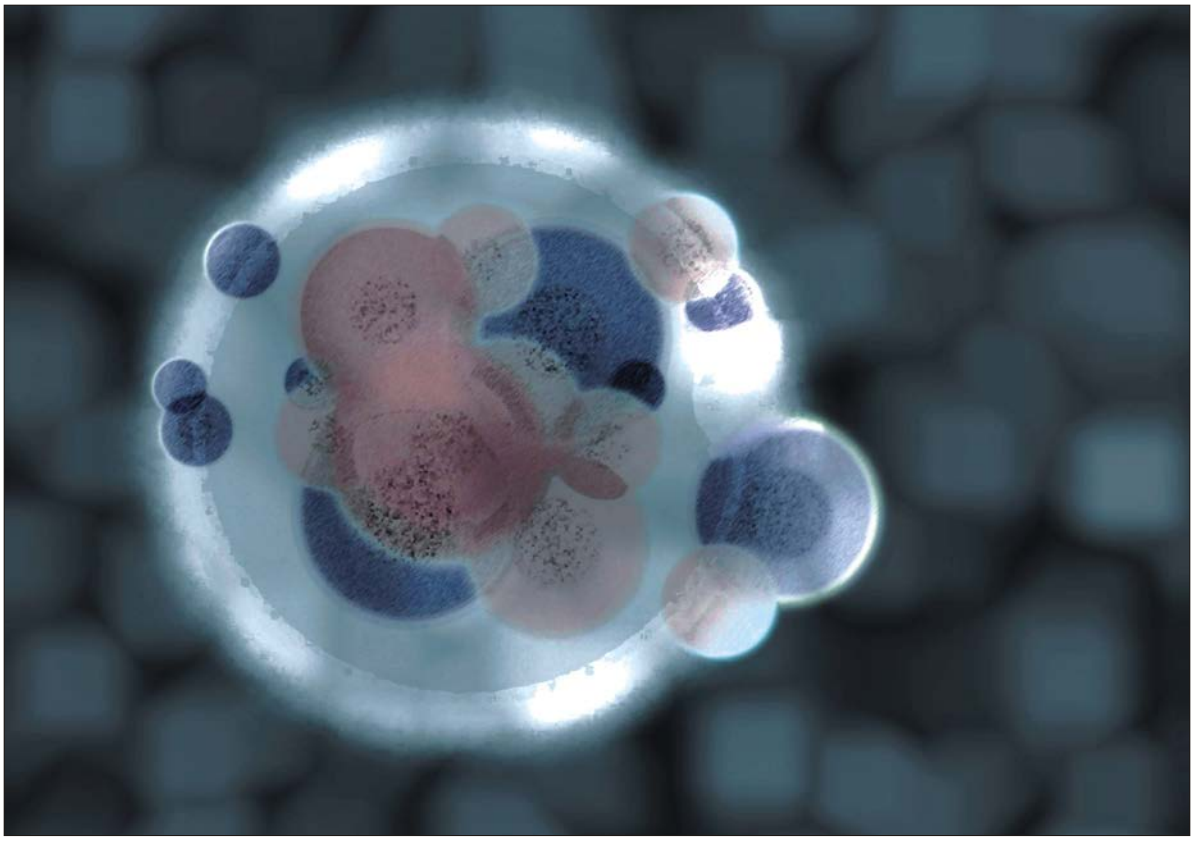

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Study of Prevalence and Antimicrobial Susceptibility Pattern of Bacteria Isolated from Blood Cultures

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Abstract: The aim of present study was to investigate the type of bacteria isolated from blood cultures and determination of their antibiotic susceptibility pattern. During 18 months, 2790 blood culture samples were screened. The positive blood cultures were examined and the organisms were identified as per standard procedures. Antimicrobial susceptibility testing was performed for all isolates by use of disk diffusion technique, according to CLSI guidelines. From total blood culture samples, 155 (5.6%) were positive. The most common isolated gram negative bacilli were *Klebsiella pneumoniae* 52 (33.5%), *Eschericia coli* 32 (20.6%) and *Enterobacter* sp. 15 (9.7%) and coagulase negative staphylococci (CONS) as predominant gram positive cocci, all the isolated bacteria showed the highest degree of resistance to ampicillin (98.7%), cefalexin (70.3%) and trimethoprim- sulfamethoxazole (69.7%). Gram positive cocci were also fully resistant to penicillin. In conclusion, present study revealed that both gram positive and gram negative bacteria were responsible for bloodstream infections and most of the strains were multi-drug resistant. The most common isolated bacteria from blood cultures were *Klebsiella pneumoniae* and *E. coli*. Ciproflexacine was the most effective antibiotic against gram negative bacilli, while vancomysin was mostly effective against gram positive cocci.

Key words: Blood culture, bacteria, susceptibility testing, antimicrobial

INTRODUCTION

Bloodstream infections cause significant morbidity and mortality worldwide and are among the most common healthcare-associated infections (Karlowsky *et al*., 2004). Amongst them, neonatal sepsis is one of the most common reasons for admission to neonatal units in developing countries (Anwer *et al*., 2000; Joshi *et al*., 2000). It is also a major cause of mortality in both developed and developing countries (Bhutta and Yusuf, 1997; Orrett and Shurland, 2001).

Despite important progresses in treatment and prevention of infectious diseases, they are considered as leading causes of death and disability and worsening life quality especially for millions of people in developing countries. Bacteremia has an increasing trend in some regions of the world (Madsen *et al*., 1999). The isolated bacteria are numerous (Reacher *et al*., 2000; Cohen, 1997) and their associated diseases need urgent and invasive management with antimicrobial drugs. Rational and correct use of these agents requires understanding of common pathogens and drug resistance pattern in the region (Reacher *et al*., 2000). Nowadays, bacterial drug resistance is an important problem and due to wide variations in bacterial drug resistance, results of studies and reports in one region or in a period of time are not necessarily true for other regions or periods of time (Huang *et al*., 2002). They are related with a series of social, environmental and technological changes (Cohen, 1997).

The surveillance of bloodstream pathogens in a hospital is important in monitoring the spectrum of microorganisms that invade the bloodstream and the types of organisms associated with a particular clinical discipline. Such data are often used to determine empiric antibiotic therapy and also to alert clinicians to emerging pathogens that may pose a threat to the community.

Bloodstream bacterial infections have been shown to have mortality rates of between 20 and 50%. An association between the type of bloodstream organism and prognosis of the patient has been shown, with the

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isolation of enterococci, gram negative bacteria and fungi being associated with increased mortality (Karunakaran *et al*., 2007).

Rapid detection and identification of clinically relevant microorganisms in blood cultures is very essential and determination of antimicrobial susceptibility pattern for rapid administration of antimicrobial therapy has been shown to reduce the morbidity and mortality associated with bloodstream infections (Mehta *et al*., 2005). So, this study was undertaken to investigate the type of bacteria isolated from cases of bloodstream infections and determination of their antibiotic susceptibility pattern.

MATERIALS AND METHODS

We studied 2790 blood culture samples collected from different wards in Imam Khomeini teaching hospital, Ahwaz, Iran, during 18 months from October 2006 to March 2008. The samples were collected in standard blood culture bottles (Padtan Teb, Tehran, Iran) containing Trypticase soy broth and were incubated at 37°C for up to 7 days or until growth was detected. Bottles were observed macroscopically daily for visible evidence of bacterial growth such as turbidity. Gram stain was performed for macroscopically positive blood samples and subcultures were made onto Blood agar, Chocolate agar and MacCankey agar (Himedia, Mumbai, India). The media were incubated at 37°C for 24 h. The positive blood cultures were examined after then and the necessary biochemical tests were conducted and the organisms identified as per standard procedures (Forbes *et al*., 2002). All the negative blood cultures were kept incubated for 21 days before discarding. Antimicrobial susceptibility testing were performed for isolated organisms by Kirby-Bauer's disk diffusion according to Clinical and Laboratory Standards Institute (CLSI) (2002) guideline. Antibiotic disks were used and the susceptibility pattern of isolated pathogens to commonly used antibiotics were then reported. The antibiotic disks (Padtan Teb, Tehran, Iran) and their concentrations per disk (μg) comprised: penicillin (10), ampicillin (10), cefalexin (10), trimethoprim-sulfamethoxazole (25), cefotaxime (10), gentamycin (10), amikacin (10), ciprofloxacine (30), ceftriaxone (30) and vancomycin (30).

RESULTS

From total blood culture samples screened during the study period, 155 (5.6%) were positive for bacterial growth. These were belonged to 79 adults (51%) comprising 44 male patients (28.4%) and 35 (22.6%) females and also 76 (49%) newborn infants.

The microorganisms which were recovered from blood cultures were: 134 (86.5%) gram negative bacilli and 21 (13.5%) gram positive cocci.

The most common isolated gram negative bacilli were *Klebsiella pneumoniae* 52(33.5%) of which 30 (57.7%) were isolated from newborns, *Eschericia coli* 32(20.6%) and *Enterobacter cloacae* 15(9.7%) and the least common were *Serratia marcescens*, *Citrobacter freundii* and *Proteus mirabilis* each, one cases (0.64%). Among gram positive cocci, coagulase negative staphylococci (CONS) were predominant organisms as shown in Fig. 1.

Klebsiella pneumoniae and *E. coli* were the most common organism isolated from all patients, *Klebsiella pneumoniae* with the highest percentage in newborns (Fig. 2).

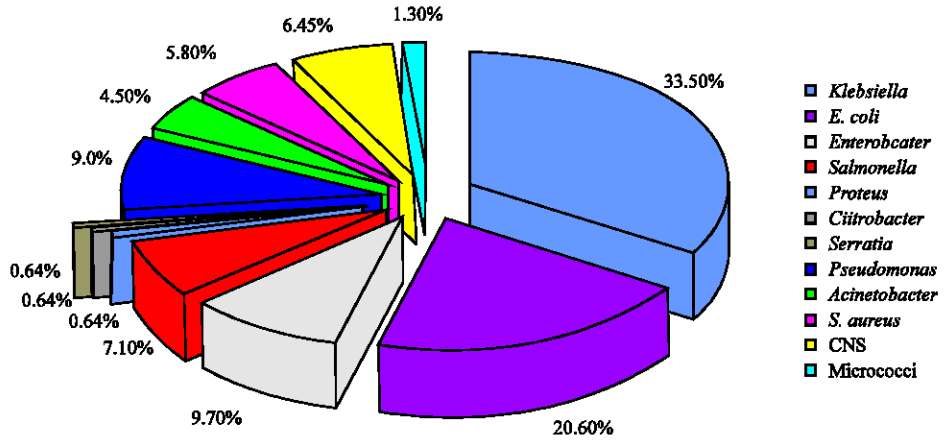

Fig. 1: Frequency of bacteria isolated from blood cultres. CNS: Coagulase negative staphylococci

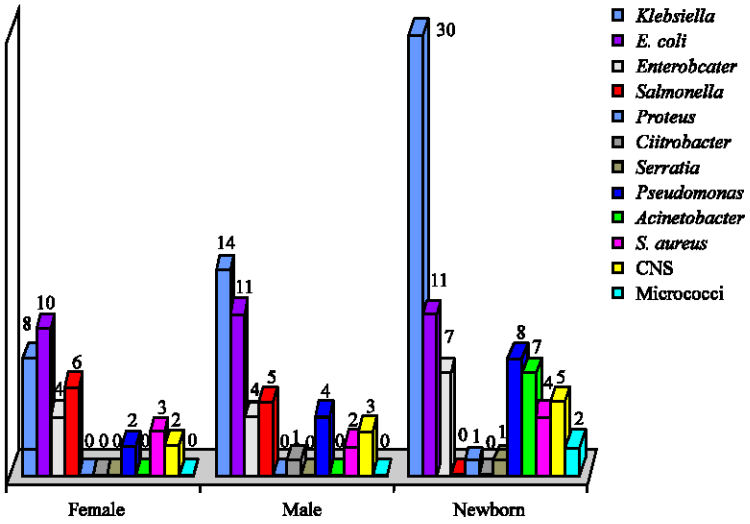

Fig. 2: Frequency and distribution of bacterial isolates from blood cultures in adults and newborns. CNS: Coagulase negative staphylococci

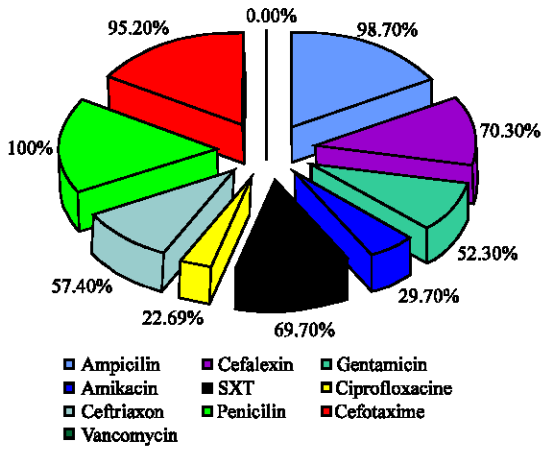

Fig. 3: Antibiotic resistance patten of bacteria isolated from blood cultures. CNS: Coagulase negative staphylococci

Based on the results from susceptibility testing, all the bacteria recovered from blood culture showed the highest degree of resistance to ampicillin (98.7%), cefalexin (70.3%) and trimethoprim-sulfamethoxazole (69.7%). The gram negative bacilli showed high degree of resistance to commonly used antibiotics with the highest to ampicillin (98.5%), cefalexin (71.6%) and trimethoprim-sulfamethoxazole and comparatively low resistance to ciprofloxacine (22.4%) and amikacin (28.4%). Gram positive cocci were fully resistant to the penicillin and ampicillin and were highly resistant to cefotaxime (95.2%), but were fully sensitive to vancomycin (Fig. 3).

DISCUSSION

Study of bacteriological profile with antibiotic susceptibility pattern plays an important role in effective management of bacteremia cases (Anbumani *et al*., 2008). This study revealed that 155 (5.6%) out of 2790 total samples screened were positive for the presence of bacteria. Sobhani *et al*. (2004) were examined 4800 cases of blood cultures and among them there was 311 positive cultures with the relative frequency of 6.5%. Their study was similar to present study in detecting the low rate of bacteremia. Besides present findings was in agreement with study of Anbumani *et al*. (2008) with reported frequency of positive blood cultures as 7.89%. In their study, the number of detected positive blood cultures was 357 cases among the total of 4521 tested samples. In controversy to present study, the frequency of positive blood cultures was higher in some other reported studies. Mehta *et al*. (2005) were detected 567 positive blood cultures among the total of 5704 screened samples and reported a frequency of 9.94% positive blood cultures. The detection rate of positive blood cultures was 509 among 2542 tested blood samples with frequency of 20.02% in study of Arora and Devi (2007). Roy *et al*. (2002) were examined 728 blood samples for neonatal septicemia and out of these, 346 were positive for bacterial growth. So, in their study the frequency was reported as 47.5% which was quite high. The high frequency of positive blood cultures was reported as 33.9% in another study (Khanal *et al*., 2002).

The number of obtained blood cultures was 3 for adults and 2 for newborn in a 24 h period in this study.

Present detection rate were as approximately 97 and 95%, respectively. The variation in blood culture positivity is related to different factors such as the number and amount of blood cultures taken for screen as reported by Lee *et al.* (2007). They believed that for achieving a detection rate of >99% as many as four blood cultures may be Cockerill *et al.* (2004). Similar comment was made by other investigators that more than three blood cultures are needed for 99% test sensitivity. The system and type of blood culture medium formulation used for bacterial detection are other factors affecting the final bacterial yields. Besides, another alternative explanation for lower level bacteremia as seen in this study, may be related to effective antibiotic therapy prior to taking blood for culture.

Gram negative bacteria were the most common organisms isolated in present study accounting 86.5% of total isolated bacteria and amongst them *Klebsiella pneumoniae* and *E. coli* were the most predominant organisms. There are earlier studies in agreement to present findings, which reported *Klebsiella pneumoniae* as the most common cause of bacteremia (Kumar *et al.*, 2004; Kang *et al.*, 2005). In most of the studies, gram negative bacilli have taken over the gram positive organisms especially in hospital settings. Mehta *et al.* (2005) have been reported the incidence of 80.96% for gram negatives and 18% for gram positives which was similar to present findings. The prevalence of CONS was not high in present study. This is the major contaminant in blood culture as previously reported by Lee *et al.* (2007). The incidence of CONS was reported as 33% of total blood culture tested by Karunakaran *et al.* (2007).

Most of the gram negative bacilli in present study were multi-drug resistant. The most common resistance was seen to ampicillin in all isolated bacteria. Other studies have also reported similar multi-drug resistance for their isolated gram negatives (Kumar *et al.*, 2004; Arora and Devi, 2007). About the gram positive cocci, while, *S. aureus* isolates were fully sensitive to vancomycin, amikacin and ciprofloxacine and the majority were also sensitive to gentamycin and cefalexin, the CONS isolates were highly resistant and except for vancomycin and partial sensitivity to ciprofloxacine, showed resistance to all tested antibiotics. Overall, vancomycin showed the highest activity against *S. aureus* and CONS as other investigators have been reported by Mehta *et al.* (2005). Since, the CONS strains are normally acquired from the hospital, which the nosocomial strains show more antibiotic resistance compared to community counterparts, this finding may explain the high resistance pattern of our isolated CONS.

Based on the results from present study, it is concluded that both gram positive and gram negative bacteria were responsible for bloodstream infections and most of the strains were multi-drug resistant. The most common isolated bacteria from blood cultures were *Klebsiella pneumoniae* and *E. coli*. Ciproflexacine and vancomycin were the most effective antibiotics against gram negative bacilli and gram positive cocci, respectively. To reduce the incidence of bloodstream infections appropriate use of antibiotics according to the standard antimicrobial susceptibility testing is essential.

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