Original Article

Frequency of Pathogens and Antimicrobial Susceptibility of Bacteria Isolated from Bloodstream Infections

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ABSTRACT

Background and Objectives: Blood stream infections cause significant morbidity and mortality in the world. In this study, we aimed at describing the organisms responsible for septicemia in 20062007- and their antimicrobial susceptibility that might lead to proper selection of antimicrobial agents in hospitalized patients with suspected sepsis.

Materials and Methods: Microbiology reports of 1753 blood specimens collected from inpatients of a referral hospital in Iran were retrospectively reviewed. Specimen culture, bacterial identification, and disk diffusion susceptibility testing were performed according to The Clinical and Laboratory Standards Institute guidelines. Data were analyzed by SPSS, X^2 Test and the fisher exact Test.

Results: Coagulase-negative *Staphylococci accounted* for most isolated bacteria (24.61%), in both genders. The second and third most frequent isolated bacteria in adults were *staphylococcus aureus* and *Stenotrophomonas maltophilia*, and in children were *Klebsiella pneumoniae* and *S. aureus*. Among the all Gram – positive cocci, vancomycin was the most effective agent. The most effective antibiotic varied among the Gram-Negative isolates, for example 95.65% of *S. maltophilia* were susceptible to co-Trimoxazole, and amikacin were suitable antibiotic in 85.29% of *E. coli*.

Conclusion: As the degree of antibiotic resistance rate for blood stream pathogens is alarming, it is mandatory to monitor the susceptibility of these isolates in order to avoid inappropriate use of antibiotics in hospital wards

Key Words: Anti Bacterial Agents, Blood, Infection, Frequency, Iran

Introduction

Blood stream infections (BSI) are important cause of morbidity and mortality worldwide (1, 2). For example in the USA, it is estimated that about 1020%- of nosocomial infections are due to BSI (3). Similarly, in Iran, BSI is one of the most common health care- related infections. It is completely clear that appropriated antimicrobial therapy of BSI is crucial in decreasing morbidity and mortality of this kind of infection (4). Although in Iran, antimicrobial resistance is a frequent cause of treatment failure of BSI.

Severely ill patients with potentially blood stream infections are usually treated empirically before the susceptibility results of their blood cultures are ready. Initial therapy with proper antibiotic agent is of critical significance for the survival of the patient (5). For selecting the choice of empiric therapy many factors should be kept in mind including the kind of pathogen that is most probably the cause the infection according to age, gender and risk factors and its probable antibiotic susceptibility pattern. Subsequently after isolation of a pathogen and receiving the results of susceptibility testing, the initial antibiotic therapy may change, however those information become available at least after 24 – 48 hours.

Surveillance of antibiotic resistance is essential to monitor the resistance situation. Surveillance programs revealed a trend of increasing antimicrobial resistance among frequent pathogens of BSI such as coagulase-negative *Staphylococci* and S*taphylococcus aureus* (6).

In Iran, like many other countries, an organized national surveillance programs are not stabilized and information about the prevalence of BSI pathogens and their antimicrobial susceptibility patterns is not available. For each geographical region, epidemiological studies are essential to demonstrate the previously mentioned data and for controlling the local spread of bacterial resistance. The final advantage of such programs is preventing the distribution of antimicrobial resistance in that geographical area (7, 8).

In this study, we aimed at describing the organisms that were responsible for septicemia in 20062007- and their antimicrobial susceptibility that may lead to proper selection of antimicrobial agents in hospitalized patients with suspected sepsis.

Materials and Methods

In a retrospective cross sectional study, data from Microbiology Laboratory reports of blood cultures from Imam Khomeini Hospital complex, Tehran, Iran were controlled and considered during 2 years (2006 – 2007). This complex is a general and referral hospital, and affiliated to the Tehran University of Medical Science, Iran. This hospital admits patients from all parts of Iran and includes a wide spectrum of socioeconomic levels patients.

The standard blood culture method (i.e. two 10-20 ml blood samples drawn with optimal time interval of 30 to 60 min between cultures) was performed in this hospital. Microbiology reports of all isolates, which were separated from aerobic blood culture bottles, were reviewed. Septicemia is defined as presence of bacteria in the blood, as confirmed by culture that is associated with clinical manifestation and systemic response. All types of microorganisms and susceptibility testing were recorded. However, data regarding the clinical significance of each isolate and the BSI if was community or hospital acquired, were not available. Isolated bacteria were microbiologically identified using standard biochemical identification methods (9,10). Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method (High Media, India) based on The Clinical and Laboratory Standards Institute (CLSI) (11, 12). Quality control organisms (American Type Culture Collection or ATCC species, including *P. aeruginosa*, *E. coli* and *S. aureus*) were utilized to ensure the accuracy of the susceptibility testing. Considering the high rate of BSI mortality, bacterial isolates that showed intermediate susceptibility to an antibiotic were categorized as resistant isolates for statistical analysis.

Data were analyzed by SPSS software (version 11.5).

Results

In a retrospective cross sectional study during two years, 1753 bacteria were identified from blood culture. It was found a male predominance between two genders, i.e. M=1011 and F=742 or M/F sex ratio=1.36.

We divided the patients into two groups, children (<12 years old, Mean Age = 8.8yr) and adults (≥12years old, Mean Age = 47.2 yr), and evaluated the frequency of each isolated organism in total and according to those age groups.

Totally, coagulase-negative *Staphylococci* accounted for most isolated bacteria (24.61%), in both genders and age groups. The other isolated microorganisms of blood cultures were *S. aureus* (16.43%), *S. maltophilia* (12.57%), *E. coli* (7.73%), *K. pneumoniae* (6.99%), *Acinetobacter* Spp. (6.71%), *Enterococci* (5.8%), *yeast* (4.14%), and others (15.02%) including *Streptococcus* Sp., *P. aeruginosa*, *Enterobacter* Spp.,

Serratia Spp. and *Proteus mirabilis*.

Meanwhile, age and sex distribution pattern of each microorganism were different (Tables 1 and 2, respectively). The second and third most frequent isolated bacteria in adults were *S. aureus* and *S. maltophilia*, and in children were *K. pneumoniae* and *S. aureus*. The rate of susceptibility to antimicrobial agents for Gram -Positive and Gram-Negative bacteria is summarized in Table 3 and 4, respectively. Among the all Gram – positive cocci, vancomycin was the most effective agent, although more than 50% of *Enterococci* showed resistance to this antimicrobial agent. In Gram-Negative bacilli, the rate of susceptibility to ceftriaxone and piperacillin / tazobactam was very low. The most effective antibiotic varies among the Gram-Negative isolates, for example, 95.65% of *S. maltophilia* were susceptible to co-trimoxazole, or amikacin were suitable antibiotic in 85.29% of *E. coli*.

Table 1: Frequency of occurrence of bacterial species or groups isolated from blood cultures of hospitalized patients (based on age)

Organism	Age < 12 years		Age > 12 years	
	No.	Percent	No.	Percent
Coagulase negative *Staphylococcus*	30	44.11	403	23.83
Staphylococcus aureus	9	13.24	280	16.56
E. coli	3	4.41	133	7.87
Klebsiella pneumoniae	14	20.59	109	6.45
***Acinetobacter* SPP.**	2	2.94	116	6.87
Streptococcus pneumoniae	2	2.94	-	-
Proteus mirabilis	1	1.47	12	0.71
***Serratia* SPP.**	1	1.47	38	2.25
Stenotrophomonas maltophilia	1	1.47	220	13.01
***Enterobacter* Spp.**	2	2.94	38	2.25
Salmonella typhi	1	1.47	-	-
***Pseudomonas* SPP.**	-	-	42	2.48
Enterococcus	-	-	117	6.92
***Brucella* SPP.**	-	-	29	1.71
Yeast	2	2.94	72	4.26

Table 2: Frequency of occurrence of bacterial species isolated from blood cultures of hospitalized patients (based on sex)

Organism		Sex		Total
		Female	Male	
Enterobacter Spp.	No	16	24	40
	Percent	40.0	60.0	100.0
Klebsiella pneumoniae	No	50	73	123
	Percent	40.7	59.3	100.0
Serratia Spp.	No	16	23	39
	Percent	41.0	59.0	100.0
Staphylococcus aureus	No	111	175	286
	Percent	38.8	61.2	100.0
Coagulase negative Staphylococcus	No	174	236	310
	Percent	42.43	57.56	100.0
Enterococcus	No	40	33	73
	Percent	54.8	45.2	100.0
Acinetobacter baumanni	No	32	44	76
	Percent	42.1	57.9	100.0
E.coli	No	71	64	135
	Percent	52.6	47.4	100.0
Stenotrophomonas maltophilia	No	65	156	83
	Percent	29.0	71	100.0
Others	No	167	183	350
	Percent	47.7	52.3	100.0
Total	No	742	1011	1753
	Percent	42.3	57.7	100.0

Table 3: Susceptibility of Gram-positive isolated bacteria to antibiotics

Antibiotics	Coagulase negative Staphylococci	Staphylococcus aureus	Enterococcus	Non hemolytic Streptococci
Amikacin	62.12	45.67	8.82	5.56
Vancomycin	94.69	92.39	48.04	77.78
Co-trimoxazole	36.26	49.48	7.84	33.33
Gentamicin	40.18	41.52	5.88	16.67
Ciprofloxacin	25.17	28.72	8.82	5.56
Clindamycin	37.88	48.44	2.94	33.33
Erythromycin	21.71	40.83	10.78	38.89
Oxacillin	1.39	9.69	0	0
Gloxacillin	36.49	35.29	5.88	22.22
Amoxicillin	0	0.69	27.45	38.89
Ampicillin	0.23	2.42	23.53	44.44

Table 4: Susceptibility of Gram Negative isolated bacteria to antibiotics (Percent)

Antibiotics	*Stenotrophomonas maltophilia*	*E. coli*	*Klebsiella* SPP.	*Acinetobacter* SPP.	*Pseudomonas* SPP.
Amikacin	73.91	85.29	16.26	31.36	60.47
Co-trimoxazole	95.65	33.82	26.83	33.90	6.98
Gentamicin	45.65	41.91	10.57	21.19	32.56
Ciprofloxacin	54.35	21.32	20.33	8.47	39.53
Ceftazidime	46.38	25.74	11.38	15.25	6.98
Imipenem	44.20	38.24	43.90	22.88	30.23
Ceftriaxone	1.45	26.47	7.32	3.39	6.98
Piperacillin / tazobactum	21.74	13.24	12.20	11.86	16.28

Discussion

Blood stream infection (BSI or septicemia) is potentially life threatening (13). The mortality rate of blood stream infection is approximately 27% (2). Isolation and identification of the infectious agent is necessary for proper antibiotic therapy. Delayed or inappropriate antibiotic therapy may cause resistance (14). The impact of specific etiologic agents on the outcome of BSI has been well documented and it clears the need for a better understanding of the spectrum of pathogens causing both nosocomial and community-acquired BSI (2,6). It is abundantly clear that in every hospital surveillance programs are necessary to identify changes in the spectrum of microbial pathogens causing serious infection and to monitor changes in antimicrobial resistance patterns in all infections including BSI (1519-).

In our study, coagulase negative *Staphylococci* were the most frequent cause of BSI in both children and adults followed by *S. aureus*, *S. maltophilia* and *E.coli* in adults, and *K. pneumoniae*, *S. aureus* and *E. coli* in children.

Similar studies in US and Canada (20) reveal the predominance of *S. aureus* (22.8%) followed by *E. coli* (18.8%) and coagulase negative *Staphylococci* (13.3%). In other hand, European arm of SENTRY study (21) found out that *E. coli* (20%), *S. aureus* (17.6%) and coagulase negative *Staphylococci* (17.1%) constituted the three most common causes of blood stream infectious agents.

Elouennass *et al.* (22) worked on the epidemiology and susceptibility profile of blood culture isolates in an intensive care unit and showed that the most frequently identified species were *Acinetobacter baumanni* (13.63%), *S. epidermidis* (12.6%), *S. aureus* (11.9%), and *P. aeruginosa* (7%). *Enterobacteriaceae* accounted for 25.54%.

In a clinical hematology unit, Elouennass *et al.* (23) identified that the most frequently species were *S. epidermidis* (36.4%), *E.coli* (8.7%), *P. aeruginosa* (6.8%) and *S. aureus* (4.9%).

According to antimicrobial susceptibility in Gram-Positive isolated bacteria, we found that more than 90% of *Staphylococci* were vancomycin susceptible. This value was decreased for *Enterococci* and Non-hemolytic *Streptococci*. Except for vancomycin, the two most effective antibiotics for coagulase negative *Staphylococci* were amikacin and gentamycin, for *S. aureus* were co-trimoxazole and clindamycin, for *Enterococci* were amoxicillin and ampicillin and for non- hemolytic *Streptococci* were ampicillin and erythromycin. There was no susceptibility to amoxicillin in coagulase negative *Staphylococci*, as well as no oxacillin susceptible *Enterococci* or non-hemolytic *Streptococci*.

In our study, the most prevalent isolated Enterobacteriaceae, *E. coli*, showed the greatest susceptibility to amikacin and gentamycin. For *S. maltophilia*, which was the third most frequent isolated bacteria and especially was seen in the adults, co-trimoxazole and amikacin were the most effective agents. Fluit *et al.* (21) revealed that all *Staphylococci* remained fully susceptible to vancomycin. In addition, they found that aminoglycoside compounds (in particular, amikacin) provided the best coverage against Enterobacteriaceae, with at least 93.4% of isolates fully susceptible.

Elouennass *et al.* (22) found out that the rate of methicilin resistant *S. aureus* was 52.94%, Enterobacteriaceae were resistance to third generation

cephalosporin in 42.6% and the resistance rate for *P. aeruginosa* to the third generation cephalosporin and imipenem was 16.6% and 10.5%, respectively.

Rahbar *et al*. (24), in a 2-year retrospective study in Urmia, Iran reported that *P. aeruginosa* was the predominant isolate and antibiotic resistances of isolated bacteria were generally quite high.

The changing spectrum of microbial pathogens within the hospital environment and the alarming microbial resistance to antibiotic drugs confirm the need for local surveillance programs and for national programs to monitor the prevalence of resistance (21, 25). Resistance data received from surveillance programs can use as important information for the understanding of the pattern of antibiotic resistance and encourage the physician to discontinue the misuse of antibiotics, a key point in controlling the occurrence and spread of resistance (20).

Conclusion

As the degree of antibiotic resistance rate for blood stream pathogens is alarming, it is mandatory to monitor the susceptibility of these isolates in order to avoid inappropriate use of antibiotics in hospital wards. It is obviously clear that this purpose can really achieve by the close correlation between physicians and laboratory.

Acknowledgments

References

1. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol 2003;41(8):3655-60.

2. Pittet D, Li N, Woolson RF, Wenzel RP. Microbiological factors influencing the outcome of nosocomial bloodstream infections: a 6-year validated, population-based model. Clin Infect Dis 1997;24(6):1068-78.

3. Report of the ASM task force on antibiotic resistance. Antimicrob Agents Chemother 1995;Suppl:123-.:1-23.

4. Nosocomial infection rates for interhospital comparison: limitations and possible solutions. A Report from the National Nosocomial Infections Surveillance (NNIS) System. Infect Control Hosp Epidemiol 1991;12(10):609-21.

5. Mandell G, Bennett J, Dolin R. principles and practice of infectious disease. 5 ed. Philadelphia, PA: Churchill Livingstone; 2000.

6. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, *et al*. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 1997;24(4):584-602.

7. Diekema DJ, Pfaller MA, Jones RN, Doern GV, Winokur PL, Gales AC, *et al*. Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. Clin Infect Dis 1999;29(3):595-607.

8. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1997, issued May 1997. A report from the NNIS System. Am J Infect Control 1997;25(6):477-87.

9. Isenberg H. Clinical microbiology procedures handbook. 2 ed. Washington, DC: American Society for Microbiology; 1992.

10. Murray P, Baron E, Pfaller M, Tenover F, Yolken R. Manual of Clinical Microbiology. 7 ed. Washington, DC: American Society for Microbiology; 1999.

11. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A4. Wayne, PA: NCCLS; 1997

12. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved Standard M2-A6. Wayne, PA: NCCLS; 1997.

13. Kieft H, Hoepelman AI, Zhou W, Rozenberg-Arska M, Struyvenberg A, Verhoef J. The sepsis syndrome in a Dutch university hospital. Clinical observations. Arch Intern Med 1993 Oct 11;153(19):2241-7.

14. Leegaard TM, Bevanger L, Jureen R, Lier T, Melby KK, Caugant DA, *et al*. Antibiotic sensitivity still prevails in Norwegian blood culture isolates. Int J Antimicrob Agents 2001;18(2):99-106.

15. Paul SM, Finelli L, Crane GL, Spitalny KC. A statewide surveillance system for antimicrobial-resistant

bacteria: New Jersey. Infect Control Hosp Epidemiol 1995;16(7):385-90.

16. Archibald L, Phillips L, Monnet D, McGowan JE, Jr., Tenover F, Gaynes R. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis 1997;24(2):211-5.

17. Jones RN. The emergent needs for basic research, education, and surveillance of antimicrobial resistance. Problems facing the report from the American Society for Microbiology Task Force on Antibiotic Resistance. Diagn Microbiol Infect Dis 1996;25(4):153-61.

18. Jones RN, Kehrberg EN, Erwin ME, Anderson SC. Prevalence of important pathogens and antimicrobial activity of parenteral drugs at numerous medical centers in the United States, I. Study on the threat of emerging resistances: real or perceived? Fluoroquinolone Resistance Surveillance Group. Diagn Microbiol Infect Dis 1994;19(4):203-15.

19. Osterholm MT, MacDonald KL. Antibiotic-resistant bugs: when, where, and why? Infect Control Hosp Epidemiol 1995;16(7):382-4.

20. Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). Antimicrob Agents Chemother 1998;42(7):1762-70.

21. Fluit AC, Jones ME, Schmitz FJ, Acar J, Gupta R, Verhoef J. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. Clin Infect Dis 2000;30(3):454-60.

22. Elouennass M, Sahnoun I, Zrara A, Bajjou T, Elhamzaoui S. Epidemiology and susceptibility profile of blood culture isolates in an intensive care unit (2002-2005). Med Mal Infect 2008;38(1):18-24.

23. Elouennass M, Foissaud V, Trueba F, Doghmi K, Malfuson JV, Fagot T, *et al*. [A 7-year survey of strains identified in blood cultures in a clinical hematology unit]. Med Mal Infect 2004;34(2):62-9.

24. Rahbar M, Gra-Agaji R, Hashemi S. Nosocomial blood stream infections in Imam Khomeini Hospital, Urmia, Islamic Republic of Iran, 1999-2001. East Mediterr Health J 2005;11(3):478-84.

25. Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000. Int J Antimicrob Agents 2005;26(5):373-9.