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Short Communication

THE PREVALENCE OF BACTERIA ISOLATED FROM BLOOD CULTURES OF IRANIAN CHILDREN AND STUDY OF THEIR ANTIMICROBIAL SUSCEPTIBILITIES

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Abstract

Infections cause significant mortality and morbidity in children. Although there are various antibiotics that can be used to combat bloodstream infection, resistant strains have subsequently emerged. A prospective study was carried out for one month period in 55 hospitals from 12 provinces representing different geographical areas in Iran. The aim was to determine the pattern of pathogens and their antimicrobial sensitivity among children. Of 1143 blood specimens tested from various hospitals, bacteria were isolated from 715 of children from one month to three years old age. The predominant isolated pathogens from blood specimens were Gram-positive bacteria in 301 (65.25%) patients, mainly 198 (65.78%) coagulase-negative *Staphylococci*. The Gram-negative bacteria were 140 (31.74%). About 66% of the Gram-positive bacteria were coagulase-negative *Staphylococci*, while the gram-negative bacteria were mainly (*Escherichia coli, Enterobacter spp, Klebsiella pneumoniae,* and *Pseudomonas spp.*). The majority of *S. aureus* were resistant to Oxacillin, Co-trimoxazole, Cephalotin, and Ampicillin. About two – third of the *E. coli* and *K. pneumonia* were resistant to Cephalotin.

Keywords:

Blood Stream Infection, *S. aureus*, Antibiotic, Resistance, Children.

Introduction

Bloodstream infections (BSI) are potentially life-threatening and require rapid identification and also antibiotic susceptibility testing of the causative agent in order to facilitate specific antimicrobial therapy (1). Despite advances in antimicrobial therapy and supportive care, bacterimia continues to be a major cause of morbidity and mortality among children. In developing countries, more than 14 million deaths of children under five years of age (2). occur during the childhood, with infections accounting for up to 70% of total mortality for this age group (3-4).

Around the world is estimated 10 million children under the age of 5 years die each year, the vast majority (90%) in a mere 42 countries. Of the major causes of death among children, are infections such as newborn sepsis (5). The organisms responsible for bacteremia vary across geographical boundaries. Organisms like *E. coli , Klebsiella spp., Staphylococcus aureus*, Coagulase negative *staphylococci* (CoNS), *Pseudomonas spp., Salmonella spp*. and *Acinetobacter spp*. are potential pathogens in bacterimia because of their frequent isolation and multi-drug resistance which has reached worrying levels (6-7).

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Furthermore, few epidemiological studies of bacterimia undertaken in the Middle East and in other developing countries (8 -9) have shown important differences in the pattern of antibiotic susceptibility of gens compared with studies in patho- European and American countries (10-12). The emergence of antimicrobial resistance is recognized as a major contributor to excess morbidity and healthcare costs in developed countries. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (13-14). For practising physicians, clinical microbiologists and public health officials, knowledge of local antimicrobial resistance patterns is essential to guide empirical and pathogen-specific therapy. This informa tion is also critical for optimal decisions regarding hospital formulary and infection control policies, for the rational formulation of public healthcare policies, and national and international research agendas in that area. Unfortunately, data regarding endemic antimicrobial resistance are unavailable in many parts of the world, especially from areas where over-the-counter antibiotic use is common.

In the USA alone, 10–20% of nosocomial infections are estimated to involve the bloodstream, resulting in 90 000 fatal cases each year. Appropriate antimicrobial treatment of BSIs is critical in decreasing morbidity and mortality due to BSIs (15).

In developed countries, nationwide surveillance programmes such as the National Nosocomial Infections Surveillaance (NNIS) System monitor the prevalence of bacterial pathogens and their antimicrobial resistance patterns and periodically publishes reports (16).

Unfortunately, in many parts of the world, particularly Iran, such national surveillance programmes are absent and information regarding the prevalence of BSI pathogens and their antimicrobial susceptibility patterns is scarce.

To shed some light on the frequency and susceptibility patterns of endemic BSI pathogens in Iran, we investigated the predominant pathogens responsible for blood infection and antimicrobial susceptibility patterns of these isolates from children in 55 hospitals representing different geographical areas in Iran during one month period (July to August), 2006.

Materials and methods

Study design

It is a routine practice in our standard hospitals, to disinfect skin with 70% alcohol followed by 2% povidone-iodine before collecting blood samples. Blood samples were transferred to blood culture media (Saba Company, Iran) and immediately transported to the hospital's microbiology laboratory. All blood cultures were incubated at 35 °C for at least 2 weeks. Negative bottle cultures were discarded.

Organism isolation and identification

Isolates were identified by gram staining and conventional biochemical tests (17).

Susceptibility testing

Antimicrobial susceptibility testing was done by disc diffusion method according to the Clinical and Laboratory Standard Institute (18) using the following antibiotics from Patan Teb, Iran: Oxacillin, Penicillin, Erythromycin, Cipr- ofloxacin, Ceftriaxone, Co-trimoxazole, Gentamicin, Cephalotin, Vancomycin, Amikacin, Cephradine, and Ampicillin.

Statistical analysis was performed using SPSS (Version 9) by Chi- square.

Results

Frequency of occurrence among blood culture microorganisms

A total of 441 bacteria were isolated from 715 blood samples taken from children (less than one month to five years old) who refered to 55 different hospitals in Iran (Fig. 1) during July to August 2006.

Table 1 shows the most frequently isolated bacterial pathogens causing BSI in these hospitals. In all hospitals, the average number of Coagulase negative *staphylococci* and *E. coli* isolates were 198 (65.78%) and 60 (42.85%), respectively. Others isolates were *S. aureus* 73 (24.30%) and *Enterobacter sp* 38 (27.20%). These four group of organisms accounted for approximately 83.67% of all BSI in the 55 hospitals in Iran. *K. pneumoniae*, *S. pneumoniae*, and *P. aeruginosa*, were also frequently reported species at all hospitals.

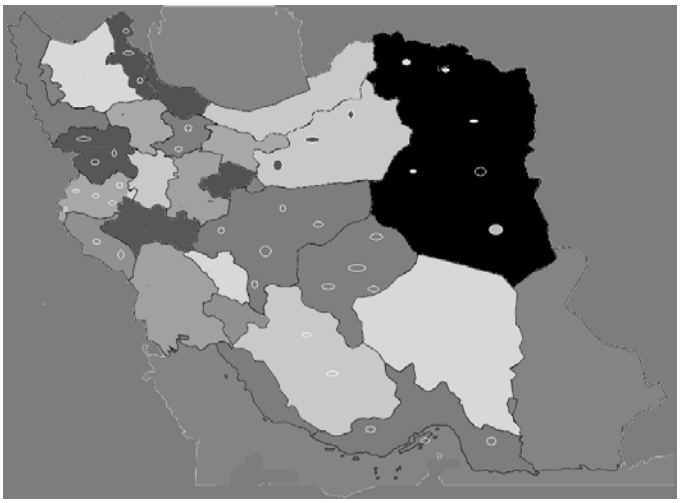

Fig. 1: Locations of hospital participants in Iran.

Table 1: Bacterial species recovered from blood cultures*

Gram negative bacteria	**Number of strains (%)**
E. coli	60 (42.9)
Enterobacter sp	38 (27.2)
Klebsiella pneumoniae	21 (15.0)
Pseudomonas aeruginosa	14 (10.0)
Other Gram negative bacteria	7.0 (5.0)
Total Gram negative bacteria	140 (100)
Gram positive bacteria	
Coagulase negative Staphylococci	198 (65.8)
Staphylococcus aureus	73 (24.3)
Streptococcus pneumoniae	21 (7.0)
Other Gram positive bacteria	9.0 (3.0)
Total Gram positive bacteria	301 (100)

*The etiological agents were isolated and identified based on Gram staining and conventional biochemical tests.

Antimicrobial susceptibility of bacterial isolates

The results of susceptibility testing of Gram-positive isolates from blood cultures are summarized in Table 2. In vitro susceptibility testing of the 140 Gram-negative isolates against ciprofloxacin and amikacin revealed that 78% and 69% were susceptible (Table 3). Of 14 *Ps. Aeruginosa*, 85.8%, 79.8 % and 78.6% were resistant to Co-trimoxazole, Ampicillin and Cephradine, respectively.

Table 2: Antimicrobial resistance pattern of Gram-positive bacteria isolated from blood cultures of children in Iran at 55 Hospitals

Antibiotics	Coagulase negative *staphylococci*	*S. aureus*	*S. pneumoniae*
	% of isolates resistant to antimicrobial agents		
Oxacillin	31.9	63.1	47.7
Penicillin	59.0	65.8	52.4
Erythromycin	64.2	59.0	Not done
Ciprofloxacin	10.2	27.4	Not done
Ceftriaxone	33.4	17.9	Not done
Co-trimoxazole	54.1	71.3	57.2
Gentamicin	59.0	Not done	71.5
Cephalotin	67.2	75.4	71.5
Vancomycin	14.2	28.8	19.1
Amikacin	32.4	28.8	Not done
Ampicillin	78.3	89.1	33.4

Antimicrobial susceptibility testing was done by disc diffusion assay according to the Clinical and Laboratory Standard Institute (CLSI 2005) using antibiotics from Patan Teb Company, Iran.

Table 3: Antimicrobial resistance pattern of Gram-negative bacteria isolated from blood cultures of children in Iran at 55 Hospitals

Antibiotics	*E. coli*	*Enterobacter sp*	*K. pneumoniae*	*Ps. aeruginosa*
	% of isolates resistant to antimicrobial agents			
Ciprofloxacin	23.4	21.1	19.1	Not done
Amikacin	31.7	Not done	9.6	35.8
Co-trimoxazole	73.4	65.8	71.5	85.8
Cephradine	60.0	Not done	66.7	78.6
Cephalotin	85.0	55.3	85.8	71.5
Ampicillin	75.0	65.8	66.7	79.8%
Ceftriaxone	56.7	42.2	66.7	Not done
Gentamycin	36.7	47.4	57.2	71.5
Erythromycin	65.0	60.6	69.0	64.3

Antimicrobial susceptibility testing was done by disc diffusion assay according to the Clinical and Laboratory Standard Institute (CLSI 2005) using antibiotics from Patan Teb Company, Iran.

Discussion

According to global surveillance reports, bloodstream isolates are the best candidates for the study of antimicrobial susceptibility of human bacterial pathogens (19, 21). Unfortunately, data regarding frequency of isolation and resistance to antimicrobial agents are scarce in Iran.

In the current study, coagulase-negative staphylococci were the most common followed by *Staphylococcus aureus* as in others (22, 23). *S. aureus* and *E. coli* were identified in many studies as the two most common blood culture isolates from hospitalized patients in the United States and Europe (24, 25).

In 2005, Mamishi et al. (22) reported a predominance of gram-positive isolates 72% from children in a Children's Medical Center, Tehran: the most common of which was Coagulase-negative staphylococci which accounted for 48.4%. As shown in Table 1 our results agree generally with those previously published (25, 26).

The need for reliable and comprehensive data regarding antimicrobial susceptibility patterns of bloodstream isolates specific to Iran prompted this investigation. Our data bring to light the fact that a serious problem of antimicrobial resistance exists among bloodstream isolates in Iran.

The microbiological spectrum of septicaemia among children shows marked geographical variations. There have been many instances of gram-negative and gram positive outbreaks among children around the world (25, 26).

Among Gram-positive isolates, CoNS, *S. pneumoniae* and *S. aureus* were only 14.2%, 19.1% and 28.8% resistant to vancomycin. Resistance to Co-trimaxazole among *Escherichia coli*, the most common Gram-negative isolate was 73.4%, and to cephalotin and Ampicillin were 85 % and 75% respectively.

Oxacillin-resistant *S. aureus* are extremely important causes of bloodstream infections and evidence has been presented that oxacillin-resistant *S. aureus* (Table 2) are increasing globally among bloodstream isolates and among isolates from other anatomical sites (20, 27).

In conclusion, it is essential to evaluate prospectively the distribution of bacterial isolates from blood, and given the high level of resistance to used antibiotics, there is need for clinical trials to determine the most feasible combination of antibiotics (cheapest, most effective, given orally) for the management of bacteremia among Iranian children. Our report draws attention to the importance of the awareness of physicians in identifying resistant bacteria during treatment of children with BSI and underscores the need for devising a national strategy to control the spread of resistance in Iran.

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References

1. Berit EC, Maria PS, Jörg G, Salima M, Martin K, Oleg K. Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. J. Clin. Microbiol. 2006; 44(7): 2389-397.
2. UNICEF. The state of the world's children. Oxford, Oxford University Press, 1994.
3. Mylotte JM, Tayara A. Blood cultures: clinical aspects and controversies. Eur. J. Microbiol. Infect. Dis. 2001; 9: 157–63.
4. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bactere-

mia and fungemia. Clin. Microbiol. Rev. 1997; 10: 444-65.
5. Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS. Bellagio Child Survival Study Group. How many child deaths can we prevent this year? *Lancet.* 2003; 362: 65-71.
6. Castagnola E, Caviglia I, Pistorio A, Fioredda F, Micalizzi C, Viscoli C. Blood stream infections and invasive mycoses in children undergoing acute leukaemia treatment. Eur. J. Cancer. 2005; 41(10): 1439 – 445.
7. Wise R, Hart T, Cars O, Streulens M, Helmuth R, Huovinen P. Antimicrobial resistance is a major threat to public health. Brit. Med. J. 1998; 317: 609–10.
8. Haque KN, Chagia AH, Shaheed MM. Half a decade of neonatal sepsis, Riyadh, Saudi Arabia. J. Trop. Pediatrics. 1990; 36: 20–23.
9. Dawodu A, Alumran K, Twum-Danso K. A case control study of neonatal sepsis: Experience from Saudi Arabia. J. Trop. Pediatrics. 1997; 43: 84–88.
10. Hadziyannis AS, Stephanou I, Dimarogona K, Pantazatou A, Fourkas D, Filiagouridis D, Avlami A. Blood culture results during the period 1995–2002 in a Greek tertiary care hospital. Clin. Microbiol. Infect. 2004; 10: 667-70.
11. Elward AM, Fraser VJ. Risk factors for nosocomial primary bloodstream infection in pediatric intensive care unit patients: a 2-year prospective cohort study. Infect. Cont. Hosp. Ep. 2006; 27(6): 553-60.
12. Stover BH, Shulman ST, Bratcher DF, Brady MT, Levine GL, Jarvis. Nosocomial infection rates in US children's hospitals' neonatal and pediatric intensive care units. Am. J. Infect. Control. 2001; 29(3): 152-57.
13. Shlaes DM, Gerding DN, John JF, Craig WA, Bornstein DL, Duncan RA. Society for healthcare epidemiology of America and infectious diseases society of America joint committee on the prevention of antimicrobial resistance. Clin. Infect. Dis. 1997; 25: 584–99.
14. 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). Antimicrob. Agents. Ch. 1997; 42: 1762–770.
15. American Society for Microbiology Report of the ASM Task Force on Antibiotic Resistance. Antimicrob. Agents. Ch. 1995; 1: 1–23.
16. 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: 609–21.
17. Forbes BA. Bailey & Scott's diagno stic microbiology, 10th ed. St. Louis, Missouri, Mosby. 1998: 283–304.
18. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial disk susceptibility tests. NCCLS documents M 100 – SIS, 940 West Valley Road. Wayne, PA, USA, 2005; 19087.
19. Fluit A, Jones M, Schmitz F, 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: 454-60.
20. Diekema DJ, Pfaller MA, Jones RN. Trends in antimicrobial susceptibility of bacterial pathogens isolated from patients with bloodstream infections in the USA, Canada and Latin America. SENTRY Participants Group. Int. J. Antimicrob. Ag. 2000; 13: 257–71.

21. Von Eiff, Becker K, Machka K, Stammer HPG. Nasal carriage as a source of *Staphylococcus aureus* bacteraemia. New. Engl. J. Med. 2001; 344: 11–16.
22. 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. Ag. 2005; 6(5): 373-79.
23. Amita J, Indranil R, Mahendra KG, Mala K, Agarwal SK. Prevalence of extended-spectrum ß-lactamase- proucing gram-negative bacteria in bacteremiacaemic neonates in a tertiary care hospital. J. Med. Microbiol. 2003; 52: 421-25.
24. Weinstein M, Reller L, Murray J, Lichtenstein K. The clinical signifycance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. Laboratory and epidemiologic observations. Rev. Infect. Dis. 1983**;** 5: 35–53.
25. Yu JL, Wu SX, Jia HQ. Study on antimicrobial susceptibility of bacteria causing neonatal infections, a 12 Year Study(1987 - 1998). Singapore Med. J. 2001; 42(3): 107-10.
26. Ni-Chung L, Shu-Jen C, Ren-Bin T, Be-Tau H. Neonatal bacteremia in a Neonatal Intensive Care Unit: analysis of causative organisms and antimicrobial susceptibility. J. Chin. Med. Assoc. 2004; 67: 15-20.
27. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program 1997–1999. Clin. Infect. Dis. 2001**;** 32: 114–32.