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Frequency of Isolated Bacteria and Fungi from Bloodstream of 3212 Patients Hospitalized at Milad Hospital, Tehran, Iran 2009-2010

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Bloodstream infections (BSIs) are one of most significant cause of mortality and morbidity worldwide, even in community-acquired or hospital-acquired infections. Unfortunately, until now there is not any comprehensive study to survey the frequency of pathogens isolated from BSIs in Iran. The aim of this study was to determine epidemiological features of nosocomial BSIs in the Milad hospital, Tehran, Iran.

This study is a retrospective study on survey of 3212 blood cultures during 2009 to 2010. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI). All analysis was performed using SPSS 16.

Our study revealed that 66% of BSIs were caused by gram-negative bacteria, while 24.1% of BSI caused by gram positive bacteria, the rest of them (9.9%) was infected by yeast. Among gram negative bacteria, *Enterobacter cloacae* (26.4%) and *Escherichia coli* (24%) were more prevalent organisms, while among gram positive bacteria *Staphylococcus aureus* (47.8%) and Coagulase negative *Staphylococci* (CoNS) (16.8%) were more prevalent. In fact, this study indicates the importance of establishment of a comprehensive program in Iran or even in Middle East upon the antimicrobial resistance rate.

Key words: Bloodstream, Bacteria, Fungi, Tehran.

Nosocomial infections are among infections that become clinically evident after 48 hours of hospitalization[1]. Even among community-acquired or hospital-acquired infections, nosocomial infections bloodstream infections (BSIs) are one of most significant cause of morbidity and mortality worldwide[1,2]. Since 1990s there were many plans, such as Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) and National Nosocomial Infection Surveillance (NNIS), to investigate the nosocomial and non-nosocomial infections in progressive countries[3-6]. Albeit in some cases each

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pathogen caused BSI separately[7-13].

Unfortunately, until now there have not been any comprehensive epidemiologically program to survey the frequency of pathogens isolated from BSIs, and their antimicrobial susceptibility in Iran, similar to what have been analyzed in other studies (14). Although in some hospitals there were reports about the bacterial frequency and antimicrobial susceptibility[15].

At this retrospective study we aim to demonstrate the epidemiological features of community-acquired and nosocomial BSIs in the Milad hospital as one of most prominent clinical centers in Iran.

MATERIAL AND METHODS

This study was designed as a retrospective survey of 3212 blood culture specimens prepared during 2009 to 2010. As we have not any control over the collection of specimens, we excluded those blood stream isolates were a potential skin contaminants (e.g. *Diphtheroids*, *Bacillus* species, or *Micrococcus*) had been cultured, except the specimens which the same bacteria was isolated in two successive cultures (3). All bacterial and fungal isolates were microbiologically identified in the microbiology department of Milad central laboratory using standard biochemical identification methods[16]. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method and zone diameter were measured following CLSI criteria[17]. Quality controls methods were routinely in place at the microbiology department of Milad central laboratory. Owing to the high risk of BSI morbidity and mortality, bacterial isolates that showed intermediate susceptibility to an antimicrobial agent were categorized as resistant isolates for data analysis and presentation[15]. All analysis was performed using SPSS 16 software and χ^2-test or Fisher's exact test. $P<0.05$ were considered statistically significant.

RESULTS

Results obtained from 3212 blood cultures have been done during 2009-2010, showing that 371 (11.6%) of cases of patients were infected. The result of our study revealed that 53.4% of BSIs did occur in men. Approximately 121 cases (32.6%) of BSIs were derived from Intensive Care Units (ICU) such as General ICU, Emergency ICU, Open heart ICU, Pediatrics ICU and Neonates ICU, the number of infected cases with gram positive bacteria, gram negative bacteria or fungi were 35, 75 and 11, respectively.

Totally our results, as shown in table 1 data indicated that 66% of BSIs were related to gram negative bacteria, while 24.1% of infected blood culture was caused by gram positive bacteria, and the rest of them (9.9%) were infected by yeast. Among gram negative bacteria, *Enterobacter cloacae* and *Escherichia coli* were more prevalent showing a frequency of 26.4% and 23.6%, respectively and among gram positive bacteria *Staphylococcus aureus* with frequency of 47.8% and thereafter Coagulase-negative *Staphilococci* (CoNS) with frequency of 16.8% were more prevalent. Among fungi, nonalbicans *candida* with a frequency of 81.1% was the most prevalent.

At this study, referencing to CLSI, we used 24 antibiotics for antibiogram testing (table 2). Investigation of gram positive bacteria antibiogram indicated that Vancomycin and Chloramphenicol were most effective antibiotics with 94.4% and 92% sensitive cases, respectively .All of the isolated *S.aureus*, CoNS and *Enterococcus faecalis* were susceptible to Vancomycin, while 55.6% of isolated *Enterococcus faecium* were resistant to Vancomycin (Vancomycin Resistant *Enterococci* VRE). Frequency of CoNS and *S.aureus* sensitivity for Chloramphenicol were 100% and 93%, respectively. We found that 54.1% of isolated *S.aureus* was methicillin resistant *S.aureus* (MRSA).

The isolated gram negative bacteria indicated that these bacteria were more sensitive to Imipenem (IPM) and secondly to Ciprofloxacin with frequency of 79.7% and 70.3%, respectively. *E.cloacae* and *E.coli* were sensitive to IPM with frequency of 97.9% and 97.6%, respectively. Results of antibiotic susceptibility pattern data for *Acinetobacter baumanni* indicated Colistin (100%) and Imipenem (42.9%) are the more effective agents.

It is noteworthy that during this study there were not any significant difference in antibiotic resistance in the first six month of the year compared to the second six month of the year (data is not showed).

Table 1. Frequency of isolated microorganisms from BSIs

Gram positive isolated bacteria	ICU	Non ICU	TOTAL
Staphylococcus aureus	20	23	43
Enterococcus faecalis	4	7	11
Enterococcus faecium	4	5	9
Streptococcus viridans	1	7	8
Staphylococcus warneri	-	5	5
Staphylococcus pasteuri	3	4	7
Streptococcus pneumoniae	-	2	2
Streptococcus pyogenes	1	-	1
Streptococcus agalactia	-	1	1
Streptococcus milleri	1	-	1
Staphylococcus capitis	1	-	1
Staphylococcus hominis	-	1	1
Gram negative isolated bacteria			
	ICU	Non ICU	TOTAL
Enterobacter cloacae	6	59	65
Escherichia coli	8	50	58
Acinetobacter baumanni	29	10	39
Stenotrophomonas maltophilia	4	18	22
Burkholderia cepacia	4	16	20
Pseudomonas aeruginosa	8	5	13
Klebsiella pneumoniae	10	3	13
Providencia rettgeri	2	1	3
Haemophilus influenzae	-	2	2
Enterobacter aerogenes	-	2	2
Citrobacter koseri	-	2	2
Acinetobacter spp.	-	1	1
Klebsiella ozaenea	1	-	1
Klebsiella planticola	1	-	1
Providencia stuartii	1	-	1
Sphingomonas spp.	1	-	1
Moraxella spp.	-	1	1
Isolated fungi			
	ICU	Non ICU	TOTAL
Candida spp.	10	20	30
Yeast (No *candida albicans* & SPP.)	-	5	5
Candida albicans	1	-	1

DISCUSSION

Reviewing reports from other countries showed that BSIs occurred more frequently in men than in women[18]. Also data indicated proximally that only 30% of BSIs isolated from ICU are clinically and epidemiologically valuable for further studying[19]. Our data as some of other international studies indicated that the frequency of BSIs with gram negative bacteria is more prevalent than gram positive bacteria[5, 14].

At our study, among isolated gram positive bacteria, *S.aureus* and secondly CoNS were more frequent, which was similar to the data achieved from other studies[5,14]. About frequency of gram negative bacteria, there were various reports, but mostly was mentioned as one of the important isolated bacteria –these reports mentioned *E.coli* as the first or the second most frequent isolated bacteria[4, 14, 15].In the field of gram positive frequency our results were not consistent with other studies. The most recently studies indicated that most common gram positive pathogens causing BSI were cooagulase-negative staphylococci. Other studies performed in our country also confirmed this finding. In a study

Table 2. Percentage of antibacterial susceptibility among gram positive and gram negative bacteria

	Gram positive bacteria						Gram negative bacteria						
	S.aureus	*CoNS*	*E. faecalis*	*E. faecium*	Others	*E.cloacae*	*E.coli*	*A. baumannei*	*S. aeroginosa*	*B. maltophilia*	*P. cepacia*	*K. pneumonia*	Others
AM[1]	10.3	7.7	90.9	22.2	80.0	4.6	10.7	2.8	0.0	0.0	0.0	0.0	7.1
AN[2]	45.5	75.0	NT	NT	NT	51.7	86.0	28.6	22.7	63.2	30.8	10.0	61.5
AZM[3]	44.1	0.0	NT	NT	84.6	NT	NT	NT	NT	NT	NT	NT	NT
C[4]	95.2	100	81.8	66.7	100	52.5	72.7	2.6	23.8	15.0	0.0	53.8	42.9
CAZ[5]	NT	NT	NT	NT	NT	37.5	69.0	2.8	18.2	5.0	23.1	7.7	40.0
CC[6]	14.6	7.7	NT	NT	38.5	NT	NT	NT	NT	NT	NT	NT	NT
CF[7]	NT	NT	NT	NT	NT	4.8	52.5	0.0	0.0	0.0	0.0	0.0	7.7
CP[8]	47.6	28.6	NT	NT	77.8	92.3	74.6	23.1	100	75.0	69.2	30.8	66.7
CRO[9]	32.6	14.3	NT	NT	90.0	42.2	67.8	5.3	0.0	20.0	0.0	15.4	40.0
CS[10]	NT	NT	NT	NT	NT	100	100	100	NT	NT	NT	100	NT
CT[11]	NT	NT	NT	NT	NT	45.3	69.5	5.6	0.0	5.3	0.0	15.4	57.1
CTX[12]	NT	NT	NT	NT	NT	38.7	67.2	0.0	0.0	10.0	0.0	15.4	46.7
CZ[13]	NT	NT	NT	NT	NT	3.3	50.0	0.0	0.0	0.0	0.0	0.0	8.3
E[14]	42.9	0.0	9.1	22.2	83.3	NT	NT	NT	NT	NT	NT	NT	NT
Gm[15]	45.9	27.3	NT	NT	NT	53.1	79.3	41.0	31.8	57.9	30.8	7.7	53.8
IPM[16]	NT	NT	NT	NT	NT	97.9	97.6	42.9	0.0	92.9	44.4	85.7	87.5
OX[17]	54.1	50.0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
P[18]	2.4	0.0	18.2	0.0	72.7	NT	NT	NT	NT	NT	NT	NT	NT
RA[19]	57.1	66.7	0.0	0.0	85.7	NT	NT	NT	NT	NT	NT	NT	NT
SXT[20]	85.7	15.4	NT	NT	22.2	49.2	70.2	15.4	95.5	63.2	12.5	30.8	40.0
TE[21]	25.6	35.7	18.2	22.2	58.3	30.2	57.6	12.8	0.0	63.2	23.1	23.1	35.7
TOB[22]	NT	NT	NT	NT	NT	47.5	62.2	32.4	10.5	35.3	23.1	7.7	50.0
V[23]	100	100	100	44.4	100	NT	NT	NT	NT	NT	NT	NT	NT
XM[24]	NT	NT	NT	NT	NT	4.9	33.3	0.0	0.0	0.0	0.0	0.0	0.0

1.Ampicilin, 2.Amikacin, 3.Azithromycin, 4.Chloramphenicol, 5.Ceftazidime, 6.Clindamycin, 7.Cephalotin, 8.Ciprofloxacin, 9.Ceftriaxone, 10.Colistin, 11.Ceftizoxime, 12.Cefotaxime, 13.Cefazolin, 14.Erythromycin, 15.Gentamycin, 16.Imipenem, 17.Oxacillin, 18.Penicillin, 19.Rifampin, 20.Trimethoprim Sulfomethoxazol, 21.Tetracycline, 22.Tobromycin, 23.Vancomycin, 24.Cefuroxime NT: Not Tested

done by Rahbar *et al* showed that Gram-positive cocci, included coagulase-negative staphylococci, *Staphylococcus aureus*, *Streptococcus pneumoniae* and other Gram-positive cocci, accounted for 42.3% of isolates. Gram-negative bacilli were responsible for another 42.3% of isolates. One reason for controversy among these finding could be due to number of blood culture taken for each patient, unfortunately in our study only one blood specimens per patient had been taken cultured. We had many episodes of Cones isolates but because of probability contamination we excluded these cases from our study. With regard to the susceptibility of *S.aureus* to Vancomycin (100% sensitive) our report about, it completely matched the data reported from Europe (SENTRY) (4) but it is was different than previous report from one of the Iran medical center's that they had isolated *S.aureus* resistant to Vancomycin with frequency of 21%. This finding maybe related to admission of antibiotic before sampling or in methods of identification of antimicrobial susceptibility in previous study. At this study our finding about *E.faecium* and its susceptibility to Vancomycin resemble the data reported by SENTRY study[4]. Our achievement about the frequency of susceptibility to Chloramphenicol for CoNS and *Enterococcus- spp*. was similar to data published in USA, Canada and Latin America but the susceptibility of *S.aureus* to Chloramphenicol in our finding was more prominent than previous studies[14].

Unfortunately, until now there has not been not any coordinated survey about frequency of isolated bacteria from BSIs in clinical centers in Iran, so we believe that this study can be used as the base of a cohort study to highlight epidemiological trend and frequency of bacteria and its antimicrobial susceptibility pattern in Iran or maybe even in Middle East. This cohort study not only can be used as a source to recognize local infections for some of special important infections such as Acinetobacter spp. in order to prevent extension of multidrug resistance bacteria but also it can be used to decrease technical deviation and personal errors in medical laboratories (9,10,12).Perhaps, the reason of differences occurred in significance of antimicrobial resistant pattern is due to the deficiencies in specimen collection and the duration of the study(13, 14).

CONCLUSIONS

In fact, our study indicates that blood cultures, specimen collection and the antimicrobial drug resistance should be performed by standard methods. Surveillance studies must be established in the country and the data should be evaluated periodically. Certainly these data could be used for appropriate use of antibiotic therapy and decrease the morbidity and mortality ratio caused by nosocomial infections.

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