

TRENDS IN ETIOLOGY AND ANTIMICROBIAL PATTERNS IN NEONATAL SEPSIS. A DESCRIPTIVE STUDY IN A TERTIARY CARE HOSPITAL, LAHORE

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ABSTRACT

Objective: The main objective of study is to determine the etiology and antimicrobial patterns of neonatal sepsis.

Place and Duration of Study: Postgraduate Medical Institute, Lahore and duration of study was 6 months (1st July' 14 -31st Dec' 14).

Materials and Methods: 95 positive blood cultures with clinical signs and symptoms were included in this study in PGMI, Lahore. The blood cultures were taken before the start of antimicrobial therapy. Blood culture reports were assessed for identification by standard methods. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar using CLSI protocols.

Results: Out of the 450 blood samples, 95 (21.1%) were culture positive. Among 95 positive blood cultures, Gram negative organisms were recovered from 56 (58.9%) followed by Gram positive organisms 36 (37.8%) whereas only 3(3.1%) blood cultures were positive for *Candida* spp. Among Gram negative organisms (n= 56) *Pseudomonas* spp 16 (28.5%) was the most common isolate followed by *Escherichia coli* 13(23.2%), *Klebsiella pneumoniae* 10 (17.8%). Among Gram positive organisms (n=36), Coagulase Negative *Staphylococci* (CoNS) was the most frequently isolated organism 24 (66.6%) followed by *Staphylococcus aureus* 10 (27.7%). In *Escherichia coli* and *Klebsiella pneumoniae* 100% resistance was seen among ceftazidime, ceftriaxone, cefotaxime and aztreonam. Variable pattern of resistance was seen among other members of enterobacteriaceae, non-fermenters and Gram positive organisms.

Conclusion: It is concluded that Gram negative organisms were the main cause of neonatal sepsis. *Pseudomonas* spp and Coagulase negative *Staphylococci* (CoNS) were the principle pathogens isolated from Gram negative and Gram positive organisms.

Key Words: Neonatal sepsis, neonates, Gram negative bacteria, Antimicrobial susceptibility patterns.

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INTRODUCTION

Neonatal sepsis, also termed sepsis neonatorum, is a clinical state of bacteremia characterized by systemic signs of infection in the first four weeks of life and is also documented by a positive blood culture [1].

National Neonatal Forum of India has defined neonatal sepsis as follows [2]

1. Probable (clinical) sepsis: It is found in an infant having suggestive of septicemia if any one of the following criteria are present: such as

- a) Existence of predisposing conditions:

Maternal fever, foul smelling liquor, prolonged rupture of membranes (>24 hrs), The septic screen would be positive due to the presence of two of the four parameters namely, TLC (< 5000/mm), band to total polymorphonuclear cells ratio of >0.2, absolute neutrophil count < 1800/mm³, C-reactive protein (CRP) >1mg/dl and micro ESR > 10 mm-first hour.

- b) Radiological evidence of pneumonia.
2. Culture Positive Sepsis: In an infant having a clinical picture suggestive of septicemia, pneumonia or meningitis, if either of the following criteria are present:

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- a) Isolation of pathogens from blood or CSF or urine or abscess
- b) Pathological evidence of sepsis in the autopsy.

Neonatal sepsis can be devastating, leading to high mortality and morbidity in newborns, and is considered as a global health challenge [3]. The incidence of neonatal sepsis shows a marked variation in geographical regions and is around 1-10/1,000 live births in industrialized nations but it was outlined three times more in underdeveloped countries [4]. Pakistan is one of the five countries which gave its contribution to 49% of child death and has the highest neonatal mortality rate (53/1000 live births) [5]. Based on a record of UNICEF, neonatal mortality rate is 27/1000 live births [6].

Neonatal sepsis can be categorized into two types depending upon their onset of origin, early onset (EOS) and late onset (LOS) [1]. EOS is defined as the appearance of signs and symptoms with the corresponding positive culture within 72 hours. However, LOS is defined as the origin of signs or symptoms after 72 hours [7].

The organisms accountable for neonatal sepsis have altered with time period and there is a marked variation from region to region. Antibiotic resistance has been outlined as a major threat in the management of neonatal sepsis [8]. Different studies have been carried out on neonatal sepsis which showed tremendous rise of resistance to the first line antibiotics. The greatest challenge today is the emerging disaster of neonatal sepsis in combination with antimicrobial resistance to commonly used antibiotics [9].

Immediate start of antimicrobial regimen is mandatory in neonatal septicemic patients, and conclusions must be made on knowing the detailed background of organisms and their antimicrobial trends in neonatal intensive care units. It is very important to conduct such type of studies in every

hospital on regular basis to determine the pattern of micro-organisms prevalent in that area. So, we can make an antibiogram policy which will help in providing empirical treatment options to our patients.

MATERIALS AND METHODS

This study was carried out on 450 blood samples from clinically suspected septicemic neonates. The aim of the study was to determine etiological pattern in neonatal septicemia in a tertiary care hospital, Lahore and duration of study was 6 months (1st July' 14 -31st Dec' 14).

The samples were collected from patients admitted in Pediatric Department, Lahore General Hospital, Lahore. To collect blood samples, pediatric tryptic soya broth blood culture bottles were provided to the medical house staff in pediatric emergency of the hospital. Thorough instructions about aseptic blood collection technique for culture were given to the house staff. All the samples were collected before start of any antimicrobial drugs in the hospital. One sample of blood i.e 0.5-1ml was drawn and then inoculated into 9 ml of tryptic soya broth.

Pediatric blood culture bottles were brought to Microbiology Laboratory of Post Graduate Medical Institute (PGMI) and were placed in an incubator at 35 ± 2 °C overnight. First subculture from broth bottles was done on Blood agar, Mannitol salt agar and MacConkey agar plates. The sub culture plates were incubated at 35 ± 2 °C overnight and observed next day for any visible growth. If no growth occurred then second and third subculture were done at day 4 and 7. The blood culture bottles were incubated for a period of seven days in case of negative subculture.

Preliminary identification was based on Gram staining, catalase test, oxidase test and motility by hanging drop method. Biochemical tests were put up for identification of organisms. All the catalase positive and oxidase negative rods were subjected to triple sugar iron, citrate utilization, urease, indole, motility, methyl red and voges proskaur tests.

Catalase positive Gram positive cocci were identified by growth on Mannitol salt agar, Coagulase test (slide and tube method), and Deoxyribonuclease tests. Gram positive cocci with catalase test negative were further grouped by Streptococcal grouping latex kit UK. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar using CLSI protocols [10,11].

Statistical analysis was done by IBM SPSS 20. Categorical variables were expressed using frequencies. Frequencies were calculated and demonstrated as graphs and charts.

RESULTS

Duration of study was 6 months (1st July'14-31st Dec'14) and 450 samples of blood of clinically diagnosed patients of neonatal septicemia were collected and processed in the laboratory. Out of the 450 blood samples, 95 (21.1%) were culture positive shown in Figure 1.

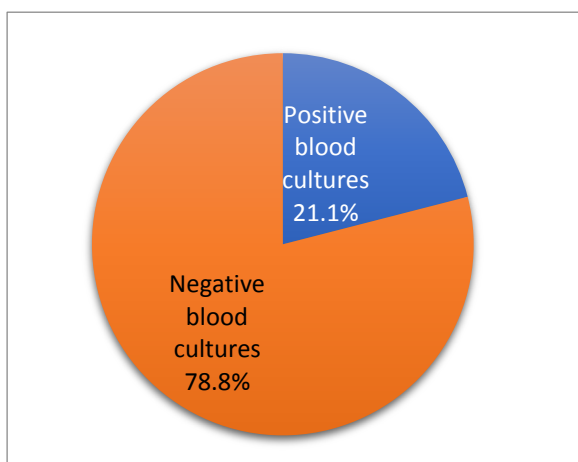


Figure-1: Blood culture results from patients with suspected neonatal septicemia (n=450).

Among 95 positive blood cultures, Gram negative organisms were recovered from 56 (58.9%), mostly belonging to Enterobacteriaceae family followed by Gram positive organisms 36 (37.8%) whereas only 3(3.1%) blood cultures were positive for *Candida* spp. Distribution of Gram positive, Gram negative and fungal isolates among positive blood cultures (n=95) is shown in Figure 2.

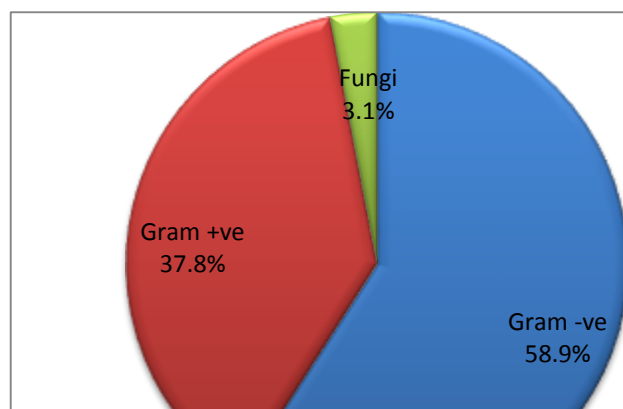


Figure-2: Distribution of Gram positive, Gram negative and fungal isolates among positive blood cultures (n=95).

Among Gram negative organisms (n= 56) *Pseudomonas spp* 16 (28.5%) was the most common isolate followed by *Escherichia coli* 13(23.2%), *Klebsiella pneumoniae* 10 (17.8%), *Acinetobacter spp* 8(14.2%), *Enterobacter spp* 6 (10.7%) and *Citrobacter spp* 3 (5.3%) shown in table-1.

Table-1: Breakup of Gram negative organisms in suspected cases of neonatal septicemia (n=56).

S, No	Organism isolated	Number (n=56)	Percentage %
1.	<i>Pseudomonas spp</i>	16	28.5%
2.	<i>Escherichia coli</i>	13	23.2%
3.	<i>Klebsiella pneumoniae</i>	10	17.8%
4.	<i>Acinetobacter spp</i>	8	14.2%
5.	<i>Enterobacter spp</i>	6	10.7%
6.	<i>Citrobacter spp</i>	3	5.3%

Among Gram positive organisms (n=36), Coagulase Negative *Staphylococci* (CoNS) was the most frequently isolated organism 24 (66.6%) followed by *Staphylococcus aureus* 10 (27.7%) and Group D *Streptococci* 2 (5.5%) as shown in table-2.

Table-2: Breakup of Gram positive cocci in suspected cases of neonatal septicemia (n=36).

S/No	Organism isolated	Number (n=36)	Percentage %
1.	Coagulase Negative <i>Staphylococci</i> (CoNS)	24	66.6%
2.	<i>Staphylococcus aureus</i>	10	27.7%
3.	Group D <i>Streptococci</i>	2	5.5%

The most commonly isolated organism was Coagulase Negative *Staphylococci* (CoNS) 24(25.2%) followed by *Pseudomonas* spp 16 (16.8%), *Escherichia coli* 13 (13.6%), *Klebsiella pneumoniae* 10 (10.5%) and *Staphylococcus aureus* 10 (10.5%). The rest are shown in Figure-3.

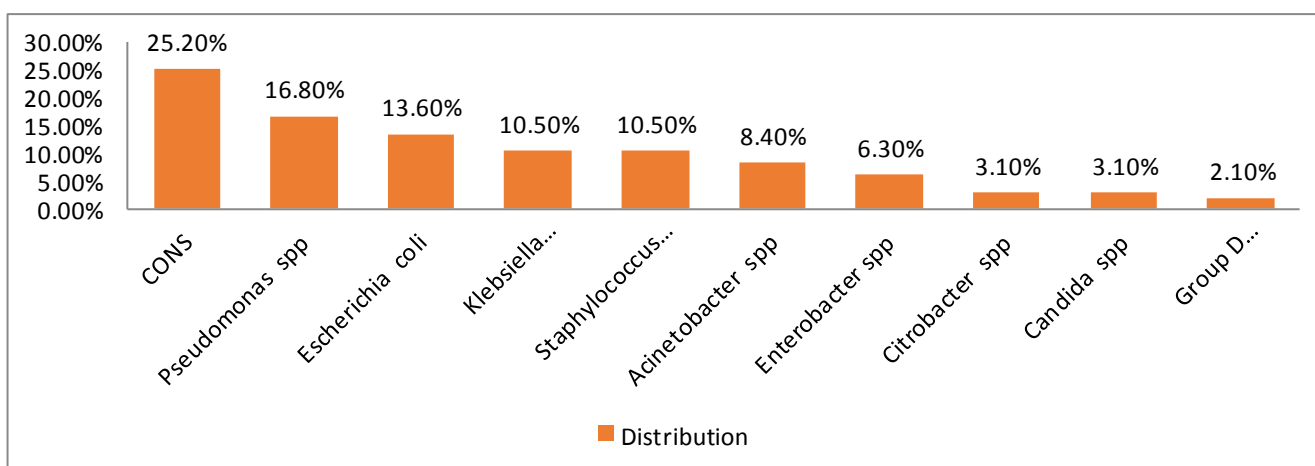


Figure-3: Distribution of different micro-organisms recovered from positive blood cultures (n=95).

In present study, patterns of antimicrobial susceptibilities were studied in different organisms isolated from suspected cases of neonatal septicemia. Susceptibility testing was carried by modified Kirby Bauer disc diffusion method [9-12].

Frequency of resistance to different antibiotics among enterobacteriaceae is shown in Table-3. In our study 100% resistance was seen among ceftazidime, ceftriaxone, cefotaxime and aztreonam in *Escherichia coli* and *Klebsiella pneumoniae* in neonatal septicemic patients. In *Enterobacter* spp, 100% resistance was seen against cefipime and gentamicin and *Citrobacter* spp showed 100% resistance among aztreonam and ciprofloxacin.

Table-3: Frequency of resistance (%) to different antibiotics among enterobacteriaceae.

Organisms	AMC	TZP	FOX	CAZ	CRO	CTX	FEP	IPM	ATM	CN	CIP	SXT
<i>Escherichia coli</i> (13)	92	31	77	100	100	100	69	0	100	69	62	46
<i>Klebsiella pneumoniae</i> (10)	90	30	60	100	100	100	60	0	100	40	60	50
<i>Enterobacter</i> spp (6)	83	83	83	83	67	67	100	33	67	100	67	67
<i>Citrobacter</i> spp (3)	67	67	67	67	67	67	67	33	100	67	100	67

(AMC-Amoxycillin-clavulanic acid, TZP-Piperacillin-tazobactam, FOX-Cefoxitin, CAZ-Ceftazidime, CRO-Ceftriaxone, CTX-Cefotaxime, FEP-Cefipime, IPM-Imipenem, ATM-Aztreonam, CN-Gentamicin, CIP-Ciprofloxacin, SXT- Trimethoprim-sulfamethoxazole)

Frequency of resistance to different antibiotics among non-fermenters is given in table 4. In our study, *Pseudomonas* spp showed the highest resistance among gentamicin (68.7%) and *Acinetobacter* spp showed 100 % resistance to ceftazidime, ceftriaxone and cefotaxime.

Table-4: Frequency of resistance (%) to different antibiotics among non-fermenters.

Organisms	PRL	TZP	CAZ	CRO	FEP	IPM	ATM	CN	CIP	DO	SAM	TGC
<i>Pseudomonas</i> spp	6	6	31	56	56	19	56	69	25	13	25	50
<i>Acinetobacter</i> spp	-	50	100	100	75	38	-	63	63	-	-	-

(PRL-Piperacillin, TZP-Piperacillin-tazobactam, CAZ-Ceftazidime, CRO-Ceftriaxone, FEP-Cefipime, IPM-Imipenem, ATM-Aztreonam, CN-Gentamicin, CIP-Ciprofloxacin, DO-Doxycycline, SAM-Ampicillin-Salbactam, TGC-Tigecycline)

Table-5 shows the frequency of resistance to different antibiotics among Gram positive organisms. In our study, Coagulase Negative *Staphylococci* (CoNS) showed 79.1% resistance to trimethoprim-sulfamethoxazole followed by 70.8% to penicillin and ciprofloxacin. *Staphylococcus aureus* showed 90% resistance to penicillin. In Group D *Streptococcus*, 100% resistance was seen among penicillin. None of the Gram-positive isolates were resistant to LZD.

Table-5: Frequency of resistance (%) to different antibiotics among Gram positive organisms.

Organisms	PG	FOX	CN	E	CIP	DA	LZD	SXT
CoNS	71	38	54	63	71	21	0	79
<i>Staphylococcus aureus</i>	90	20	50	60	50	40	0	40
Group D <i>Streptococci</i>	100	-	-	-	-	-	0	-

(PG-Penicillin, FOX-Cefoxitin, CN-Gentamicin, E- Erythromycin, CIP-Ciprofloxacin, DA-Clindamycin, LZD-Linezolid, SXT-Trimethoprim-sulfamethoxazole)

DISCUSSION

Neonatal sepsis has a major contribution in causing neonatal mortality and morbidity in our country [4]. Main focus of study is to determine the etiology and antimicrobial susceptibility patterns in neonatal sepsis in a tertiary care hospital, Lahore. 450 samples of blood of clinically diagnosed patients of neonatal septicemia were processed. Culture positivity rate in the present study was 21% which is similar with the reports in few studies i.e 24% and 22.4% [12,13]. However, a study was conducted in Bangladesh which showed lower culture positivity rate as compared to our study [14]. Much higher rate of culture positivity is also shown in other studies with the figures of 40.5% and 61.3% [15,16].

In our study, Gram negative organisms were recovered from 56 (58.9%), mostly belonging to Enterobacteriaceae family. Gram positive organisms marked 36 (37.8%) whereas only 3(3.1%) blood cultures were positive for *Candida* spp. The etiology of pathogens causing sepsis is comparable to those observed in other studies in which Gram-negative group accounts for more than 80% of total isolates [8,14]. There are studies conducted by other researchers whose findings are in contrast with our study in which Gram-positive organisms were substantially high in suspected cases of neonatal sepsis [17].

The results of our study showed that among Gram negative organisms most cases were due to

Pseudomonas spp 16(28.5%) followed by *Escherichia coli* 13(23.2%), *Klebsiella pneumoniae* 10 (17.8%), *Acinetobacter* spp 8 (14.2%), *Enterobacter* spp 6(10.7%) and *Citrobacter* spp 3(5.3%). The similar patterns were also reported in India [18]. Another study was conducted in Quetta in which the most common organisms were *Pseudomonas* spp (21.4%) and *Klebsiella pneumoniae* (21.4%) [19].

In our study, among Gram positive organisms, CoNS was the most frequently isolated organism which were 66.6% followed by *Staphylococcus aureus* 27.7% and Group D *Streptococci* 5.5%. This finding is also supported by other studies in which CoNS was the most common pathogen in neonatal intensive care units [15].

In present study, we also studied patterns of antimicrobial susceptibilities in different organisms isolated from suspected cases of neonatal septicemia. Susceptibility testing was carried by modified Kirby Bauer disc diffusion method. In our study, 100% resistance was seen among ceftazidime, ceftriaxone, cefotaxime and aztreonam in *Escherichia coli* and *Klebsiella pneumoniae*. In *Enterobacter* spp, 100% resistance was seen against cefipime and gentamicin and *Citrobacter* spp showed 100% resistance among aztreonam and ciprofloxacin.

Studies were conducted in 2015 which showed 100% resistance to ampicillin and variable pattern of resistance against different antibiotics in *Escherichia coli* and *Klebsiella pneumoniae* respectively [15,20]. 100% resistance was also observed among cefipime and ciprofloxacin in *Citrobacter* spp in India [21].

In our study, *Pseudomonas* spp showed the highest resistance to gentamicin (68.7%) and *Acinetobacter* spp showed 100 % resistance to ceftazidime, ceftriaxone and cefotaxime. Another study was carried out in Rawalpindi who reported maximum 66.7% resistance to aztreonam [20]. Eighty

percent resistance was observed among penicillin in a study conducted in India [22].

Coagulase Negative *Staphylococci* (CoNS) showed 79.1% resistance to trimethoprim-sulfamethoxazole followed by 70.8% to penicillin in our study. *Staphylococcus aureus* showed 90% resistance to penicillin. In Group D *Streptococcus*, 100% resistance was seen among penicillin. None of the Gram-positive isolates were resistant to linezolid.

CONCLUSION

Our study concludes that Gram negative organisms are the main cause of neonatal sepsis in our setup. *Pseudomonas* spp and Coagulase negative *Staphylococci* (CoNS) are the principle pathogens responsible for neonatal sepsis. The antibiogram established in the light of my study will help in providing empirical treatment options to our patients.

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AUTHORS CONTRIBUTION

Sarah Qadeer: Entire research work, sample collection, analysis, write-up.

Iffat Javed: Planning of research, literature review, help in write-up.

Sohaila Mushtaq: literature review, sample analysis

Muhammad Saeed Anwar: Concept, overall supervision

REFERENCES

1. Stoll BJ. Pathogenesis and epidemiology. In: Behrman RE, Kliegman RM, Jenson HB (edi). Nelson Textbook of Paediatrics 19th

- Ed. Philadelphia PA; Saunders. 2011; 623-40.
2. Indian Council of Medical Research New Delhi. National Neonatal Perinatal Database. Report 2002-2003. NNPD Network. Available at http://www.newbornwhocc.org/pdf/nnpd_report_2002-03.PDF Accessed Jun, 2005.
 3. Tsai MH, Chu SM, Lee CW, Hsu JF, Huang HR, Chiang MC, et al. Recurrent late-onset sepsis in the neonatal intensive care unit: incidence, clinical characteristics and risk factors. *Clinical Microbiology and Infection*. 2014 Nov 30; 20(11):0928-35.
 4. Mustafa MA, Korejo R, Shahid A, Nasim S. Infection remains a leading cause of neonatal mortality among infants delivered at a tertiary hospital in Karachi, Pakistan. *The Journal of Infection in Developing Countries*. 2014; 8(11): 1470-5.
 5. Aijaz N, Huda N, Kausar S. Disease burden of NICU, at a tertiary care hospital, Karachi. *J Dow Univ Health Sci Karachi*. 2012; 6(1): 32-5.
 6. UNICEF. Rwanda-About Us-RapidSMS-Maternal and Newborn Consultant Child Survival and Development Section;2013: 1-4.
 7. Shane AL, Stoll BJ. Recent developments and current issues in the epidemiology, diagnosis, and management of bacterial and fungal neonatal sepsis. *American journal of perinatology*. 2013; 30(02): 131-42.
 8. Khan QA, Iqbal H, Rehman H. Trends in patterns of resistance among micro organisms causing neonatal sepsis in Peshawar. *J Postgrad Med Inst*. 2012; 26(2):165-9.
 9. Thaver D, Ali SA, Zaidi AK. Antimicrobial resistance among neonatal pathogens in developing countries. *Pediatr Infect Dis J*. 2009; 28(1): 19-21.
 10. Winn WC. *Koneman's color atlas and textbook of diagnostic microbiology*. Koneman EW, editor. Lippincott Williams & Wilkins; 2006.
 11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty third informational supplement update. CLSI document M100-S22 U. Clinical and Laboratory Standards Institute, Wayne, PA. 2014.
 12. Ojukwu JU, Abonyi LE, Ugwu J, Orji IK. Neonatal septicemia in high risk babies in South-Eastern Nigeria. *Journal of Perinatal Medicine*. 2006; 34(2): 166-72.
 13. Mhada TV, Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. *BMC Public Health*. 2012; 12(1): 904.
 14. Raha BK, Baki MA, Begum T, Nahar N, Jahan N, Begum M. Clinical, bacteriological profile & outcome of neonatal sepsis in a tertiary care hospital. *Medicine Today*. 2014; 26(1): 18-21.
 15. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of neonatal sepsis and implicated pathogens: a study from Egypt. *BioMed research international*. 2015;4: 2015.
 16. Kheir AEM, Khair RA. Neonatal sepsis: Prevalence and outcome in a tertiary neonatal unit in Sudan. *Time J Med Sci Rep Res*. 2014; 2(1): 21-25.
 17. Jan AZ, Zahid SB, Ahmad S. Sensitivity pattern of bacterial isolates in neonatal

- sepsis: A hospital based study. Khyber Med Univ J. 2013; 5(4): 207-212.
18. Swarnkar K, Swarnkar M. A Study of early onset neonatal sepsis with special reference to sepsis screening parameters In A tertiary care centre of Rural India. The Internet Journal of Infectious Diseases. 2012; 10(1):1-8.
 19. Goheer L, Khattak ZS. Early onset neonatal sepsis (Risk factors & clinic-bacteriological profile). Pak Pediatr J. 2014; 38(4):205-10.
 20. Liaquat I, Khan NS, Naveed A. Bacteriological spectrum of Neonatal Sepsis. JPMC. 2015; 19(1):29-32.
 21. Nayak S, Rai R, Kumar VK, Sanjeev H, Pai AKB, Ganesh HR. Distribution of microorganisms in neonatal sepsis and antimicrobial susceptibility patterns in a tertiary care hospital. Arch Med Health Sci. 2015; 2(2):136-139.
 22. Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M. Incidence of neonatal sepsis in tertiary care hospital: an overview. Int J Med Sci Public Health. 2013; 2(3):548-552.