

Comparative Study of Antibacterial Susceptibility Patterns of the Bacteria Isolated from Patients' Blood Samples over Two Periods

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Abstract: Problem statement: Due to continuous changes in the frequencies and antibacterial susceptibility patterns of nosocomial pathogens, periodical surveillance of these fluctuations could help the clinicians to treat hospitalized patients more efficiently whenever empirical therapies need to be considered. This study was conducted to compare the prevalence of the bacteria recovered from bloodstream samples by Bactec 9240, over the two periods of 2001-2004 and 2005-2008 and to evaluate their antibacterial susceptibility patterns. **Approach:** Totally, 3622 culture positive blood samples were analyzed over the periods. Antibacterial susceptibility patterns of the isolates were determined by standard disk diffusion (Kirby-Bauer) method. The data were compared in terms of quantity and quality of the pathogens and based on their distributions in three main wards. **Results:** Changes were observed in bacterial composition and frequencies of them, between the two periods. Compared with the first period, increased frequencies of antibiotic resistant bacteria such as *S. aureus*, *enterococci*, *acinetobacter* and *pseudomonas* were noticed in the second period. Most patients were admitted to pediatrics, followed by adults and neonates wards. Increased antibiotic resistance of the majority of the bacteria in the second period indicates the decreased efficacy of corresponding antibiotics. However, overall efficacy of some antibiotics such as ciprofloxacin and amikacin against Gram positive bacteria preserved. **Conclusion:** Trend of composition of the bacteria from first to second period could suggest the domination of antibiotic resistant bacteria over the sensitive ones. Appropriate strategies including strict control measures and rational prescription of the effective antibiotics may retard the trend accordingly. Vancomycin and imipenem were the most active antibiotics against Gram positive and negative bacteria. Combination of these two antibiotics is highly recommended for empirical therapy.

Key words: Comparative study, blood samples, antibiotic resistance, control measures, antibacterial susceptibility patterns, *S. aureus*, standard disk diffusion, Methicillin Resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*

INTRODUCTION

Rapid and reliable detection of microorganisms from the blood is one of the most critical functions of a diagnostic microbiology laboratory. It is well known that the isolation of microorganisms is a gold standard for accurate detection of etiological agents of infectious diseases (Paolucci *et al.*, 2010). Furthermore, early detection of bloodstream infections could prevent implantation of microorganisms into vital organs such as the brain, heart or kidneys (Bakowski *et al.*, 2008). To detect blood infections, different techniques and instruments have been innovated. One of such detection systems is bactec, which is widely accepted as a rapid

and accurate method for the detection of bloodstream infections (Cermak *et al.*, 2011). In this study, blood samples were processed in bactec 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). This system is noninvasive and automated blood culture systems with continuous monitoring have introduced a technology that reduces the time needed to detect positive blood cultures as well as decreases the specimen handling (Kim and Han, 2010; Bert *et al.*, 2010).

The purpose of this study was to compare the frequencies and antibacterial susceptibility patterns of the bacteria isolated from blood samples of the hospitalized patients during two four year periods, using bactec 9240. Hopefully, this comparison can help

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adopt a new strategy to prevent the emerging antibiotic resistant isolates in the hospitals. Prediction of the predominant bacteria and determination of the effective clinical antibiotic therapy can help promote the treatment and save on the patient's management.

MATERIALS AND METHODS

This study was conducted to compare the bacteria isolated from blood samples over the two periods, 2001-2004 and 2005-2008, at Nemazee Hospital, affiliated with Shiraz University of Medical Sciences, Shiraz, southern Iran. This hospital is a tertiary care facility with 1000 beds and located in Fars province. Suspicious patients to blood infections admitted to three main hospital wards including, neonates, pediatrics and adults were enrolled in the study. Ten and 3 ml blood samples from pediatric/neonate and adult patients were taken under the supervision of specialized physician and inoculated to bactec bottles peds plus/F or adult plus aerobic/F, aseptically. An indication for patient blood infection was confirmed by the specialized physician in each ward.

The bottles were incubated in bactec system, as recommended by the manufacturer for 7 consecutive days. The negative bottles were then removed from the instrument. During the seven day incubation, when system alerted for positive results, 3-5 drops of blood culture samples taken with 1ml sterile syringe, were inoculated on the blood and chocolate agars containing 5% whole sheep blood and then incubated aerobically at 37°C overnight. The pure cultures were then stained by Gram's method. Identification of bacteria in positive cultures was carried out according to standard biochemical tests and subculturing to appropriate media.

Sensitivity of identified bacteria to the different antibiotics was determined, according to standard disk diffusion (Kirby-Bauer) method using Mast Co (Mast Co, Merseyside, UK) or Difco (BBL, USA) disks. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as the control for antibiotic susceptibility determination. Antibacterial susceptibility was interpreted as recommended by National Committee for Clinical Laboratory Standards NCCLS (National Committee for Clinical Laboratory Standards).

Difference in frequency of bacteria over the two periods of study was analyzed by *Chi-square* (SPSS version 15 software) and Fisher's exact tests. The significant level was defined as $p < 0.05$.

RESULTS

During the periods 2001-4 and 2005-8, 9407 and 10400 blood specimens were received and processed, of which 1122 (11.9%) and 2500 (24%) were grown in bactec apparatus, respectively. Totally, from 19807 blood samples, 3622 (18.2%) were positive during the whole periods of the study. Seven top list isolated bacteria from the blood samples were as follows: coagulase negative staphylococci, *Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa*, *bacillus* spp, *enterobacter* spp and *acinetobacter* spp. Coagulase negative staphylococci and *bacillus* spp, are normal flora of the skin and the possibility of blood samples contamination with them during sampling should be considered. Fluctuation in frequencies of some important pathogenic isolates such as *Pseudomonas aeruginosa*, *acinetobacter* spp, *enterococci* spp and *brucella* spp. was statistically significant. *Staphylococcus aureus* and *E.coli* were Gram positive and Gram negative true pathogenic microorganisms, isolated from the patients with the highest frequencies. As shown in Table 1, during the 8 year period of study some bacteria such as fusobacter, *Neisseria meningitidis*, peptococci, *Listeria monocytogenes*, *campylobacter* spp and hafia were isolated at very low quantities (1; 0.1%). Comparison of fluctuation in antibiotic resistance of the Gram negative and Gram positive bacteria indicates that in the second period (2005-8), bacteria acquired more resistance to the tested antibiotic, compared to the first period (2001-4). This finding for some bacteria such as: *acinetobacter*, *Pseudomonas aeruginosa*. *S. aureus* and *enterococci* spp were remarkable. Table 2 and 3 present the detailed comparison of bacterial resistance patterns over the two periods. Comparison of the isolates from the patients admitted to the three above-mentioned wards, revealed that frequencies (%) of bacteria in the patients with bloodstream infections in pediatrics ward was higher, compared to the other two wards (Table 4). Changes in frequencies of some bacteria such as *S. aureus*, *E.coli*, *P. aeruginosa* and *brucella* spp were statistically significant. Reduced efficacy of the antibiotics against Gram positive bacteria was observed in the second period, while for Gram negative bacteria the efficacy of majority of antibiotics preserved. Overall reduction of *in vitro* activity of cephalexin and clindamycin against Gram negative and ceftazidime against Gram positive bacteria was considerable. Figure 1 and 2 illustrate the changes in sensitivity of Gram negative and positive bacteria to the five effective antibiotics during the periods of investigation.

Table 1: Frequencies of isolated microorganisms from bactec 9240 over the periods 2001-2004 and 2005-2008

Microorganisms	Total Frequency 2001-2004 (%)	Total Frequency 2005-2008(%)	P value
<i>*Coagulase Negative Staphylococci</i>	523 (47)	1295 (51.8)	0.09
<i>Staphylococcus aureus</i>	132 (12)	250 (10)	0.15
<i>E.coli</i>	64 (6)	112 (4.5)	0.13
<i>Pseudomonas aeruginosa</i>	52 (4.5)	50(2.0)	0.000018
<i>Bacillus spp</i>	52 (4.5)	60(2.4)	0.0005
<i>Enterobacter spp.</i>	50 (4)	70 (2.8)	0.012
<i>Acinetobacter spp.</i>	40 (3.5)	165 (6.6)	0.00005
<i>Streptococcus viridans</i>	34 (3)	45 (1.8)	0.022
<i>Enterococcus spp.</i>	29 (2.5)	115(4.6)	0.005
<i>Klebsiella spp.</i>	29 (2.5)	55(2.2)	0.49
<i>Brucella spp.</i>	28 (2.4)	20(0.8)	0.00005
<i>Streptococcus pneumoniae</i>	20 (1.6)	23(0.9)	0.029
<i>*Diphtheroid</i>	16 (1.3)	70 (2.8)	0.014
<i>cedecia davisae</i>	12(0.46)	NA	
<i>Salmonella spp.</i>	11 (1)	5 (0.2)	0.001
<i>Haemophilus influenzae.</i>	8 (0.8)	15 (0.60)	0.7
<i>Candida spp</i>	6 (0.6)	30 (1.2)	0.064
<i>Proteus spp</i>	5(0.5)	-	NA
<i>Oligella spp</i>	4 (0.4)	30 (1.2)	0.015
<i>Morganella</i>	-	4(0.16)	NA
<i>*Micrococcus spp</i>	3 (0.3)	10(0.4)	0.75*
<i>Streptococcus spp.</i>	3 (0.3)	25 (1.0)	0.02
<i>Serratia spp</i>	3 (0.3)	25(1.0)	0.02
Gram positive anaerobic org	2 (0.2)	-	NA
Gram negative rod	2(0.2)	5(0.2)	1*
<i>Nocardia</i>	-	2 (0.08)	NA
<i>Citrobacter</i>	-	2 (0.08)	NA
<i>Morexella</i>	-	2 (0.08)	NA
<i>Fusobacter</i>	1 (0.1)	-	NA
<i>Neisseria meningitidis</i>	1 (0.1)	-	NA
b hemolytic sterp group A&B	1 (0.1)	2 (0.8)	1*
<i>Peptococci</i>	-	1(0.04)	NA
<i>Listeria monocytogrenes</i>	1 (0.1)	-	NA
<i>Campylobacter spp</i>	1 (0.1)	-	NA
<i>Hafnia</i>	1 (0.1)	-	NA
Total	1122 (100)	2500(100)	NA

*: Analyzed with Fisher's exact tests. Significant values are printed in bold

Table 2: Comparison of antibiotic resistance patterns of important Gram negative bacteria recovered from bactec 9240 over periods 2001-4 and 2005-8

Bacteria	Period: a or b: frequency	Percentage of antibiotic resistance													
		GM	CN	SXT	CXM	CRO	IMI	CIP	AK	CAZ	AP	CPM	C	CFM	CTX
<i>Acinetobacter spp</i>	a: 40	17	39	38.00	ND	ND	17	41	39	ND	ND	ND	ND	ND	ND
	b: 165	46	74.6	43.50	82	47	32.5	17	25	48	86.7	ND	45.7	85.7	62.5
<i>E. coli</i>	a: 64	4.5	53.3	76.00	37.9	ND	2.5	16.8	9.1	24	74	ND	ND	ND	ND
	b: 112	57	78	75.50	63.5	64.5	0	45	17	47.5	98	48.6	72	65.9	71
<i>Pseudomonas spp</i>	a: 52	24.3	ND	93.80	100	ND	ND	10.8	ND	ND	ND	ND	ND	ND	ND
	b: 50	33.3	81	81.00	95	58	12.5	26	17	41.5	87	43.5	75	91	ND
<i>Enterobacter</i>	a: 50	ND	ND	39.00	44	ND	0	ND	11	54	78	ND	ND	ND	ND
	b: 70	42.5	78.7	31.66	68.7	66	0	14.5	26	52.4	86	50	41	65.5	59.5
<i>Klebsiella spp</i>	a: 29	23.5	28.6	26.60	18.2	ND	16.7	9.1	26.8	54	36.4	ND	ND	ND	ND
	b: 55	42	60	53.00	80	56	0	33	24	58	95	38	55.5	ND	ND
<i>Salmonella spp</i>	a: 11	11	0	11.00	ND	ND	0	0	ND	6	ND	ND	ND	ND	ND
	b: 5	0	20	33.30	ND	20	0	0	0	0	ND	0	0	ND	25
<i>Brucella spp</i>	a: 28	10	80	44.50	ND	25	0	10	16.7	66.7	33.3	ND	ND	ND	ND
	b: 20	0	62.5	50.00	25	5.5	0	0	0	31.5	ND	ND	ND	ND	ND
<i>Haemophilus influenzae</i>	a: 8	6	25	38.00	25	9	0	5.2	6	15	25	5.3	15	16	15
	b: 15	11.1	33.3	44.40	35	11.1	0	11.1	11.1	22.2	33.3	11.1	ND	22.2	22.2

Period a: 2001-2004; Period b: 2005-2008, Abbreviations: GM; gentamicin, CN: cephalixin, SXT; co-trimoxazole, CXM; ceforaxime, IMI; imipenem, CIP; ciprofloxacin, Ak; amikacin, CAZ; ceftazidime, AP; ampicillin, CPM, cefepeme, C; chloramphenicol, CFM; cefixime, CTX; cefotaxime, ND; not determined

Table 3: Comparison of antibiotic resistance patterns of important Gram positive bacteria recovered from bactec 9240 over the periods 2001-4 and 2005-8

Bacteria	Period: a or b: frequency	Percentage of antibiotic resistance						
		GM	VA	CN	SXT	CC	CIP	C
<i>S. epidermidis</i>	a: 523	36.6	1.0	19	62.2	38.1	21.2	11.8
	b: 1295	46.7	1.3	21	60	45	40.2	18.5
<i>S. aureus</i>	a: 132	30.6	0.0	25.9	34	18.5	20.5	6.8
	b: 250	33.5	0.0	27	31.6	28.5	22.4	6.6
<i>Enterococci Spp.</i>	a: 29	22.0	1.6	ND	50	ND	33.0	ND
	b: 115	73.2	18.5	70	76.3	72.4	56.0	40
<i>Streptococcus viridans</i>	a: 34	79.0	3.0	ND	ND	ND	29.0	7
	b: 45	80.4	5.8	15.1	63.7	28.7	30.0	12.5
<i>Streptococcus Pneumonia</i>	a: 20	86.7	0.0	0	62.5	7.7	0.0	17.8
	b: 23	66.7	0.0	11.1	66.7	11.1	11.1	ND

Abbreviations , Period a: 2001-2004; Period b: 2005-2008,: GM; gentamicin, VA; vancomycin,CN; cephalixin, SXT; co-trimoxazole, Ap; ampicillin, CC; clindamycin, CIP; ciprofloxacin, C; chloramphenicol, ND; not determined

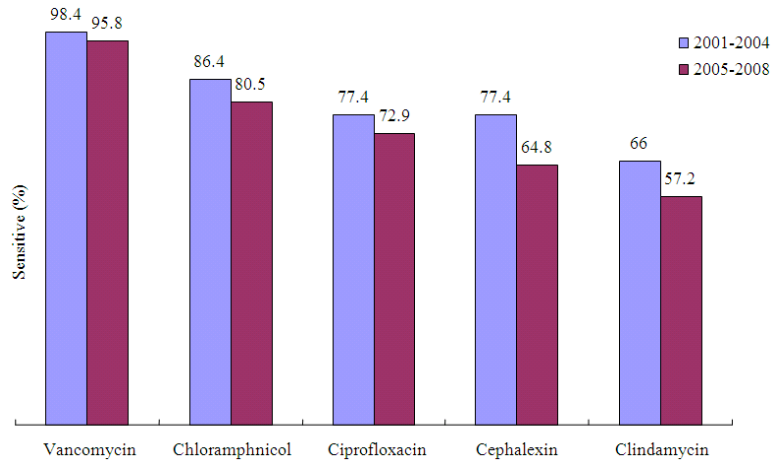


Fig. 1: Comparison of Gram positive sensitivity profiles to five effective antibiotics over the periods 2001-2004 and 2005-2008

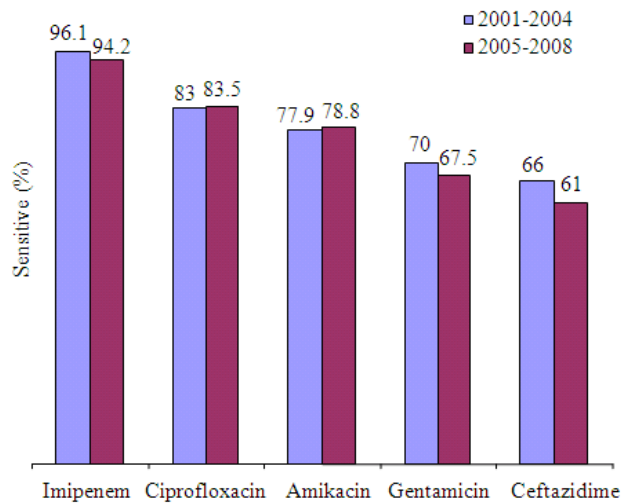


Fig. 2: Comparison of Gram negative sensitivity profiles to five effective antibiotics over the periods 2001-2004 and 2005-2008

Table 4: Comparison of frequencies (%) of the bacteria recovered from bactec 9240, based on hospital wards admission over the two periods (2001-2004 and 2005-2008)

Bacteria	Periods: a & b:		Frequency (%)		Frequency (%)		Frequency (%)	
	Frequency (%)	P value	in neonate ward	P value	in pediatrics ward	P value	in adult ward	P value
<i>*Coagulase Negative Staphylococci</i>	a: 523 (47)	0.00000	80 (60)	0.1700	321 (49)	0.00000	122 (36)	0.2000
	b:801 (32)		147 (18.4)		420(52.4)		234(29.2)	
<i>Staphylococcus aureus</i>	a: 132 (12)	0.00000	11 (8.2)	0.002*	66 (10)	0.00000	55 (16.2)	0.0700
	b:122(4.88)		5(4.0)		27(22.2)		90(73.8)	
<i>E.coli</i>	a: 64 (6)	0.00000	4 (3)	0.470*	29 (4.5)	0.49000	31 (9.1)	0.0040
	b:64 (2.6)		5(7.8)		75(39)		34(53.2)	
<i>Pseudomonas aeruginosa</i>	a: 52 (4.5)	0.00000	4 (3)	0.210*	29(4.5)	0.00000	19(5.6)	0.0018
	b:28(1.12)		3(0.7)		10(35.7)		15(53.6)	
<i>*Bacillus spp</i>	a: 52 (5.5)	0.00000	10 (7.5)	0.002*	17 (2.8)	0.11000	25 (7.3)	0.0000
	b:37(1.48)		4(10.8)		23(62.2)		10(27)	
<i>Enterobacter spp.</i>	a: 50 (4)	0.00000	8 (6)	0.010*	25 (3.9)	0.00000	17 (5)	0.0250
	b:37(1.48)		4(10.8)		15(40.5)		18(48.7)	
<i>Acinetobacter spp.</i>	a: 40 (3.5)	0.99000	1 (0.7)	1.000*	31 (4.8)	0.04400	8 (2.3)	0.0220
	b:89(3.56)		4(4.5)		43(48.3)		42(47.2)	
<i>Streptococcus viridans</i>	a: 34 (3)	0.02000	2 (1.4)	1.000*	30 (4.6)	0.00030	2 (0.7)	0.1700
	b:45(1.8)		6 (13.3)		26(57.8)		13 (28.9)	
<i>Enterococcus spp.</i>	a: 29 (2.5)	0.67000	6 (4.4)	0.030*	10 (1.5)	0.97000	13(3.8)	0.1400
	b:71(2.84)		3(4.2)		22(31)		46(64.8)	
<i>Klebsiella spp.</i>	a : 29 (2.5)	0.00700	5 (3.7)	0.140*	11 (1.7)	0.02000	13 (3.8)	0.2900
	b:33(1.32)		4(12.1)		9(27.3)		20(60.6)	
<i>Brucella spp.</i>	a: 28 (2.4)	0.00000	0	-	16 (2.5)	0.00002	12 (3.5)	0.0003*
	b:10(0.4)		0		6(60)		4(40)	
<i>Streptococcus pneumoniae</i>	a: 20 (1.6)	0.00000	0	-	19 (2.9)	0.00002	1 (0.3)	-
	b:9(0.36)		0		9(100)		0	
<i>*Diphtheroid</i>	a: 16 (1.3)	0.24000	2 (1.4)	1.000*	5 (0.8)	0.07000	9 (2.6)	0.9900
	b:50(2)		4(8)		26(52)		20(40)	
<i>Salmonella spp.</i>	a: 11 (1)	0.00002*	0	-	7 (1)	0.00200*	4 (1.2)	0.2100*
	b:5(0.2)		0		2(40)		3(60)	
<i>Haemophilus influenzae.</i>	a: 8 (0.8)	0.03000*	0	-	8 1.2)	0.00300*	0	-
	b:5(0.2)		0		5(100)		0	

*: Analyzed with Fisher's exact test, significant values printed in bold. Period a: 2001-2004; Period b: 2005-2008

DISCUSSION

It has been proposed that frequencies and antibacterial susceptibility patterns of nosocomial pathogens continuously change, which demands periodical surveillance of their fluctuations (Japoni *et al.*, 2009; Sepehri *et al.*, 2010)

In the present study, frequencies (%) of bacteria isolated from the blood samples of patients, changed in quality and quantity from first to the second period. Continuous changing of nosocomial pathogens has been previously reported (Starnes *et al.*, 2008). Amsterdam *et al.* (2010) Patients may be infected in the community and transfer these bacteria to hospitals, as happened for methicillin resistance *S. aureus* or vice versa (Song *et al.*, 2011). Exchanging of resident flora in the hospital with circulating bacteria in the community, may gradually reduce the efficacy of the prescribed antibiotics in outpatients and render them ineffective (Zhang *et al.* 2010; Moskowitz *et al.*, 2010). Nevertheless, this flow does not reduce the nosocomial resistance rate due to continuous emerging of resistant isolates which may happen under selective pressure of consuming antibiotics in the hospitals (Ozer *et al.*, 2011).

Increased frequencies of some antibiotic resistant bacteria such as *acinetobacter* and *entrococci*, have been observed from first to second periods, which demands appropriate attention and action to be taken. Change in the ecology of nosocomial bacteria from antibiotic sensitive to antibiotic resistant bacteria may revert by frequent application of strong antiseptics and disinfectants along with strong control measures (Sepehri *et al.*, 2009); Special attention should be taken to educate personnel for frequent hand washing, correct using of gloves to prevent cross-contamination of other patients, wearing gown and mask (Sepehri *et al.*, 2009).and preventing of close contact of patients admitted to infectious wards with visitors (Xerry *et al.*, 2010). Of course, hospital personnel are more important in cross-contamination of patients. Therefore, routine surveillance of personnel of the hospitals and suspending from duty the healthy carrier individuals, carrying antibiotic resistant bacteria such as Methicillin Resistant *S. aureus* (MRSA), Vancomycin Resistance Entrococci (VRE) and *pseudomonas* until they become decontaminated, might prove effective (March *et al.*, 2010; Askarian *et al.*, 2009). The data also support the hypothesis that determination of antibiotic sensitivity patterns in periodic intervals should be

mandatory in each region, for choosing appropriate antibiotic therapy (Japoni *et al.*, 2009; Sepehri *et al.*, 2010).

The number of positive cultures in the pediatric ward was high, compared with adults and neonates wards. This is partly due to the number of beds in pediatrics ward which admit a wide range of patients with ages ranging from 6 months to 16 years old. Moreover, children at pre or primary school ages may have not received appropriate hygienic cares, as compared with adults. Besides, neonates' hygiene is continuously supported and monitored by their mothers.

Overall efficacy of antibiotics reduced in the second study period (2005-8). Continuous prescription of antibiotics in hospitals and clinics can give rise to the emerging antibiotic resistance due to clonal selection (Velickovic-Radovanovic *et al.*, 2011). It is documented that due to continuous emerging of resistant isolates, the efficacy of antibiotics decreases gradually, particularly those administered for bloodstream infections (Amsterdam *et al.*, 2010) Bakowski *et al.*, 2008) However, in the present study, *in vitro* efficacy of two antibiotics (ciprofloxacin and amikacin) preserved. Present results show that imipenem and vancomycin are highly active against Gram negative and positive bacteria. These results are in concordance with other reports However, it should not be expected that this activity continues for a longtime, as it has been observed during this study and reports from other centers (Jamal *et al.* 2010; Grant *et al.*, 2010). Nevertheless, ciprofloxacin and chloramphenicol are alternative antibiotics with lower efficacies.

CONCLUSION

Present findings reveal the etiology of infectious diseases of the blood over two periods. The recovered bacteria with high frequency from blood samples at pediatrics ward indicates that special attention should be paid to such wards both in prevention and treatment aspects. Vancomycin and imipenem were the most active antibiotics which could cover the majority of Gram positive and negative bacteria. Therefore, an administration of the combination of these two antibiotics is highly recommended for empirical therapy. To maintain the efficacy of a few effective antibiotics, strict control measures should be implemented.

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