds to the virulence profile of staphylococci isolated from blood stream infections and that the *ica* genes are important virulence markers for clinically significant CONS isolates. The better agreement between the CRA plate tests with the molecular detection of *ica* genes indicates the former as a reliable test for the phenotypic characterization of virulence of clinical isolates.

[Salwa S. Seif El-Din, Moustafa S. El-Rehewy, Mohammed M. Ghazaly, Mohammed H. Abd-Elhamid. **Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute**. Journal of American Science 2011;7(1):674-686]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Biofilm, bacteraemia, coagulase negative staphylocci, cancer.

1. Introduction:

Blood stream infections (BSI) remains the main cause of morbidity and death in patients undergoing treatment for cancer. Approximately 10%-30% of all febrile neutropenic cancer patients are bacteremic at presentation. Pediatric cancer patients are more susceptible to infections especially in patients with haematological malignancy (Lee *et al*., 1990; Rahiala *et al*., 1998). Cancer patients are predisposed to BSI due to changes in both cell mediated and humoral immunity that is related to primary tumour and subsequent treatment. Therefore, pediatric cancer patients need to be treated early and promptly with proper antibiotics until the blood culture results are available. Cancer patients are more susceptible to infection associated with health care because of their compromised immune system, use of invasive technologies, surgical operations and chemotherapy. Institutions that provide care for cancer patients are expected to have higher rates of nosocomial infections than general care hospitals (Kelly *et al.*, 2010).

There is a shift of microbial spectrum of cancer patients from Gram negative to Gram positive species. Factors that contribute to this shift may be intensive chemotherapy that leads to damage of mucosal barriers, that increases the risk of infection with oral and gastrointestinal flora. In addition the use of implantable intravenous catheters can facilitate entry of organisms colonizing the skin into the blood stream and thus increase the rate of staphylococcal infections (Viscoli *et al*., 1999).

The production of capsular polysaccharides by coagulase negative staphylococci is considered as a virulence factor. Staphylococcal biofilm is mediated by the polysaccharide adhesion (PIA), and referred to product of the *icaABCD* gene cluster that encodes for the N-acetyl glucosaminyl transferase enzyme. N-acetyl glucosaminyl transferase enzyme catalyzes the synthesis of the capsular polysaccharide (b-1,6-glucosamineglycan) from N-acetyl glucosamine. Ziebuhr *et al*. (1997, 1999) detected the *ica* locus in 85% of coagulase-negative staphylococci causing invasive infections, but only in 6% of contaminating strains, and proposed targeting the *ica*-locus as a diagnostic marker for pathogenicity in staphylococci.

The capacity of both *S. epidermidis* and *S. aureus* to form biofilm is an important virulence factor in the development of device related infections. This represents a serious clinical problem, given that the majority of hospitalized patients undergo procedures for the insertion of foreign devices, from catheters to artificial heart valves, etc. Moreover, patients susceptible to device-related infections are often colonized with hospital-acquired, multiple antibiotic- resistant organisms and may be further compromised by serious underlying disease or trauma. Significantly, the majority of biofilm-mediated device-related infections are caused by either *S. epidermidis* or *S. aureus* (O'Gara & Humphreys, 2001).

The aims of this study were to monitor the prevalent aerobic microorganisms causing bacteremia in the pediatric oncology department at the South Egypt Cancer Institute (SECI). Also to determine the antimicrobial susceptibility of bacterial isolates and the isolated staphylococci were examined for biofilm production by using qualitative congo red agar plate test (CRA test) and quantitative microtitre plate assay (MTP). The presence of *icaA* and *icaD* genes was determined by polymerase chain reaction. Also to examine biofilm formation by skin and nasal staphylococcal isolates from healthy care workers at the pediatric oncology ward and to assess the relationship between biofilm production and pathogenicity of staphylococci.

2. Patients and Methods

This prospective study was carried out in Pediatrics Oncology Department, at SECI and the Medical Microbiology & Immunology Department, Faculty of Medicine Assuit University during the period from January 2008 to December 2009.

Group (A) included 100 pediatric cancer patients in South Egypt Cancer Institute. Out of 139 fever episodes; number of episodes in males was 86 and in females was 53 and their age ranged between 1-12 years with mean of 6.73± 2.028 years. Full medical history and complete physical examination were performed in search for any septic focus. The study was approved by the Institutional Ethical Committee and patients' consents were obtained from the parents before collection of specimens. The data collected included age, diagnosis, type of chemotherapy, surgery, absolute neutropenic count (ANC) (patients having ANC raised above 500 X 10^9/L were not excluded from the study), grade of fever, ICU admission and type of empirical antibiotic therapy. Exclusion criteria were fever due to chemotherapy and patients under empirical antibiotic therapy. Blood cultures were obtained from group A.
Group (B) included 25 healthy volunteers from healthcare members at the Pediatrics Oncology Department, at SECI as a control group for studying biofilm formation in staphylococci skin and nasal isolates taken from group B.

Two to five milliliters of peripheral blood were aseptically collected from neonates and children up to 10 years of age and 10 ml from children more than 10 years and inoculated into blood culture bottles. Blood cultures (Oxoid) were transported immediately to the Microbiology Laboratory. Blood agar, nutrient agar, mannitol salt agar and MacCkoncy's agar were used for isolation of the organisms. Staphylococcal isolates were preserved in tryptic soya broth with glycerol (15% v/v) at -80°C. Identification of the organisms and antibiotic susceptibility testing was carried via MicroScan WalkAway system 96 (Dade Behring Inc., MicroScan Inc., West Sacramento, CA95691, USA) which is a conventional overnight incubation system that uses the reference broth microdilution method.

Phenotypic characterization of biofilm formation: Congo Red Test:

Congo red test was performed as previously described by Freeman *et al*. (1989) in triplicate and results were interpreted by two different investigators. The medium composed of brain heart infusion broth (37 gm/l), sucrose (5 gm/l), agar (10 gm/l) and congo red dye (0.8 gm/l). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from

other medium constituents and was then added when the agar had cooled to 55°C. Plates were inoculated with test organism and incubated at 37°C for 24-48 hours aerobically. On CRA, biofilm-producing strains form black colonies with a dry crystalline consistency, while biofilm non producing strains form pink colonies (Arciola *et. al.*, 2001).

Microtitre Plate Test:

Quantitative determination of biofilm production was carried out as described by Aricola *et al*. (2002). Briefly, overnight grown bacteria in trypticase soya broth (TSB) were diluted (1:100) and 200 ml portions were inoculated into sterile 96-well flat bottom polystyrene microtiter plates. Incubation was carried out at 35°C for 22-24 h before removal of the cultures. The wells were washed 3 times with phosphate buffered saline (PBS, pH, 7.2), air dried and stained with 0.25% crystal violet for 1 min. The optical density of the wells was measured at 570 nm using micro ELISA auto reader (Stat Fax – 2100, AWARENESS Technology Inc.). An optical density of 0.240 was chosen to distinguish biofilm producers from those that did not form biofilm. Biofilm positive and negative strains of *S. epidermidis* were included in each plate as was a negative control of medium without bacteria. The tests were carried out in quadruplicate and all strains were tested on at least two different days. Strains that had given reading values of more than 0.240 were considered strong biofilm forming, Strains that had given readings values more than 0.120 and less than 0.240 were considered weak biofilm forming strains. While strains that had given reading values of less than 0.120 were considered non-biofilm forming strains (Arciola *et al.*, 2002).

PCR for the detection of ica*A* and ica*D* genes:

Bacterial DNA was extracted from a staphylococcal pure colonies grown on blood agar and suspended in nutrient broth using QIAamp Mini DNA extraction kit (QIAGEN Incorporation) according to the manufacturer's instructions. PCR for *icaA* and *icaD* genes was performed using the method described by Arciola *et al*. (2001 a). For the detection of *icaA* gene 5-TCTCTTGCAGGAGCAATCAA-3 was used as the forward primer (corresponding to nucleotides 4796–4815) and 5-TCAGGCACTAACATCCAGCA-3 was used as the reverse primer (corresponding to nucleotides 4964-4983). For *icaD*, 5-ATGGTCAAGCCCAGACAGAG-3 was used as the forward primer (corresponding to nucleotides 5422–5441), and 5-CGTGTTTTCAACATTTAATGCAA-3 was used as the reverse primer (corresponding to nucleotides 5616–5597). Reaction mixtures (50 µl) contained 25 µl PCR master mixture, 1 µl of each primer (0.1-0.5 µM final concentration), 18 µl RNA ase free water & 5 µl of template DNA. Amplifications were performed with the following thermal cycling profile an initial denaturation at 94°C for 2 min., followed by 30 cycles of amplification (denaturation at 94°C for 1 min., primer annealing at 60°C for 1 min., and extension at 72°C for 2 min.) and a final extension for 4 minutes. Amplicons for *icaA* and *icaD* produced fragments of 188 and 198 bp, respectively. The amplified product sizes were estimated by comparison with 100 bp DNA ladder (QIAGEN Incorporation).

Statistical Analysis:

Statistical analyses were conducted using the SPSS package version 17. Mean values and standard deviation were used for data description. Differences in the distribution of individual parameters among patient subsets were analyzed using the T test for categorized variables and Pearson chi-square was used to measure the concordance between *ica* genes and CRA positivity. *P*-value was two-tailed and was considered significant at a level of ≤0.05.

3. Results

During a two years period extending from Jan 2008 to Dec. 2009, Group A: were 100 pediatric cancer patients who suffered from 139 febrile episodes at the SECI. They were 86 males and 53 females with a mean age ± 6.73 ± 2.03 years. Group B: were 25 healthy care volunteers as a control. Table (1) summarizes the characteristics of the pediatric cancer patients involved in this study regarding tumor type, presence or absence of neutropnia, ICU admission or surgical intervention. All patients were using intravenous catheter and were treated empirically by third generation cephalosporins and amikacin.

Bacteriology:

In group A: out of the 139 febrile episodes, 63 blood cultures were negative (45.3%) and 76 blood cultures were positive (54.7%). Gram negative bacteremia represented 52.6% with 40 isolates in which 11 were (14.5%) *Klebsiella*, 8 (10.5%) *Proteus*, 7 (9.2%) *E- Coli*, 3 (3.9%) *Enterobacter*, 4 (5.3%) *Yersenia pseudotuberculosis*, 4 (5.3%) *Shigella* and 2 (2.6%) *Salmonella.*

Gram positive bacteraemia represented 47.4% with 36 Staphylococcal isolates. *S. aureus* were 14 strain (18.5%) and coagulase negative staphylocooci (CONS) were 22 (28.9%) isolates. CONS were distributed as 9 (11.9%) *S. epidermidis*,

5 (6.6%) *S. hominis*, 3 (3.9%) *S. simulans*, 2 (2.6%) *S. haemolyticus*, 2 (2.6%) *S. warneri* and 1 (1.3%) *S. xylosus*.

Group B: 29 Staphylococcal isolates were collected in which 12 were nasal isolates and 18 were skin isolates. They were 8 *S. aureus* and 21 CONS. Table (2) shows number, percent and species identification of isolates collected from blood cultures from febrile pediatric cancer patients using MicroScan WalkAway System.

Antimicrobial susceptibility patterns for *Staphylococci aureus* isolates exhibited 92.8% susceptibility to ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, gatifloxacin, 85.7% to azithromycin, gentamycin, tetracycline and trimethoprim. However high resistance pattern 71.4% was recorded in amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, cefotriaxone, cephalothin, imipenem and oxacillin. Vancomycin resistance was detected in 42.8% (6/14) of the isolates. Similarly coagulase negative staphylococci exhibited 54.5% resistance to amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, cefotriaxone, cephalothin, imipenem and oxacillin. CONS were still 90.9% susceptible to vancomycin, synercid, rifampin, gatifloxacin, motifloxacin and 86.4% to levofloxacin, and clindamycin.

Among gram negative bacilli, 9/11 *Klebssiella* species were resistant to cefotazidime, ticracillin and to azotrenem, 8/11 were resistant to cefoperazone, cefotizoxime and piperacillin. Highest resistance pattern was contributed with azotrenem (78%) followed by cefotaxime (73%). The least resistance pattern for 40 Gram negative isolates was recorded with amikacin (5/40, 12.5%) and imipenem (7/40, 17.5%).

Biofilm formation:

Biofilm production assessed by CRA reveled that 8/14 (57%) strains of *S. aureus* were biofilm positive and 11/22 strains (50%) of CONS were biofilm forming. All strains of the control group were non-biofilm forming. Quantitative biofilm production determined by microtiter plates assay (MTP) showed that 8 (57%) strains of *S. aureus* were biofilm producers, 7 (50%) strains were strong biofilm producers with readings > 0.240, one (7%) strain was weak biofilm producer, with readings > 0.120 and <0.240. and 6 (43%) strains were non-biofilm producers with readings >0.120. In CONS, 9 (41%) strains were biofilm forming (6 (27%) strains, were strong biofilm producers, and 3 (14%) was weak biofilm producers) and 13 (59%) strains were non-biofilm producers (Fig. 1).

The results of these two phenotypic tests showed that two strains of CONS that had been proved to be biofilm forming with the CRA test appeared to be non-biofilm forming with the spectrophotometer detection. All strains of the control group were non-biofilm forming with OD ≤ 0.120.

Out of 22 CONS strains, 12 strains (53%) show the presence *icaA* gene at 188-bp and 10 strains (46%) were negative for *icaA* gene, while out of 14 *S. aureus* strains, 10 strains (70%) show the presence *icaA* gene and 4 strains (30%) were negative for *icaA* gene (Fig. 2).

The results of *icaA* gene at 188-bp were the same when testing the *icaD* gene amplification product at 198- bp. Staphylococcal strains of group B were negative for both *icaA and icaD* genes.

Table (3) presents comparison between the results from the genotypic testing of biofilm presenting genes *icaA & icaD* via PCR versus those results revealed via phenotypic biofilm CRA and MTP assay. There was 10 strains of *S. aureus icaA* & *icaD* positive versus 8 strains CRA positive & 8 strains MTP positive. Similarly, 12 strains of CONS were *icaA* & *icaD* positive versus 11 strains CRA positive & 9 strains MTP positive. On the other hand there was one strain of *S. epidermidis* positive biofilm on MTP with negative *icaA* and *icaD* gene on PCR. Considering biofilm slime on CRA as gold standard; sensitivity of MTP was 89.5% and specificity was 100%. Taking biofilm slime on CRA as gold standard; sensitivity of PCR was 94.7% and specificity was 76.5%.

Figure (3) demonstrates the relation between biofilm formation and antimicrobial susceptibility pattern showed a higher percent of resistance in biofilm positive isolates i.e.; 15 strains out of 19 biofilm positive strains were resistant to imipenem on the other hand only 7 out of 17 biofilm negative strains were resistant to imipenem. Table (4) shows the clinical characteristics of *ica* gene positive cases compared to those who were negative. No significant differences were encountered between type of tumour, neutropenia or ICU admission among *ica* positive and negative cases.

Table 1:Characteristics of the 139 febrile episodes in 100 pediatric cancer patients.

Characteristics	(N)	(%)
Sex		
Male	86	61.9
Female	53	38.1
Tumour type (Type of chemotherapy)		
ALL (MTX high dose)	51	36.7
AML (ADR + AraC)	36	25.9
NHL (COPADAM)	20	14.4
Wilms tumor (SIOP)	8	5.8
Bone tumors (Platinum based)	3	2.2
Rhabdomyosarcoma (VAC)	5	3.6
Germ cell tumors (PEP)	3	2.2
Neuroblastoma (OPEC-OJEC)	13	9.4
Surgery		
Yes	36	25.9
No	103	74.1
Neutropenia		
Yes	53	38.1
No	29	20.9
Profound	57	41
IV Catheter		
Yes	139	100
Grade of Fever		
Low	48	34.5
High	91	65.5
Empirical Therapy		
3rd Generation + Amikacin	139	100
ICU Admission		
Yes	87	62.6
No	52	37.4

Table 2: Isolated organisms of bacteremia in pediatric cancer patients.

Organisms	(N)	(%)
Gram negative	**40**	**52.6%**
Klebsiella pneumonia	10	13.2
Klebsiella ornithinolytica	1	1.3
Proteus penneri	8	10.5
E. coli	7	9.2
Enterobacter cloaca	3	3.9
Enterobacter aerogenes	1	1.3
Yersinia pseudotuberculosis	4	5.3
Shigella dysenteria	4	5.3
Salmonella Arizona	2	2.6
Gram positive	**36**	**47.4%**
Staphylococcus aureus	14	18.5
Staphylococcus epidermidis	9	11.9
Staphylococcus hominis	5	6.6
Staphylococcus simulans	3	3.9
Staphylococcus haemolyticus	2	2.6
Staphylococcus warneri	2	2.6
Staphylococcus xylosus	1	1.3
Total	**76**	**100%**

Table 3: Biofilm formation in blood stream *Staphylococcal* strain when tested on CRA, MTP and PCR.

		S. aureus	*S. epidermidis*	*S. hominis*	*S. simulans*	*S. haemolyticus*	*S. warneri*	*S. xylosus*	Total
CRA	**Positive**	8	5	4	1	0	1	0	**19**
	Negative	6	4	1	2	2	1	1	17
MTP	**Strong**	7	3	2	1	0	0	0	**13**
	Negative	6	4	2	2	2	2	1	19
	Weak	1	2	1	0	0	0	0	**4**
icaA gene	**Positive**	10	4	4	1	1	1	1	**22**
	Negative	4	5	1	2	1	1	0	14
icaD gene	**Positive**	10	4	4	1	1	1	1	**22**
	Negative	4	5	1	2	1	1	0	14

CRA, congo red agar, MTP, microtitre plate, PCR, polymerase chain reaction

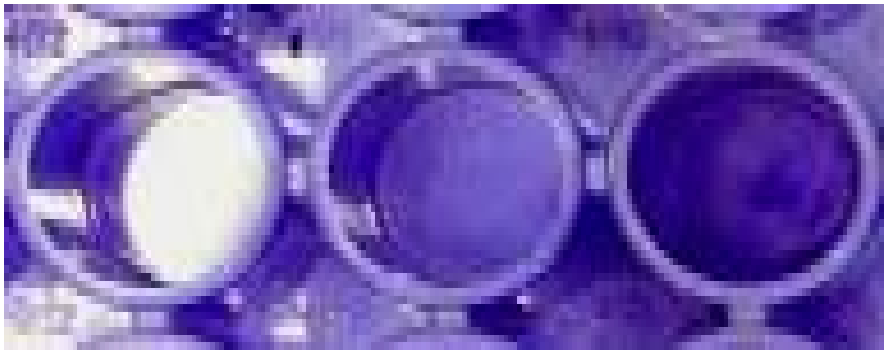
Table 4: The relation between biofilm formation and antimicrobial resistance pattern.

	Biofilm CRA		Biofilm MTP			*ica* genes	
	Positive (19)	Negative (17)	Strong (12)	Week (4)	Negative (20)	Positive (22)	Negative (14)
Amox/K Clav	15	7	11	1	10	16	6
Amp/ Sulbactam	15	7	11	1	10	16	6
Azithromycin	8	2	7	1	2	8	2
Cefazolin	15	7	11	1	10	16	6
Cefepime	15	7	11	1	10	16	6
Cefotaxime	15	7	11	1	10	16	6
Ceftriaxone	15	7	11	1	10	16	6
Cephalothin	15	7	11	1	10	16	6
Chloramphenicol	4	1	3	1	1	4	1
Ciprofloxacin	4	1	4	0	1	4	1
Clindamycin	8	4	6	0	6	9	3
Erythromycin	8	2	7	1	2	8	2
Gatifloxacin	2	1	2	0	1	2	1
Gentamicin	8	3	6	1	4	7	4
Imipenem	15	7	11	1	10	16	6
Levofloxacin	3	1	3	0	1	3	1
Moxifloxacin	2	1	2	0	1	2	1
Ofloxacin	4	1	4	0	1	4	1
Oxacillin	15	7	11	1	10	16	6
Rifampin	4	4	3	0	5	6	2
Synercid	4	4	3	0	5	6	2
Tetracycline	6	3	5	1	3	7	2
Trimeth/Sulfa	7	3	6	1	3	8	2
Vancomycin	4	4	3	0	5	6	2

CRA, congo red agar, MTP, microtitre plate

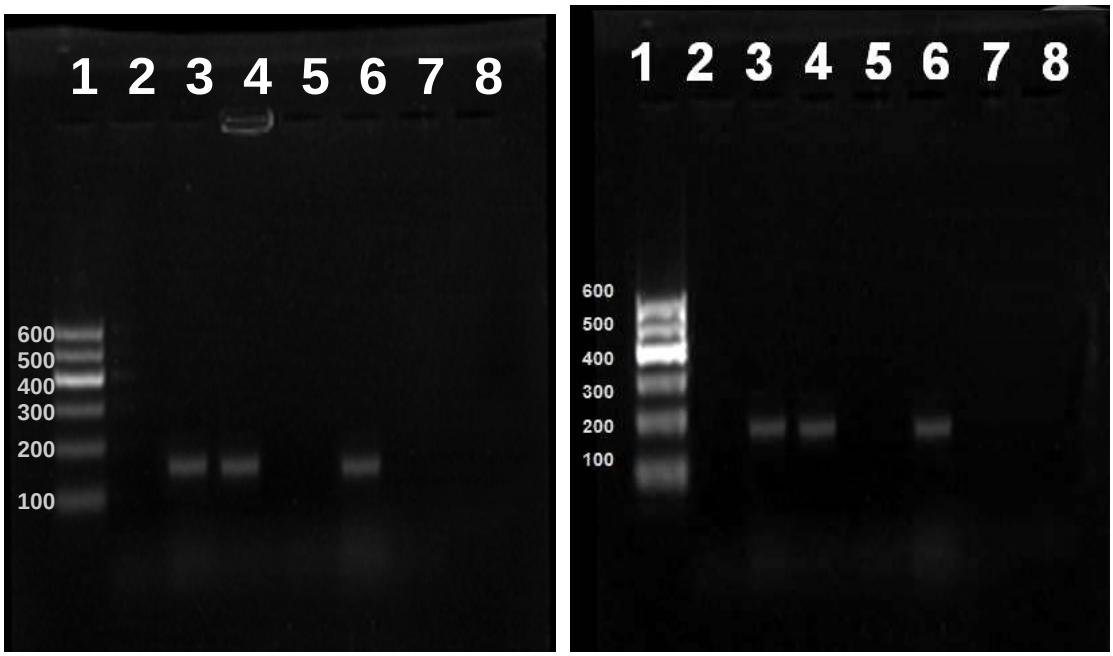
Table 5: Clinical characteristics and *ica* gene.

		ica genes		*P*-value
		Positive N (%)	Negative N (%)	
Sex	Male	11 (47.8)	9 (69.2)	0.21
	Female	12 (52.2)	4 (30.8)	
Tumor type	ALL (MTX high dose)	4 (17.4)	7 (53.8)	0.18
	AML (ADR + AraC)	8 (34.8)	1 (7.7)	
	NHL (COPADAM)	5 (21.7)	3 (23.1)	
	Wilms tumor (SIOP)	3 (13.)	1 (7.7)	
	Rhabdomyosarcoma (VAC)	2 (8.7)	0 (0)	
	Neuroblastoma (OPEC-OJEC)	1 (4.3)	1 (7.7)	
Surgery	Yes	10 (43.5)	4 (30.8)	0.45
	No	13 (56.5)	9 (69.2)	
Neutropenia	Yes	11 (47.8)	3 (23.1)	0.21
	No	6 (26.1)	3 (23.1)	
	Profound	6 (26.1)	7 (53.8)	
Grade of Fever	Low	9 (39.1)	7 (53.8)	0.39
	High	14 (60.9)	6 (46.2)	
ICU Admission	Yes	11 (47.8)	9 (69.2)	0.21
	No	12 (52.2)	4 (30.8)	



A Negative OD <0.120 | **B** Weak positive OD > 0.120 and < 0.240 | **C** Strong positive OD > 0.240

Figure 1: Quantitative detection of biofilm production by MTP – high, moderate and non slime producers differentiated by crystal violet staining in 96 well microtiter plates.



(A) **(B)**

Figure 2: PCR detection of *icaA* (A) and *icaD* (B) genes. Lane 1, molecular size marker (100 bp Ladder); lanes 3 ,4 and 6, *icaA* at 188 bp and *icaD* at 198 bp; lane 8, negative control (DNA template absent).

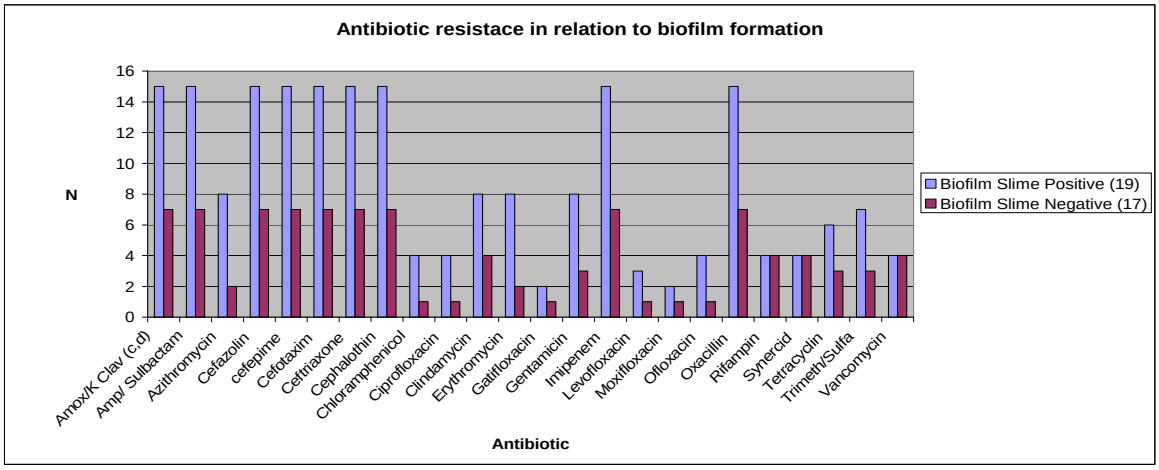


Figure 3: Antibiotic resistance pattern among biofilm producers on CRA in comparison with non biofilm producers. Biofilm forming strains are much more resistant to antibiotics (almost double the resistance pattern presented by non-biofilm forming Staphylococcal strains).

4. Discussion:

Blood stream infection (BSI) remains the major cause of morbidity and death in patients undergoing treatment for cancer; approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation (Pizzo *et al.*, 1986 and Hsin *et al.*, 2003) and with the increased use of indwelling venous access devices, catheter-associated bacteremic episodes have become more frequent (Raad and Bodey, 1992). Staphylococci are recognized as the most frequent causes of biofilm-associated infections (Vuong and Otto 2002). In this study bacteremia represented 54.7%, this percent is higher than that stated in literature which range between 10% to 30% (Alexander *et al.*, 2005). According to the global reports, the prevalence of bacteremia in patients with cancer ranged between 5.7-44%. (Kim *et al.*, 2005 and Hosseini *et al.*, 2006). El-Mahallawy *et al.* (2006) performed a prospective cohort study on pediarric cancer patients at National Cancer Institute (NCI) of Cairo that revealed a 46% positive blood culture for bacteremia. The higher

figure in this study may be due to strict inclusion criteria of the patients where we excluded patients who have received empirical antibiotics therapy.

Bacterial strains isolated from blood cultures from febrile pediatric cancer patients had the following distribution: 53 % were Gram negative organisms, 47% were Gram positive; this shows a relatively high percent of Gram negative organisms. Other studies reported that Gram positive organisms accounts for 60 to 70% of bacteremias (Locus *et al.*, 1996; Hughes *et al.*, 1997; Marie *et al.*, 1998 and Hsin *et al.*, 2003). This high percent of Gram positive baceraemia presented by these workers is referred to the factors that possibly contribute to the shift in Gram-positive isolates as increased use of indwelling central venous catheters, fluoroquinolone prophylaxis, and high-dose chemotherapy inducing oral mucositis (*Paganini et al.*, 2003 and Walsh *et al.*, 2006).

In this study the relatively high percent of Gram negative bacteremia; may be explained by the use of more intensive regimens of chemotherapy and the nature of the chemotherapy used has also been reported to influence the bacterial etiology of febrile neutropenia; the use of more specific agents with less cytotoxic potential and, therefore, less mucosal toxicity can lead to a reduction in infections due to Gram-negative organisms. The use of quinolones prophylactic antibiotics in adult cancer patients in Barcelona has shown a sudden resurgence of *E-coli* bacteremia in febrile neutropenic patients. One overlooked factor may even be the regional climactic or environmental conditions that may affect the etiology (Zinner 1999 ; Ramphal 2004).

El Mehallawy and coworkers (2006) explained the high percent of Gram negative organisms forming BSIs in pediatric neutropenic patients at the NCI of Cairo is more likely to be derived from endogenous sources such as gastrointestinal tract and since the high frequency of diarrhea; also the high rate of Gram negative organism due to the nosocomial infection pattern in the institute.

In this Study the Gram negative organisms were distributed as follows: 14% *Klebsiella*, 11% *Proteus*, 9% *E- coli*, 5 % *Enterobacter*, 5% *Yersenia pseudotuberculosis*, 5% *Shigella*, and 3% *Salmonella.* El-Mahallawy and co-workers (2006) stated a similar pattern of microorganisms causing bacteremia in pediatric oncology at NCI of Cairo with predominant *Klebsiella* Species on top of Gram negative isolates. Similar pattern was presented by Ashour and El-Sharif (2009) in a study performed on cancer patients in NCI, Cairo showing the main isolated Gram-negative bacteria from all clinical specimens were *Klebsiella spp.* (31.2%) followed by *Escherichia coli* (22.2%). Also isolation of other less-frequent Gram-negative bacteria had been reported showing the low prevalence of *Salmonella*, *Shigella*, and *Yersenia* species.

El-Mahalawy *et al.* (2006) stated that it's important to recognize the importance of bacteremia due to organisms such as *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp., as they causes higher mortality rate rather than bacteremias due to Gram positive organisms. They reported that 47% of deaths were associated with GNB in contrast to 7% of Gram positive bacteremia and that *Klebsiella* was the more dangerous group with higher mortalities in 88% of hematological malignancies cases.

In the current study the Gram positive cocci were distributed as follows: 18% were *Staphylococcus aureus*, 12 % *S. epidermidis*, 7% *S. hominis*, 4% *S. simulans*, 3% *S. haemolyticus*, 3% *S. warneri*, 1% *S. xylosus*. This pattern was consistent with studies of Ramphal (2004); Kim *et al.* (2005) and Eslaminezhad *et al.* (2010). CONS were the predominant etiological pathogens of bacteraemia. Similar pattern was reported by Ashour and El-Sharif (2007).

The resistance pattern of Gram negative bacteria showed the highest resistance pattern was found with Aztereonam (78%) followed by Cefuroxime (73%) while the highest efficient antibiotics for treatment of GNB were Amikacin with resistance pattern of (13%) followed by Imipenem with (18%). This high pattern of resistance in *Klebsiella* and *E coli* species was reported by Eslaminezhad *et al.* (2010) who stated that isolates of *E. coli and Klebsiella* were multidrug resistant. Similar high resistance pattern was presented by Berjan *et al.* (2001) who found *Enterobacter/Citrobacter/Serratia* group had a 40% - 50% resistance to ceftazidime and piperacillin and 50% of *E. coli* and *Klebsiella* species were resistant to piperacillin with very little quinolone resistance.

Paul *et al.* (2007) also reported similar resistance pattern of Gram-negative bacteria to broad-spectrum beta-lactams, commonly used for empiric treatment of febrile neutropenia, increased with the length of time in hospital prior to bacteremia acquisition. Resistance to ceftazidime increased from 8% when acquired before hospitalization to 48% when acquired after 14 days in hospital.

Generally multi-drug resistance (resistance to three or more antibiotics) was observed in 40% of *S. aureus* isolates than in CONS strains (33%). Antibiotic resistance was (61%) and was related to this group of antibiotics (amoxacillin/K clavulanic, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, ceftriaxone, cephalothin, imipenem, oxacillin). This was consistent with Kim *et al.* (2005)

who isolated staphylococci blood isolates in pediatrics cancer patients and reported 84% of the isolates were resistant to penicillin and 60% were resistant to oxacillin. The lowest resistance pattern was (8%) reported with gatifloxacin and moxifloxacin and this may be explained by the restricted use of quinolones in pediatrics. Vancomycin resistant staphylococci (VRSA) was recorded in 8 strains (22%) which were 6 strains on *S. aureus* and 2 of *S. epidermidis*. This is consistent with Ashour and El-Sherif (2007) who studied the microbial spectum amd antibiotic susceptibility profile of Gram positive aerobic bacteria from cancer patients at the NCI, in Cairo revealing that 15.5% of *S. aureus* and 11% of CONS resistant to vancomycin. They attributes that the misuse of antibiotics in Egypt might have contributed to this rapid evolution of VRSA strains.

Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices and causing nosocomial infection (Aricola *et al*., 2001). Suggested mechanisms by which biofilm producing bacteria cause disease are detachment of cells from medical device biofilm causing blood stream or urinary infection, endotoxin production, resistance to immune system and generation of resistance through plasmid exchange (Donlan and Costerton, 2002).

In the present study we have assayed isolated staphylococcal strains blood isolates for qualitative biofilm forming ability by congo red agar test (CRA). There was 57% strains of *S. aureus* and 50% of CONS biofilm forming. Similar results were reported by Arciola *et. al* (2001) who found that biofilm formation among staphylococci isolated from catheter associated infection was 61 % of *S. aureus* and 49% for *S. epidermidis* isolates, and El-Mahalawy *et al.* (2009) who assayed the staphylococcal blood isolates of febrile neutropenic pediatric cancer at the NCI, Cairo presented 60% of *S. aureus* and 24.4% of CONS were CRA positive. However De Silva *et al.* (2002) reported that only 25% of the tested CONS were biofilm positive by CRA. The discrepancy may be explained by differences in locality and environmental conditions.

In the current study we have performed quantitative detection of biofilm using MTP showed that 57% of *S. aureus* strains were biofilm forming while 41 % strains of CONS were biofilm producers. de Silva *et al*. (2002) reported that 42% of *S. epidermidis* isolated from bacteraemia in neonatal intensive care unit to be positive biofilm producer using MTP. The most important advantage of the microtiter plate assay in addition to the phenotypic biofilm production information presented by CRA is the ability of this method to differentiate between weak and strong biofilm producers. This reflects the severity of the condition and so may help in the determination of suitable line of management, and also at the research level it reflects the degree of gene regulation, as the difference of the degree of biofilm production is due to the difference of PIA production and this is due to changes that occur in the regulation of *ica* operon (Handke *et al.*, 2004).

The molecular mechanism and the genetic control of the PIA synthesis in *Staphylococcal Spp.* have been identified, *icaA, icaD, icaB, icaC*, also termed intracellular adhesion operon (Götz 2002 and Gerke *et al.*, 1998), *icaR* is an additional regulatory gene presented in the same operon. However it have been definitively proven that co-transcription of at least both the *icaA, icaD* genes is required for the N-acetyl-glucosaminyl-transferase activity leading to synthesis of oligomers of no more than 20 residues. (Götz, 2002). In this study 70% of *S. aureus* strains, and 53% CONS, were *icaA* gene and *icaD* gene positive. These results were consistent with Arciola *et al.* (2001) who have shown 61 % of *S. aureus and* 49% for *S epidermidis* were positive and El-Mahallawy *et al.* (2009) also demonstrated that 50% of *S. aureus* and 18% of CONS were simultaneously *icaA* and *icaD* genes positive.

Both the *icaA* and *icaD* genes were present in all biofilm producing strains; this indicates that the presence of both genes is essential for biofilm production and confirms that both genes are part of one operon, so either the entire operon is present or abcsent. This is supported by the results of a study done by Fluckiger *et al.* (2005) who stated that the *ica* locus and biofilm formation are crucial parameters for staphylococcal colonization and survival on implants. Arciola *et al*. (2001) and El-Mahallawy *et al.* (2009) revealed that all strains bearing the *icaA* gene, a component of the *ica* locus, also bear *icaD*.

None of the commensal staphylococcal strains of the control group in this study show the ability to produce biofilm phenotypically and genotypically. These results were similar to Aricola *et al*. (2001) and de Silva *et al.* (2002). This may be explained by the fact that biofilm production is a virulence factor and that the production of PIA is an important component in the process of biofilm formation and the presence of *ica* operon plays an important rule in disease pathogenesis. Biofilm formation in *Staphylococci* is multifunctional and this ability makes strains much better able to survive in hostile environments of tissues and blood. However Eftekhar and Mirmohamadi (2010) found that 8% of the skin isolates of *S. epidermidis* obtained from health volunteers were positive for biofilm production and *icaADBC*. They conclude that *S.*

epidermidis isolates from patients with symptomatic infections are not necessarily more virulent (pathogenic) than the skin contaminants and the capacity to form biofilms in *vivo* is influenced by environmental stimuli, expression levels of *icaADBC* or other regulatory factors independent of PIA synthesis.

One isolates of *S. epidermidis* was positive biofilm producer using MTP but with negative *ica* genes by PCR. Arciola *et al.* (2001) reported this phenomenon and studies performed by Fitzpatrick *et al.* (2005); Rohde *et al.* (2005, 2007); and Kogan *et al.* (2006) highlighted the existence of PIA/PNAG-independent biofilm mechanisms in both *S. aureus* and *S. epidermidis*. as *icaADBC*-independent biofilm mechanism. In the same time four strains (two *S. aureus* and two CONS) were positive *ica* Locus but negative in biofilm slime CRA test. Mack et al. (2000) pointed out that although *ica* genes are responsible for the synthesis of the polysaccharide component, full phenotypic expression of PIA and of biofilm functions could be conditioned by a few additional genes having a direct or indirect regulatory influence. *atlE*, *sarA*, *agrA* and *mecA* are all genes that have been hypothesized potentially to modulate or affect PIA functionality. The appearance of phase-variant bacteria with a complete set of *ica* genes but a slime-negative phenotype, even if relatively infrequent, has been evidenced in several studies during culture on CRA. Finally, it has to be considered that the control of slime production can involve genetic mechanisms capable to alter *ica*-expression acting at a gene level, as for instance, the insertion and precise excision of the naturally occurring insertion sequence IS256 (Ziebuhr *et al.*, 2000).

Comparison of biofilm formation by the two phenotypic methods CRA test and MTP and *ica* gene carriage showed that there were was better agreement between the presence of the *ica* operon and CRA (94.7%) compared to the results obtained for *ica* gene carriage in relation to MTP method (89.5%). These results agree with Aricola *et al.* (2005). El-Mahallawy *et al.* (2009) found a strong agreement between *ica* gene positivity and the ability to produce slime by CRA test ($P < 0.001$). The CRA test is easier to perform with lesser cost.

In the present study, isolates expressing *ica* genes were exhibiting more resistant to broad spectrum antibiotics. This supports that biofilm adds to the virulence profile of *Staphylococcal* strains isolated from blood stream infection. The results were consistent with (De silva *et al.*, 2002, Fux *et al.*, 2004, El-Mahallawy *et al.*, 2009 and Bose et al., 2009). The latter group studied biofilm formation and antibiotic susceptibility on *staphylococci* isolated from different clinical materials and found that there was a significant and clinically relevant higher resistance to conventional antibiotics in biofilm producers than non-biofilm forming. In this study we have found 8 strains (6 strains on *S. aureus* and 2 of *S. epidermidis*) were resistant to vancomycin and this result was supported by the result reported by Bose et al. (2009) where they found that the two strains of *Staphylococci aureus* were vancomycin intermediate resistant *S. aureus* (VISA) and also were biofilm producers. Souli *et al.* (1998) explained that this resistance to vancomycin by biofilm producers may be due to entrapment vancomycin in the extra cellular mucopolysaccharides because of their high molecular weight.

Gilbert *et al*. (2002), reported that biofilm producers were to be 10-1000 times less susceptible towards antibiotics than are the equivalent cells growing planktonically. Also Keren et al. (2004) explained this issue as bacterial populations produces persister cells that neither grow nor die in the presence of antibiotics and that persisters are largely responsible for high levels of biofilm tolerance to antimicrobials. Biofilm hampered penetration of antimicrobial and the concentrations required to eradicate biofilm producing bacteria are higher than those required to eradicate strains that did not produce biofilm (Curtin *et al.*, 2003).

There is association between biofilm production with persistent infection and antibiotic failure. Hence in infection caused by biofilm producing staphylococci, the differentiation with respect to biofilm phenotype might help to modify antibiotic therapy and to prevent infection related to biomedical devices. A suitable and reproducible method is necessary for screening of biofilm in healthcare setting and CRA test is recommended as it is easier to perform, cheap. CRA is a method that could be used to determine whether an isolate has the potential for biofilm production or not. The better agreement between the CRA plate test with the molecular detection of *ica* genes indicates the former as a reliable test for the phenotypic characterization of virulence of clinical isolates.

The continuous evolution of antimicrobial resistanece patterns is bacteria necessiates a comprehensive policy for infection control in hospitals in order to decrease the risk of nosocomial infection in cancer patients.

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5. References:

1. Alexander S, Pizzo, Philip A and Poplack, David G (2005). Principles & Practice of Pediatric Oncology, 5th Edition.
2. Arciola CR, Montanaro L and Baldassarri L (2001). Presence of *icaA* and *icaD* Genes and slime production in a collection of staphylococcal strains from catheter-associated infections. Journal of Clinical Microbiology, 39 (6): 2151-2156.
3. Arciola CR, Campoccia D and Montanaro L (2002). Detection of biofilm-forming strains of *S. epidermidis* and *S. aureus*. Expert Rev Mol Diagn., 2 (5): 478-484.
4. Aricola CR, Commpaoccia D, Baldassari L, Donati ME, Gamberini S and Montanaro L (2005). Detection of biofilm formation in *Staphylococcus epidermidis* from implant infections. Comparisons of a PCR method that recognize the presence of *ica* genes with two classic phenotypic methods. J Biomed Mater Res., 76A (2): 425-430.
5. Ashour Hossam M and El-Sharif Amany (2007). Microbial spectrum and antibiotic susceptibility profile of gram-positive aerobic bacteria isolated from cancer patients. Journal of Clinical Oncology, 25 (36): 5763-5769.
6. Ashour Hossam M and El-Sharif Amany (2009). Species distribution and antimicrobial susceptibility of gram-negative aerobic bacteria in hospitalized cancer patients. Journal of Translational Medicine, 7: 14.
7. Berjan A Collin, Helen L Leather, John R Wingard and Reuben Ramphal (2001). Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clinical Infectious Diseases, 33 (7): 947-953.
8. Bose S, Khodke M, Basak S and Mallick SK (2009). Detection of biofilm producing staphylococci: need of the hour. Journal of Clinical and Diagnostic Research December, 3 (6): 1915-1920.
9. Curtin J, Cormican M, Fleming G, Keelehan J and Colleran E (2003). Linezolid compared with eperezolid, vancomycin, and gentamicin in an *in-vitro* model of antimicrobial lock therapy for *S. epidermidis* central venous catheter-related biofilm infections. Antimicrobial Agents and Chemotherapy, 47: 3145-3148.
10. de Silva GDI, Kantzanou M, Justice A, Massey RC, Wilkinson AR, Day NPJ and Peacock SJ (2002). The *ica* operon and biofilm production in coagulase-negative staphylococci associated with carriage and disease in a Neonatal Intensive Care Unit. Journal of Clinical Microbiology, 40 (2): 382-388.
11. Donlan RM and Costerton W (2002). Biofilms Survival mechanisms of clinically relevant Microorganisms. Clinical Microbiological Review, 15(2): 167-193.
12. Eftekhar Fereshteh and Mirmohamadi Zeinab (2010). Evaluation of biofilm production by *S. epidermidis* isolates from nosocomial infections and skin of healthy volunteers. International Journal of Medicine and Medical Sciences, 1 (10): 438-441.
13. El Mahallawy H A, Abeer K. Abdulall, Tarek Mostafa, Sameh A. Loutfy and Rehab Abdel Hai (2006). The impact of microbiological factors on clinical course and outcome of FN in pediatric cancer patient; cancer patient. International Journal of Infectious Diseases, 9: 43-51.
14. El Mahallawy H A, Samah A. Loutfy, Mohamed El-Wakil, Abeer K. Abd El-Al and Hanaa Morcos (2009). Clinical implications of *icaA* and *icaD* genes in coagulase negative Staphylococci and *S. aureus* bacteremia in febrile neutropenic pediatric cancer patients. Pediatric Blood and Cancer, 52(7): 824-828.
15. Eslaminezhad Z, Ghafouri E, Farahmandina Z, Kalantari Behjat, and Safari Fereshteh (2010). Isolation, identification, and profile of antibiotic resistance of bacteria in patients with cancer. Iranian Journal of Medical Sciences, 35 (2): 109-115.
16. Fitzpatrick F, Humphreys H and O'Gara JP (2005). Evidence for *icaADBC*-independent biofilm development mechanism in methicillin-resistant *S. aureus* clinical isolates. Journal of Clinical Microbiology., 43 (4): 1973-1976.
17. Fluckiger Ursula, Ulrich Martina, Steinhuber Andrea, Do¨ringGerd, Dietrich Mack, Regine Landmann, Christiane Goerke, and Christiane Wolz (2005). Biofilm formation, *icaADBC* transcription, and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. Infection and Immunity, 73 (3): 1811-1819.
18. Freeman DJ, Falkiner FR and Keane CT (1989). New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology., 42 (8): 872-874.
19. Fux, CA, Wilson S, and Stoodley P. (2004). Detachment characteristics and oxacillin resistance of *S. aureus* biofilm emboli in an in-vitro infection model. Journal of Bacteriology 186 (14):4486–4491
20. Gerke C, Kraft A, Süssmuth R, Schweitzer O and Götz F (1998). Characterization of the N-acetyl-glucosaminyl-transferase activity involved in the biosynthesis of the *S. epidermidis* polysaccharide Intracellular Adhesion. J Biol Chem., 273 (29): 18586-18593.
21. Gilbert P, Maira-Litran T, McBain AJ, Rickard AH and Whyte F (2002). The physiology and collective recalcitrance of microbial biofilm communities. Advances in Microbial Physiology, 46, 203-256.
22. Götz Friedrich (2002). Staphylococcus and biofilms. Molecular Microbiology, 43(6): 1367-1378.
23. Handke LD, Conlon KM, Slater SR, Elbaruni S, Fitzpatrick F, Humphreys H, Giles WP, Rupp ME, Fey PD and O'Gara JP (2004). Genetic and phenotypic analysis of biofilm phenotypic variation in multiple Staphylococcus epidermidis strains. J Med Microbiol., 53: 367-374.
24. Hosseini MJ, Ranjbar R and Saadat A (2006). A study on the prevalence and etiology of fever in hospitalized patients with fever and neutropenia in Bagyiatallah Hospital during 1995-2005. Ilam Univ Med J., 14 (3): 45-48.
25. Hsin Pao Lai, Po ren Hsueh and Yee Chun Chin (2003). Bacteremia in heamatological and oncological children with febrile neutropenia: experience in tertially medical center in Taiwain, Jornal of Microbiol Immunol Infect., 36 (3): 197-202.

26. Hughes WI, Armstrong D, Bodey GP, *et al*. (1997). Guidelines for the unexplained fever. Clin Infect Dis., 25: 551-573.
27. Kelly MJ, Vivier PM, Panten TM and Schwarz CL (2010). Bacteriamia in febrile nonneutropenic pediatric oncology patients. *Pediatr* Blood Cancer, 54: 83-87.
28. Keren I, Kaldalu N, Spoering A, Wang Y and Lewis K (2004). Persister cells and tolerance to antimicrobials. *FEMS* Microbiol Lett., 230 (1): 13-18.
29. Kim YH, Lee HD and Hah JO (2005). Bacteremia in pediatric cancer patients: Causative organisms and antibiotic sensitivities. Korean Journal of Pediatrics, 48 (6): 619-623.
30. Kogan G, Sadovskaya I, Chaignon P, Chokr A and Jabbouri S (2006). Biofilms of clinical strains of Staphylococcus that do not contain polysaccharide intercellular adhesin. FEMS Microbiology Letters., 255: 11-16.
31. Lee SI, Kim JS and Kang JM (1990). A study on infection in childhood acute leukemia. Korean Journal of Infectious Diseases, 22: 33-41.
32. Locus KG, Brown AE, Armstrong D, Champman D and Heller G (1996). The identification of febrile, neutropenic children with neoplastic disease at low risk for bacteremia and complications of sepsis. Cancer, 15; 77 (4): 791-8.
33. Mack D, Rohde H and Dobinsky S (2000). Identification of three essential regulatory gene loci governing expression of *S. epidermidis* polysaccharide intercellular adhesin and biofilm formation. Infection and Immunity., 68 (7), 3799-3807.
34. Marie JP, Vekhoff A, Pico JL, Guy H, Andremont A and Richet H (1998). Neutropenic infection a review of a french febrile aplastic study group trial in 608 febrile neutropenic patients. Journal of Antimicrobial Chemotherapy, 41 (D): S57-64.
35. O"Gara JP and Humphrey H (2001). *S. epidermidis* biofilms: importance and implications. Journal of Medical Microbiology, 50: 582-587.
36. Paganini H, Staffolani V and Zubizarreta P (2003). Viridans streptococci bacteraemia in children with fever and neutropenia: A case-control study of predisposing factors. European Journal of Cancer, 39 (9): 1284-1289.
37. Paul M, Gafter-Gvili A, Leibovici L, Bishara J, Levy I, Yaniv I, Shalit I, Samra Z, Pitlik S, Konigsberger H, and Weinberger M. (2007). The epidemiology of bacteremia with febrile neutropenia: experience from a single center, 1988–2004. Israel Medical Association Journal Jun;9(6):424-429.
38. Pizzo PA, Hathorn JW and Hiemenz JW (1986). A randomized trial comparing ceftazidime alone with combination antibiotic therapy in cancer patients with fever and neutropenia. New England Journal of Medicine, 315: 552-8.
39. Raad I and Bodey G (1992). Infectious complications of indwelling vascular catheters. Clinical Infectious Diseases, 15 (2): 197.
40. Rahiala J, Perkkio M and Riikonen P (1998). Infections occurring during the courses of anticancer chemotherapy in children with ALL: a retrospective analysis of 59 patients. Pediatric Haematolology and Oncology, 15: 165-174.
41. Ramphal Reuben (2004). Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clinical Infectious Diseases, 39 (Suppl 1): S25-31.
42. Rohde H, Burdelski C and Bartscht K (2005). Induction of *S. epidermidis* biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. Molecular Microbiology, 55 (6): 1883-1895.
43. Souli M and Giamarellou H (1998). Effects of slime produced by clinical isolates of coagulase negative Staphylococci on activities of various antimicrobial agents. Antimicrobial Agents and Chemotherapy, 42 (4): 939-941.
44. Viscoli C, Castagnola E, Giacchino M, Cesaro S, properzi E and TuccI F (1999). Bloodstream infections in children with cancer: a multi-centre surveillance study of the Italian Association of Pediatric Hematology and Oncology. European Journal of Cancer, 35: 770-774.
45. Vuong C and Otto M (2002). *S. epidermidis* infections. Microbes and Infection., 4 (4): 481-489.
46. Walsh Thomas J, Roilides Emmanuel, Groll Andreas H, Gonzalez Corina and Pizzo Philip A (2006). Infectious Complications in Pediatric Cancer Patients Principles & Practice of Pediatric Oncology, 5th Edition.
47. Ziebuhr W, Heilmann C, Gotz F, Meyer P, Wilms K, Straube E and Hacker J (1997). Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *S. epidermidis* blood culture strains and mucosal isolates. Infection and Immunuity., 65 (3): 890-896.
48. Ziebuhr W, Krimmer V, Rachid S, Lossner I, Gotz F and Hacker J (1999). A novel mechanism of phase variation of virulence in *S. epidermidis:* evidence for control of the polysaccharide intercellular adhesion synthesis by alternating insertion and excision of the insertion sequence element IS256. Molecular Microbiology, 32 (2): 345–356.
49. Ziebuhr W, Lossner I, Rachid S, Dietrich K, Gotz F and Hacker J (2000). Modulation of the polysaccharide intercellular adhesin (PIA) expression in biofilm forming *S. epidermidis*. Analysis of genetic mechanisms. Advances in Experimental Medicine and Biology, 485: 151-157.
50. Zinner SH. (1999). Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. Clinical Infectious Diseases ; 29(3):490–494.

12/21/2010