6A. DISCUSSION

The pharmacotherapy of septicemia has emerged as a challenge for clinicians in developing countries like India because of increasing bacterial resistance. The data from the present study conducted at a University Hospital in Delhi between 2004-2008 gives information regarding the causative organisms of septicemia and their resistance patterns to common antibacterials. Analysis of data revealed that both the Gram-positive and Gram-negative bacteria were responsible for bloodstream infections (BSIs). From the antimicrobial resistance data, it is also clear that bacterial resistance is prevalent in Delhi region which further emphasizes the importance of antimicrobial susceptibility testing of BSIs prior to antimicrobial therapy. The present data indicates that the prevalence of bloodstream infection (BSI) pathogens in Delhi region differs from earlier reported studies in India and other foreign countries.

Pediatric cases were consequently more predominant than adult cases in the present study [Table 10]. Kumar and Rizvi (2009) from Maulana Azad Medical College New Delhi, India reported more septicemia cases in pediatrics than neonates. In a nationwide survey in United States of America, Watson, et al. (2003) reported more cases of septicemia in pediatric group of patients, which conforms the present findings. The higher occurrence in childhood septicemia has been reported from different parts of Nigeria (Angyo et al., 2001; Adegoke, 2008). The present findings revealed that the disease affects all age groups but it was noticeable that children and neonates are more vulnerable than adults as persons between years of 0-14 years were most commonly infected. The high incidence of pediatric septicemia in Delhi needs further investigation.

Septicemia was found to be clearly predominant in male as compared to female patients in the five years study period [Table 11], [Figure 2]. Study from Chandigarh, North India reported similar findings (Mehta, Dutta and Gupta, 2005). A retrospective cohort study from Los Angeles, USA, reported higher proportion of septicemia in males (56.5%) than in females (43.5%) out of 3975 septicemia patients (Nissenson, et al., 2006). Similar findings were reported from Italy that included more cases of septicemia in males 53% as compared to females 47% (Gallucci, et al., 2005). Whereas, a recent study from Chandigarh, India reported septicemia more in females
(68%) as compared to males (32%) out of 216 septicemia cases following burns (Sharma, Harish, Singh and Bangar, 2006). Young females might be more exposed to social and family stress that leads to burn victim so they are having more septicemia than males. Furthermore, the existing dowry system might be another reason for more septicemia in females than in males (Sharma, Harish, Singh and Bangar, 2006).

As per the literature females have a more active humoral and cell-mediated response than males (Grossman, 1989). Sex steroids influence sepsis. Studies have shown that 17-beta-estradiol has salutary effects on immune functions after trauma-hemorrhage (TH). Five alpha-dihydrotestosterone treated mice showed a depressed pro-inflammatory cytokine production after TH-sepsis in both splenic macrophages (SMphi) and peripheral blood mononuclear cells (PBMC). The 17-beta-estradiol treated groups showed suppressed pro-inflammatory cytokine production in the PBMC population. In summary, 17-beta-estradiol was able to prevent immune dysfunction after TH and subsequent septicemia (Dienstknecht et al., 2004).

Estrogens may be able to reduce the devastating inflammation associated with acute overactive host responses such as septic shock without compromising long term defense against infectious organisms. Prolactin present in females’ acts as immunomodulating hormone might be one of the reasons for fewer septicemias in females than in males (Zellweger et al., 1996).

Septicemia occurring largely in males than females could also reflect a gender bias in presentation to the hospital for care. Males more frequently visit the hospitals for any medical problem, being the only earning members in most of the Indian families. Population based studies would be needed to address this important question.

In the current study the incidence of septicemia (21.5%) in admitted patients was found to be higher than those reported from Chandigarh, North India (cal. 13.2%) (Kaistha, et al., 2009). Lower incidence of septicemia (12%) than the present study has been reported from 17 ICUs of European countries (Vincent, et al., 1995) and in Iran (9.1%) (Mamishi, Pourakbari, Ashtiani and Hashemi, 2005). A study from Calabar, Nigeria reported the incidence of septicemia as (45.9%) which was higher than the present study (Meremikwu, et al., 2005).

Gram-negative organisms were predominant during early two years of the study period, however the trend was changed during the later period, when Gram-positive
organisms were more predominant [Table 13]. But the studies that were conducted in Chandigarh, reported a predominance of Gram-negative organisms (Agnihotri, Kaistha and Gupta, 2004; Kaistha, et al., 2009). These changing trends clearly indicate that the microorganisms implicated in septicemia change with change of time. In Delhi region, coagulase-negative staphylococci, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were the predominant Gram-positive and Gram-negative bacteria, which constituted overall 78.6% of the entire causative organisms responsible for septicemia. A study from Iran reported coagulase-negative staphylococci as the commonest cause of septicemia (Gheibi, et al., 2008). However, other study reported *Klesiella* as the most common organism causing septicemia (Blomberg, et al., 2007). The organisms reported in the present study were similar to those reported in earlier BSIs studies. However, the predominance of the causative organisms varied according to the geographical locations (Decousser, et al., 2003; Agnihotri, Kaistha and Gupta, 2004; Mamishi, Pourakbari, Ashtiani and Hashemi, 2005; Kaistha, et al., 2009).

The trends of resistance in coagulase-negative staphylococci and *Staphylococcus aureus* to penicillin G, ampicillin, amoxicillin, cephalexin, gentamicin and erythromycin were statistically significant [Table 22]. However, the trends of resistance in these organisms to cefoxitin, cefuroxime, cefoperazone, netilmicin, ofloxacin, chloramphenicol and vancomycin were statistically non significant. Therefore, these antibacterials may be preferred to treat the BSIs caused by coagulase-negative staphylococci and *Staphylococcus aureus*.

The trends of resistance in *Salmonella typhi* and *Escherichia coli* to amoxicillin-clavulanic acid and cefaclor were statistically significant [Table 24]. However, the trends of resistance in these organisms to cephalexin, gentamicin, amikacin, ofloxacin, gatifloxacin, chloramphenicol and tetracycline were statistically non significant. Therefore, these antibacterials may be preferred to treat the BSIs caused by *Salmonella typhi* and *Escherichia coli*. The trends of resistance in *Acinetobacter* species to cephalexin, amoxicillin and gentamicin were statistically insignificant. However, the trends of resistance in *Acinetobacter* species to ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefaclor, cefotaxime, ceftriaxone, cefoperazone, amikacin, ciprofloxacin, ofloxacin, gatifloxacin, chloramphenicol and tetracycline were statistically non significant. Therefore, these antibacterials may be preferred to
treat the BSIs caused by Acinetobacter species. High resistance rates in Klebsiella species to ampicillin (99%) has been reported from Iran (Mamishi, Pourakbari, Ashtiani and Hashemi, 2005), which is accordant with the present study.

Marginally higher rates of MDR were observed in Gram-negative bacteria than in Gram-positive bacteria. Among Gram-positive bacteria coagulase-negative staphylococci showed lower rates of MDR than those in Staphylococcus aureus [Table 26]. However, some studies reported higher rates of MDR in coagulase-negative staphylococci than those in Staphylococcus aureus (The European Study Group on Antibiotic Resistance, 1987; Dornbusch and the European Study Group on Antibiotic Resistance, 1990). In Staphylococcus aureus there is presence of mobile genetic element called SCCmec, which contains the mecA resistance gene. The mecA determinant encodes PBP2a, a new penicillin binding protein with decreased affinity for oxacillin and other beta-lactam antibiotics that might be responsible for MDR in this organism (Okuma, et al., 2002; Perichon and Courvalin, 2004). Vancomycin resistance might occur due to the expression of vanA, which is associated with alteration of the vancomycin binding site in the cell wall (Severin, et al., 2004). Enterococci showed 29.4% MDR to the antimicrobials tested. This organism naturally have varying levels of resistance to penicillins, cephalosporins and to achievable serum concentrations of aminoglycosides. Cephalosporins are not active against enterococci because they have a poor affinity for enterococcal PBPs. As aminoglycosides do not readily penetrate the enterococcal cell wall, enterococci exhibit low level resistance to them (Kaye and Kaye, 2000).

Lower rates of MDR in Salmonella typhi to cephalexin, cefuroxime, cefotaxime, ciprofloxacin and chloramphenicol were observed in our study. However, studies have reported increasing multidrug resistance in Salmonella typhi to ampicillin, chloramphenicol, cotrimoxazole (Coovadia, et al., 1992), ceftazidime and ciprofloxacin (Su, Chiu, Chu and Ou, 2004). Salmonella species have been found to express a wide variety of ESBL types such as TEM, SHV, PER, OXA and CTX-M enzymes which might be one of the reasons for MDR in this organism (Bradford, et al., 1998; Ait-Mhand, et al., 2002; Casin, et al., 2003). Additionally, Salmonella strains have been detected which produce plasmid mediated AmpC type beta-lactamases (Hanson, et al., 2002). Studies have reported high level ciprofloxacin resistance in serovar Typhi due to alteration in Ser83Phe and Asp87Gly (Renuka,
Sood, Das and Kapil, 2005; Saha, et al., 2006). The Ser83 in GyrA was the predominant alteration in serovar Typhi strains from Vietnam responsible for resistance in fluoroquinolones (Chau, et al., 2007). In *Escherichia coli* more than 50% isolates were MDR to the tested antimicrobials [Table 27], majority of which were resistant to 3-5 antimicrobials predominantly ampicillin, amoxicillin-clavulanic acid, cefuroxime, cefaclor, cefotaxime and netilmicin. A very recent study from South Asia reported considerable multidrug resistance amongst ESBL producing *Escherichia coli* isolates, with 66% resistance to quinolones, trimethoprim and aminoglycosides classes of antibiotics (Stoesser, et al., 2012). Extended spectrum beta-lactamases (ESBLs) are plasmid mediated enzymes (TEM-1, TEM-2 or SHV-1) capable of hydrolysing and inactivating a wide variety of beta-lactams, including third generation cephalosporins, penicillins and aztreonam, might be involved for resistance in *Escherichia coli* to these group of antimicrobials (Turner, 2005; Rupp and Fey, 2003; Shanthi and Sekar, 2010). In *Escherichia coli*, the alteration in mar regulon (multiple antibiotic resistant) confers resistance to multiple antimicrobials (Alekshun and Levy, 1999). Studies reported that constitutive mutations in the soxR gene might be involved in multiple antimicrobials such as ampicillin, chloramphenicol and tetracycline resistance in clinical *Escherichia coli* infections (Chou, Greenberg and Demple, 1993; Koutsolioutsou, Pena-Llopis and Demple, 2005). This multiple antimicrobial resistance is a hallmark of soxRS mediated mechanisms (Joon-Hee, et al., 2009). Another study reported spontaneous gene duplication as a new mechanism for mediating multidrug resistance in *Escherichia coli* through AcrAB-TolC (Nicoloff, Perreten, McMurry and Levy, 2006). *Acinetobacter* species showed high rates of MDR, majority of which were resistant to more than 5 antimicrobials predominantly to amoxicillin, cephalaxin, cefoperazone and gentamicin. A study reported increasing MDR prevalence strains of *Acinetobacter* (Slama, 2008). Landman, et al. (2002) reported 12% of *Acinetobacter* were resistant to all commonly used antibiotics that included ampicillin-sulbactam, piperacillin-tazobactam, cefepime, ceftiraxone, ceftazidime, meropenem, imipenem, amikacin and ciprofloxacin. The AdeABC efflux pump constitutes a major mechanism of multiple antibiotic (diminished susceptibility to aminoglycosides, fluoroquinolones, chloramphenicol and tetracycline-tigecycline) resistance in *Acinetobacter* species (Peleg, et al., 2007). Mutation in the GyrA and ParC fluoroquinolone targets and over expression of efflux systems might be the possible
mechanism of resistance in *Acinetobacter* to fluoroquinolones (Dijkshoorn, Nemec and Seifert, 2007). *Pseudomonas aeruginosa* showed very high, 64.5% MDR in the present retrospective study, majority of them were resistant to 3-5 antimicrobials, including ampicillin, amoxicillin-clavulanic acid, cephalaxin, chloramphenicol and tetracycline. A study from Iran reported 73.9% MDR isolates in *Pseudomonas aeruginosa*, majority of which were resistant to three antimicrobial agents (Moniri, Mosayebi, Movahedian and Mousavi, 2005). Another study reported about 30% strains of *Pseudomonas aeruginosa* as MDR which exhibited resistance to four antimicrobial drug classes that included antibiotics like ticarcillin, carbenicillin, piperacillin, cephalaxin, ceftazidime, amikacin, tetracycline, trimethoprim-sulfamethoxazole, meropenem and imipenem (McGowan Jr, 2006). The reasons might be due to the production of metallo-beta-lactamase as seen in *Pseudomonas* species reported by an Indian study (Navneeth, Sridaran, Sahay and Belwadi, 2002). The production of carbapenemases and AmpC enzymes reported as the main cause for multidrug resistance in *Pseudomonas aeruginosa* in China (Wang, Zhou and Yu, 2005). *Klebsiella pneumoniae* showed more than 55% MDR to the tested antimicrobials. Majority of them were resistant to 3-5 antimicrobials including ampicillin, amoxicillin, cefaclor, gentamicin, chloramphenicol and tetracycline. *Klebsiella pneumoniae* isolates that are resistant to ampicillin have been described previously, with resistance caused by either the production of a plasmid mediated AmpC like beta-lactamase (Papanicolau, Medeiros and Jacoby, 1990; Leiza, et al., 1994) or the loss of OMPs, such as OmpK35 and OmpK36 (Ardanuy, et al., 1998). A high prevalence of co-existence of *bla-CTX-M-2* and *ant(2")-Ia* genes in the same genetic structure has been reported as the reason for gentamicin resistance ((Melano, et al., 2003).

The possible mechanism of resistance which might be involved in MDR may be intracellular degradation of antibiotic, hyper production of chromosomal class C enzymes, presence of multidrug efflux system, low outer membrane permeability, resistance factor with resistance transfer factor in plasmid and poor binding with cell surface receptor. Multidrug resistant pathogens represent a significant challenge for the physicians in choosing empirical as well as defined therapy for septicemia, which may lead to therapeutic failure and fatal outcome. Pathogens containing more than one distinct resistance mechanisms may be involved in MDR. Appropriate empirical
antimicrobial treatment for MDR pathogens can thus be complicated. Knowledge of international, national and local resistance rates and prevalence of MDR pathogens might assists in selection of suitable antimicrobial agents for the therapy of septicemia. Where, resistance to an antimicrobial that occurs during drug development or clinical trials, or within 2 years of general use, then that antibiotic has a high resistance potential. (Paulus, et al., 2005). The emergence of microbial resistance can be limited by using antimicrobials with the lowest resistance potential.

The mortality rate in septicemia patients progressively decreased from 18.9% in 2004 to 3.7% in 2008 during the five years’ study period [Table 28]. Similar trends of decreased mortality rate in septicemia patients were reported in a prospective study from Germany (Geerdes, et al., 1992). The reasons for the trend of reduced mortality rate might be due to early detection and treatment of the underlying infections or improved supportive care (Wheeler and Bernard, 1999). Furthermore, decreased mortality might be due to significant improvements in supportive treatment in ICUs (more specific antibiotic treatment, improved mechanical ventilation and improved monitoring of circulation).

The prevalence of bacterial strains causing septicemia and their antimicrobial resistance is widespread in our hospital. The increased risk of septicemia due to antimicrobial resistant bacteria stresses the importance of implementing rational antimicrobial prescription policies and new vaccine strategies in our hospital setting. Gram-negative organisms did not show significant resistance to amikacin. These findings of the susceptibility of bacterial strains causing septicemia will contribute greatly to establish important baseline data to form an antibiotic policy in the hospital and also provide valuable information for implementing chemotherapy both empirical as well as definitive treatment for septicemia.

These study findings will help in making decision regarding empirical prescription of antibiotic in septicemia until culture report is available. So amikacin is the drug of choice for empirical treatment of septicemia for Gram-negative bacteria, until other factors are ruled out and exact sensitivity report is available for a particular patient. Therefore, it is suggested that the valuable therapeutic agent can be prescribed after performing the gram stain. Both prudent use of antibiotics and compliance with hand hygiene and other infection control measures are essential to reduce selection and spread of multidrug resistant bacteria.