**IJPSR (2011), Vol. 2, Issue 12** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 August, 2011; received in revised form 10 September, 2011; accepted 10 November, 2011

# PREVALENCE AND SUSCEPTIBILITY PATTERN OF *E. COLI* IN LOW BIRTH WEIGHT NEONATES OF EARLY ONSET SEPSIS

R. Singh*[1], S. Singh [1], S. P. Singh [2] and D. Sachan [2]

Department of Pharmacology, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS) [1], Bareilly, Uttar Pradesh, India

Department Of Pharmacology, GSVM Medical College [2], Kanpur, Uttar Pradesh, India

**Keywords**:
*E. coli,*
NICU,
Neonatal

**Correspondence to Author:**

**Dr Rashmi Singh**

Assistant Professor, Department of Pharmacology, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS), Bareilly, Uttar Pradesh, India

## ABSTRACT

Neonatal sepsis is one of the commonest cause of neonatal mortality in the developing world which can be classified into early onset sepsis (EOS) which occurs in the first 7 days of life and late onset sepsis (LOS) which occurs ≥7 days of life. *E. coli* has been reported to be one of the significant and most common nosocomial pathogen which may cause septicemia, pneumonia and meningitis in the newborn. Most of the antibiotics which have been used extensively as life saving are rendered useless because of the emergence of resistant strains of bacterias. Therefore for determining the prevalence and antimicrobial susceptibility pattern of *E. coli* which is responsible for EOS and LOS and to establish the relationship with birth weight, a total of 229 blood samples were obtained from the neonates admitted to neonatal intensive care unit (NICU) who showed the clinical signs and symptoms of neonatal sepsis and sent for culture and sensitivity. Out of these 229, 102 showed the positive culture, among which early onset sepsis was found in 80 neonates while late onset sepsis was diagnosed in 22 neonates. The most frequent pathogen isolated from positive blood culture was *E. coli* (66.66%) and it was also the most common pathogen in low birth weight and preterm neonates of both early (59 cases- 57.84%) and late-onset (9 cases- 8.82%) sepsis and the incidence was found higher in early onset sepsis. The isolate was completely resistant to vancomycin and the resistance was higher for monotherapy of semi-synthetic penicillin group of antibiotics than their combination therapy with sulbactum. Imepenam and gatifloxacin showed the highest sensitivity (100%), followed by Piperacillin - tazobactum and ciprofloxacin, however the frequency of resistance was more common in low birth weight neonates of early onset sepsis.

**INTRODUCTION:** Neonatal sepsis is defined as a disseminated disease with positive blood culture during the first month of life and is more common in developing countries as compared to developed countries [1, 2]. It is one of the commonest cause of neonatal mortality in the developing world accounting for 30-50% of neonatal deaths per year [3, 4].

Mortality rate in neonatal sepsis differs according to the type of organism and may vary from one country to another and within a country from one hospital or

region to another [5, 6]. Neonatal sepsis can be classified into two relatively distinct illnesses, early onset sepsis (EOS) and late onset sepsis (LOS). EOS occurs in the first 7 days of life, while LOS occurs ≥7 days of life [1].

Early onset neonatal sepsis occurs in the background of several predisposing factors related to labor and delivery while late onset neonatal sepsis is transmitted through hands of care providers or hospital staff.

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. According to Neonatal infection surveillance, Group B *streptococcus* (GBS) and *E. coli* are the dominant EOS pathogens in developed countries and coagulase negative *staphylococci* (CoNS) followed by GBS and *Staphylococcus aureus* (*S. aureus*) are the dominant LOS pathogens.

Whereas, in developing countries *Klebsiella*, *E. coli*, *Pseudomonas*, *Salmonella*, *S. aureus*, CoNS, *Streptococcus pneumoniae* and *Streptococcus pyogenes* have been increasingly responsible for neonatal sepsis [7-14].

Among these *E. coli* has been reported to be one of the significant and most common nosocomial pathogen which may cause septicemia, pneumonia and meningitis in the newborn. If diagnosed early and treated aggressively with antibiotics and with good supportive care, it is possible to save most of the cases of neonatal sepsis.

But most of the antibiotics which have been used extensively as life saving are rendered useless. It may be because of the emergence of resistant strains of bacterias, or may be due to wide spread, irrational and indiscriminate use of antibiotics.

Hence, the emerging antimicrobial resistance constitutes an important problem worldwide & has become a threat to public health. So it is very necessary to conduct periodic surveillance to assess the changing sensitivity pattern of different pathogens responsible for specific infection and generate data for rationale use of antibiotics to treat septicemia and prevent the antimicrobial resistance in neonates.

Therefore, the present study was undertaken with the objectives of determining the antimicrobial susceptibility pattern of most common isolate (*E. coli*) responsible for early onset neonatal sepsis (EOS) and late onset neonatal sepsis (LOS) and to establish the relationship with birth weight.

**MATERIALS AND METHODS:** The present prospective study was conducted in Department of Pharmacology and Microbiology, GSVM Medical College, Kanpur from 16.04.08 to 16.04.10 (two years).

The subjects enrolled were the neonates admitted to neonatal intensive care unit (NICU) of pediatric department in the hospital of same college with clinical signs and symptoms suggestive of neonatal sepsis, like poor feeding, fever, hypothermia, respiratory distress, signs of gastrointestinal or central nervous system involvement.

Exclusion criterias for the study were any genetic or congenital abnormality and newborns referred from other wards. Neonates were classified according to onset of sepsis (EOS and LOS), birth weight (NBW- normal birth weight & LBW- low birth weight) and gestational age (preterm <37 weeks & term >37 weeks).

Two blood cultures, complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), blood sugar, electrolytes, cerebrospinal fluid (CSF), chest X-ray (CXR) and urine analysis/culture were tested in all the cases of suspected neonatal sepsis. Blood samples were obtained by taking all antiseptic precautions from the neonates and inoculated into Tryptone Soy Broth (TSB) for culture and incubated for one week at 37ºC and were checked daily for evidence of bacterial growth. For positive broth cultures, subcultures were made on solid media (Blood agar and McConkey agar) and incubated at 37$^{o}$C for 24 to 48 hours. The bacteria were identified by colony morphology, gram stain and standard biochemical tests [15].

Antimicrobial sensitivity test was done by using disc diffusion method (Karbibauer method). Seventeen (17) different discs of known concentration Ampicillin 10µg, Ampicillin + sulbactum10/10 µg, Cefotaxime 30 µg, Cefotaxime + sulbactum 30/10µg, Ceftazidime 30µg, Ceftazidime + sulbactum 30/10µg, Piperacillin 100µg, Piperacillin-Tazobactum 100/10µg, Ciprofloxacin 5µg, Gatifloxacin 5µg, Amikacin 30µg, Gentamicin 10µg,

Netilmycin 30µg, Tobramycin 10 µg , Imipenam 10µg, Linezolid 30µg, and Vancomycin 30µg were used following the guidelines of National Committee for Clinical Laboratory Standards (NCCLS).

The diameter of zone of inhibition was measured and compared to that of standard strain and the results were interpreted as sensitive or resistant. At the end, the data obtained were analyzed for the prevalence and sensitivity pattern of *E. coli* for different antibiotics in above mentioned groups. Statistical analysis was done by using chi-square test. P value less than 0.05 was considered as significant.

**RESULTS:** A total of 229 blood samples were obtained from the 982 admitted neonates of NICU during the study period of two years who showed sign and symptoms of sepsis. Out of these, 102(44.5%) showed the positive blood culture. The median age of proven septic cases was 10.3 ± 8.2 days, (1 to 30 days). Among 102 newborns with sepsis, 52 were male (40.63%) and 50 (49.5%) were female *(P*>0.05), 76 (81.72%) were preterm (<37 weeks) and 26 (19.11%) were term (>37 weeks) by gestational age *(P*<0.05). There were 73 (90.12%) neonates with low birth weight and 29 (19.59%) with normal birth weight *(P*<0.05) (**Table 1**).

**TABLE 1: DEMOGRAPHIC VARIABLES AT THE TIME OF PRESENTATION OF NEONATAL SEPSIS**

| Characteristics | | Total cases from which the blood samples were obtained (229) | Total cases of sepsis with positive blood culture (102) | % of neonates with positive blood culture (44.5%) | P value |
|---|---|---|---|---|---|
| Gender | male | 128 | 52 | 40.63% | >0.05 |
| | female | 101 | 50 | 49.5% | |
| Birth weight | NBW | 148 | 29 | 19.59% | <0.001 |
| | LBW | 81 | 73 | 90.12% | |
| Gestational age | >37 weeks | 136 | 26 | 19.11% | <0.001 |
| | <37 weeks | 93 | 76 | 81.72% | |
| Onset of sepsis | EOS | 105 | 80 | 76.19% | <0.001 |
| | LOS | 124 | 22 | 17.74% | |

Diagnosis of early onset sepsis was found in 80 (76.19%) neonates among them 18 were of normal birth weight and 62 of low birth weight, 68 were preterm (<37 weeks) and 12 were term (>37 weeks) while late onset sepsis was diagnosed in 22(17.74%) neonates out of which 11 had normal birth weight and another 11, low birth weight, 8 were preterm (<37 weeks) and 14 were term (>37 weeks) (**Table 2**).

**Figure 1** shows the distribution of bacterial isolates from blood cultures in which the most frequent isolate was *E. coli* (66.66%) and it was also the most common pathogen in low birth weight and preterm neonates of both early –(59 cases- 57.84%) and late-onset (9 cases- 8.82%) of neonatal sepsis but the incidence was higher in early onset sepsis (Table 2).

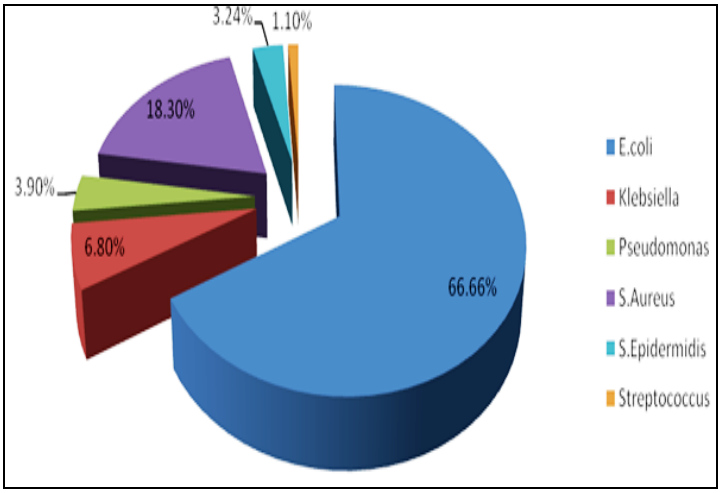


**FIG. 1: FREQUENCY DISTRIBUTION OF MICROORGANISMS ISOLATED FROM BLOOD CULTURES**

**TABLE 2: PREVALENCE OF *E. COLI* AMONG NEONATES WITH PROVEN SEPSIS ACCORDING TO BIRTH WEIGHT AND GESTATIONAL AGE**

| Cases of sepsis with positive culture 102 (44.5%) | | *E. coli* positive 68 (66.66%) | Variables | Total culture positive | *E. coli* positive |
|---|---|---|---|---|---|
| EOS | 80 (78.43%) | 59 (57.84%) | LBW | 62(60.78%) | 49(48.04%)* |
| | | | NBW | 18(17.65%) | 10(9.80%) |
| | | | <37 weeks | 68(66.66%) | 53(51.96%)# |
| | | | >37 weeks | 12(11.76%) | 5(4.9%) |
| LOS | 22 (21.56%) | 9 (8.82%) | LBW | 11(10.78%) | 2(1.96%) |
| | | | NBW | 11(10.78%) | 3(2.94%) |
| | | | <37 weeks | 8(7.8%) | 3(2.94%) |
| | | | >37 weeks | 14(13.73%) | 4(3.92%) |

*P<0.05 LBW vs NBW, # P<0.05<37 weeks vs >37 weeks

**Table 3** shows the antibiotic susceptibility pattern of *E. coli* in different groups which demonstrates that the isolate was completely resistant to vancomycin in all the groups. Resistance was higher for monotherapy of semi-synthetic penicillin group of antibiotics while combination therapy of semi-synthetic penicillins with sulbactum showed significantly higher (P< 0.01) antibacterial activity when compared to their monotherapies. Imepenam and gatifloxacin showed the highest sensitivity (100%), followed by Piperacillin-Tazobactum and Ciprofloxacin in all the groups however the frequency of resistance was more common in low birth weight neonates of early onset sepsis (Table 3).

**TABLE 3: ANTIBIOTIC SUSCEPTIBILITY PATTERNOF *E. COLI* IN LOW AND NORMAL BIRTH WEIGHT NEONATES OF EOS AND LOS**

| Micro organism | EOS | | | | LOS | | | |
|---|---|---|---|---|---|---|---|---|
| | NBW 18 | | LBW 62 | | NBW 11 | | LBW 11 | |
| | Resistant | Sensitive | Resistant | Sensitive | Resistant | Sensitive | Resistant | Sensitive |
| Ampicillin | 14 (77.77%) | 4 (22.2 %) | 60 (96.77%) | 2 (3.2%) | 7 (63.63%) | 4 (36.36%) | 8 (72.72%) | 3 (27.27%) |
| Ampicillin + sulbactum | 11 (61.1%) | 7 (38.8%) | 50 (80.64%) | 12 (19.4%) | 4 (36.36%) | 7 (63.63 %) | 6 (54.54%) | 5 (45.45 %) |
| Cefotaxime | 11 (61.1 %) | 7 (38.8 %) | 49 (79.03%) | 13 (20.96 %) | 9 (81.81%) | 2 (18.18 %) | 9 (81.81%) | 2 (18.18 %) |
| Cefotaxime + sulbactum | 8 (44.4%) | 10 (55.5%) | 38 (61.29%) | 24 (38.7 %) | 5 (45.45%) | 6 (54.54%) | 6 (54.54%) | 5 (45.45%) |
| Ceftazidime | 9 (50 %) | 9 (50%) | 35 (56.45%) | 27 (43.5%) | 4 (36.36%) | 7 (63.63%) | 5 (45.45%) | 6 (54.54%) |
| Ceftazidime + sulbactum | 6 (33.3%) | 12 (66.6 %) | 26 (41.9%) | 36 (58.06 %) | 5 (45.45%) | 6 (54.54%) | 8 (72.72%) | 3 (27.27%) |
| Piperacillin | 6 (33.3 %) | 12 (66.6%) | 23 (37.96%) | 39 (62.9 %) | 4 (36.36%) | 7 (63.63 %) | 5 (45.45%) | 6 (54.54%) |
| Piperacilli-Tazobactum | 2 (11.1%) | 16 (88.8%) | 9 (14.52%) | 53 (85.48%) | 1 (9.1 %) | 10 (90.9 %) | 2 (18.18%) | 9 (81.81%) |
| Ciprofloxacin | 2 (11.1 %) | 16 (88.8%) | 9 (14.52%) | 53 (85.48%) | 4 (36.36%) | 7 (63.63%) | 6 (54.54%) | 6 (54.54%) |
| Gatifloxacin | 0 (0%) | 18 (100%) | 0 (0%) | 62 (100%) | 0 (0%) | 11 (100%) | 0 (0%) | 11 (100%) |
| Amikacin | 8 (44.4 %) | 10 (55.5%) | 28 (45.16%) | 34 (54.83%) | 4 (36.36%) | 7 (63.63%) | 6 (54.54%) | 5 (45.45%) |
| Gentamycin | 14 (77.7 %) | 4 (22.2%) | 49 (79.03%) | 13 (20.96%) | 6 (54.54%) | 5 (45.45%) | 7 (63.63%) | 4 (36.36%) |
| Netilmycin | 11 (61.1 %) | 7 (38.8%) | 45 (72.58%) | 17 (27.41%) | 8 (72.72%) | 3 (27.27%) | 9 (81.81 %) | 2 (18.18 %) |
| Tobramycin | 15 (83.33%) | 3 (16.6 %) | 54 (87.1%) | 8 (12.9%) | 7 (63.63 %) | 4 ( 36.3%)6 | 8 (72.72 %) | 3 (27.27%) |
| Imipenam | 0 (0 %) | 18 (100 %) | 0 (0%) | 62 (100%) | 0 (0%) | 11 (100%) | 0 (0 %) | 11 (100 %) |
| Linezolid | 15 (83.33%) | 3 (16.6%) | 52 (83.87%) | 10 (16.13%) | 6 (54.54%) | 5 (45.45 %) | 7 (63.63 %) | 4 (36.36 %) |
| Vancomycin | 18 (100 %) | 0 (0%) | 62 (100%) | 0 ( %) | 11 (100%) | 0 (0%) | 11 (100 %) | 0 (0%) |

Thus, the study demonstrates that low birth weight and prematurity were associated with higher risk of early onset sepsis and the drug resistance was also more common in low birth weight neonates of early onset sepsis.

**DISCUSSION:** Sepsis is still prevalent in newborns and it is a major medical problem. The incidence of neonatal septicemia is 1-10/1000 normal live births and 1 per 250 premature live births and it is also one of the important causes of neonatal morbidity and mortality [3, 4, 5, 16].

In the present study, the prevalence of positive blood culture was 44.5% which was comparable to studies of Rahman *et al*., (2002) (62.8%) and Bhttacharjee *et al*., (2008) (48%) but it was in contrast with reports from Mohammad-Mehdi Karambin *et al*., (2010) (10.4%) [17, 18, 19]. This may be because of regional variation in prevalence of microbes.

Considering sex preponderance, no significant relationship was found between sex and sepsis though some studies have reported the male preponderance in sepsis [15].

In the present investigation, among the cases with positive blood cultures, 80 (78.43%) neonates presented with EOS and 22(21.56%) presented with LOS (Table 2) and the prevalence of early onset sepsis (EOS) was significantly higher than late onset sepsis (LOS). These findings were in consistent with the reports of other developing countries like Iran (77.5% vs.22.5%) (Movahedian *et al.,* 2006) and Bangladesh (70.7 vs. 29.3%) (Rasul *et al*., 2006), but in contrast with reports from Saudi Arabia (39% vs. 61%) (Dawodu *et al*., 1997), Pakistan (42% vs.58%) (Aftab and Iqbal, 2006) and Libya (31 vs.69%) (Misallati *et al.,* 2000) where, late onset sepsis is more common [20, 21, 22, 23, 24].

The lower frequency of LOS in this study might be either due to early administration of antibiotics or more referral of preterm labors and preterm newborns to our centre or early discharge policy in the hospital. Findings of this study indicate that the low birth weight and prematurity were associated with higher risk of sepsis *(P*<0.001) which agrees with the previous reports [19, 25, 26].

The risk factors and the clinical presentations of neonatal sepsis differ in early and late onset types as are the usual organisms responsible for each type [27]. Blood culture to isolate the offending pathogen remains the gold standard for the diagnosis of neonatal sepsis and in advanced centers, it is positive in almost 80% cases of genuine sepsis [28, 29].

Nevertheless, negative cultures do not rule out the possibility of neonatal sepsis and may be due to several reasons, e.g. administration of antibiotics before blood collection either to mother or baby, or the possibility of infection with viruses or fungi [19]. The spectrum of organisms causing neonatal sepsis in this study is similar to that reported from developing countries, with gram negative bacteria being responsible in most of the cases. *E. coli* was found to be the predominant pathogen (66.66%). These findings were also consistent with the study of Aurangzeb *et al* who revealed 112 hospitalized newborns as sepsis, 67% had positive blood culture and *E. coli* was the most frequent cause with 77.1% frequency [27]. In addition to this, the preponderance of *E. coli* in our study among low birth weight and preterm neonates of early onset sepsis was also in accordance with the results of a previous study [30]**.**

While studying the antibiotic sensitivity pattern of *E. coli* isolated from different groups, it was observed that the combination therapy of semi-synthetic penicillins (Ampicillin and Piperacillin) had significantly higher (P< 0.01) antibacterial activity when compared to their monotherapies in all the groups. A similar scenario was found in case of cephalosporins. Combinations of cephalosporins with sulbactum showed significantly greater (P< 0.01) sensitivity to *E. coli* than their monotherapies.

In yet another study, carried out in neonates by Jhoshi *et al*., has shown that the gram negative organisms were highly resistant to most of the penicillins and cephalosporins which is probably attributable to beta lactamase production. The use of combination of semi-synthetic penicillins or cephalosporins with “suicide inhibitors” of ESBL (extended spectrum beta lactamase) like sulbactam or tazobactam, Cefoperazone + sulbactam or piperacillin + tazobactam combination would be an appropriate choice in most gram negative neonatal sepsis in regions with high prevalence of organisms producing ESBL [31, 32].

Among monotherapies the organism showed markedly high sensitivity (100%) to Imipenam. The high sensitivity of *E. coli* to imipenem indicates the absence of selective pressure since the drug is rarely prescribed. These findings are similar to the results of the study of Marzban *et al*., which showed that gram negative bacilli are highly sensitive to imipenam (95%) and there was only one strain of *E. coli* in his study that was resistant to imipenam [33]. The susceptibility range for aminoglycosides group of antibiotics ranges between 12.9%-54.84%, in which only Amikacin had higher sensitivity (54.84%) as compared to others.

It has been shown by the study of Anwer SK *et al* that aminoglycosides are usually found to be effective against most of the gram negative infections so it should be reserved for severe infections only [34]. The flouroquinolone group showed better sensitivity [Ciprofloxacin (85.48%) and Gatifloxacin (100%)]. On the other hand, Vancomycin and Linezolid demonstrated a high degree of resistance (sensitivity only 0%, 16.13% respectively). One of the causes of this emerging resistance may be indiscriminate and irrational use of antibiotics.

Overall, the antimicrobial resistance in our study was found higher in early onset, low birth weight neonates which may be due to compromised immunity that favors the drug resistance. This observation is similar to the results of a study conducted in very low birth neonates.[35] Hence antibiotic resistance can cause many difficulties in the treatment of sepsis such as increase in mortality rate, duration of hospitalization and treatment expenses so the antibiotics should be judiciously used and their inappropriate use should be avoided.

**CONCLUSION:** The study shows that the prevalence of *E. coli* was very high in low birth weight and preterm neonates of early onset sepsis and there was an increased incidence of antimicrobial resistance in low birth weight neonates especially with conventional monotherapies which may be due to wide spread, indiscriminate and inappropriate use of antibiotics. Therefore, the antimicrobials must be used with confirmed indications, according to the data collected from epidemiologic studies in the concurred region and if possible on the basis of the results of culture and sensitivity tests.

To prevent the resistance and maintain antibiotic effectiveness, combination therapy should be preferred over monotherapies and because flouro-quinolones and imipenam are very effective against *E. coli*, they should be reserved for severe infections only. Antibiotics administration should not be a routine practice in the apprehension of infection and proper sterilization and aseptic practice during delivery must be given due weightage. Besides this, every hospital's drug committee should form an effective policy and should conduct regular periodic surveillance programme at the state and national level to study the changing prevalence and sensitivity pattern of infecting organisms.

**ACKNOWLEDGEMENT:** Authors are thankful to the faculty and staff of the microbiology department of the institute for their cooperation and help. Authors are also grateful to Dr V.N. Tripathi, Head of the Department of Pediatrics, for his encouragement and support.

**REFERENCES:**

1. Edwards MS: Postnatal infections. In: Fanaoff and Martins Neonatal-perinatal Medicine. Elsevier, Philadelphia, Edition 8, 2006: 791-804.
2. Vergnano S, Sharland M, Kazembe P, Wansambo CM, and Heath PT: Neonatal sepsis: An international perspective. Arch Dis Child Fetal Neonatal Ed 2005; 90: 220- 224.
3. Stoll BJ: The global impact of neonatal infection. Clinical perinatol 1997; 24: 1-21.
4. Bang AT, Reddy HM, Deshmukh MD, Baitule SB, Bang RA: Neonatal and infant mortality in the ten years (1993 to 2003) of the Gadchiroli field trial- Effect of homebased neonatal care. J Perinatol 2005; 25: 92-107.
5. Aletayeb SMH, Khosravi AD, Dehdashtian M, Kompani F, Mortazavi SM, Aramesh MR: Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a tertiary hospital. African Journal of Microbiology Research 2011; 5(5): 528-531.
6. Desinor OY, Silva JL, Menos MJ: Neonatal sepsis and meningitis in Haiti. J Trop Pediatr 2004; 50(1): 48- 50.
7. Karthikeyan G, Premkumar K: Neonatal sepsis- Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001; 68: 715 –717.
8. Tallur SS, Kasturi AV, Nadgir SD, Krishna BV: Clinico-bacteriological study of neonatal septicemia in Hubli. Indian J Pediatr 2000; 67: 169 –174.
9. Bhutta ZA, Yusuf K: Early-onset neonatal sepsis in Pakistan: A case control study of risk factors in a birth cohort. Am J Perinatol 1997; 14: 577 –581.
10. Malik AS, Pennie RA: Early onset neonatal septicaemia in a level II nursery. Med J Malaysia 1994; 49: 17 –23.
11. Muhe L, Tilahun M, Lulseged S, Kebede S, Enaro D, Ringertz S, Kronvall G, Gove S,Mulholland EK: Etiology of pneumonia, sepsis and meningitis in infants younger than three months of age in Ethiopia. Pediatr Infect Dis J 1999; 18(10): 56 –61.
12. Gatchalian SR, Quiambao BP, Morelos AM, Abraham L, Gepanayao CP, Sombrero LT, Paladin JF, Soriano VC, Obach M, Sunico ES: Bacterial and viral etiology of serious infections in very young Filipino infants. *Pediatr* Infect Dis J 1999; 18(10): 50 –55.
13. Hyde TB, Hilger TM, Reingold A, Farley MM, O'Brien KL, Schuchat A: Trends in incidence and antimicrobial resistance of early onset sepsis: Population based surveillance in San Francisco and Atlanta. Pediatrics 2002; 110: 690 –695.
14. Isaacs D: Australasian study group for neonatal infections: A ten years multicenter study of coagulase negative staphylococcal infections in Australasian neonatal units. Arch Dis Child Fetal Neonatal Ed 2000; 88: 89 –93.
15. Forbes BA, Sahm DF, Weissfeld AS: Laboratory cultivation and isolation of bacteria. In: Forbes BA, Sahm DF, Weissfeld AS eds.

Bailey and Scott's Diagnostic Microbiology. Missouri: Mosby Inc, St. Louis, Edition 12, 2007: 478- 509.
16. Schelonka R, Freij BJ, Mc Cracken GH: Bacterial and fungal infection. In: Mc Donald M., Mullet MD, Seshia M.M.K., eds. Avery's neonatology pathophysiology management of the new born. Lippincottt Williams Wikkins, Philadelphia, 2005: 1235-1273.
17. Bhttacharjee A, Sen MR, Prakash P, Gaur A, Anuprba S: Increased prevalence of extended spectrum beta lactamase producers in neonatal septicaemic cases at a tertiary referral hospital. Indian J Med Microbiol 2008; 264(4): 356-360.
18. Rahman S, Hameed A, Roghani MT, Ullah Z: Multidrug resistant neonatal sepsis in Peshhawar, Pakistan. Arch Dis Child Fetal Neonatal Ed 2002; 87(1): 52-54.
19. Karambin MM, Zarkesh M: Entrobacter, the most common pathogen of neonatal septicemia in Rasht, Iran. Iran J Pediatr 2011; 21(1): 83-87.
20. Movahedian AH, Moniri R, Mosayebi Z: Bacterial culture of neonatal sepsis. Iranian J Publ Health 2006; 35: 84-89.
21. Rasul CH, Hassan MA, Habibullah M: Neonatal sepsis and use of antibiotic in tertiary care hospital. Pak J Med Sci 2007; 23: 78-81.
22. Dawodu A, Umran K, Twum-Danso K: A case control study of neonatal sepsis: Experience from Saudi Arabia. J Trop Pediatr 1997; 43: 84-88.
23. Aftab R, Iqbal I: Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital Multan. J Coll Physicians Surg Pak 2006; 16: 216-219.
24. Misallati A, Bargathy S, Shembesh N: Blood-culture-proven neonatal septicemia: A review of 36 cases. East Mediterr Health J 2000; 6: 483-486.
25. Simiyu E: Morbidity and mortality of low birth weight infants in new born units in Kenyatta National Hospital, Nairobi. East African Med J 2004; 81: 367-384.
26. Morteno TO: Neonatal sepsis in low birth weight infants in Dr Soetomo General Hospital. Indonesian journal of tropical and infectious disease 2010; 1: 86-89.
27. Aurangzeb B, Hameed A: Neonatal sepsis in hospital born babies: Bacterial isolates and antibiotic susceptibility patterns. Journal of College of Physicians and Surgeons Pakistan 2003; 13(11): 629-632.
28. Klein JO, Remington JS: Current concepts of infections of the fetus and the newborn infant. In: Remington JS, Klein JO, eds. Infectious Diseases of the Fetus and the Newborn infant, WB Saunders, Philadelphia, Edition 5, 2001: 1-23.
29. Buttery JP: Blood cultures in newborn and children: Optimizing an everyday test. Arch Dis Child Fetal Neonatal Ed 2002; 87: 25-27.
30. Kaufman D, Karen D: Clinical microbiology of bacterial and fungal sepsis in very low birth weight infants. Clinical Microbiology Reviews 2004; 17(3): 638-680.
31. Jhoshi SG, Ghole VS, Niphadkar KB: Neonatal gram negative bacteremia. Indian Journal of Pediatrics 2000; 67(1) : 27-32.
32. Shaw CK, Shaw P, Thapalial A: Neonatal sepsis bacterial isolates and antibiotic susceptibility patterns at a NICU in a tertiary care hospital in western Nepal: A retrospective analysis. Kathmandu University Medical Journal 2007; 5(2) issue 18: 153-160.
33. Marzaban A, Hadi S, Pourmemari MH, Vahidian AA: Neonatal late onset sepsis in a NICU: Analysis of causative organisms and antimicrobial susceptibility. Research Journal of Biological Sciences 2010; 5(5): 376-379.
34. Anwer SK, Mustafa S, Pariyani S, Ashraf S, Taufiq KM: Neonatal Sepsis: An etiological study. J Pak Med Asssoc 2000; 50: 91-94.
35. Bhat YR, Lewis ESL, Vandana KE: Bacterial isolates of early onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: An audit from a center in India. Italian Journal of Pediatrics 2011; 37: 31-32.

**********