Etiology and antimicrobial resistance patterns in early and late neonatal sepsis in a Neonatal Intensive Care Unit

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ABSTRACT

Introduction. Neonatal sepsis is one of the main causes of death among newborn infants. Empirical antimicrobial treatment is based on epidemiological information and antimicrobial susceptibility tests. The objective of this study was to describe etiologic agents and their antimicrobial susceptibility among newborn infants with early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS) at a Neonatal Intensive Care Unit.

Methods. Cross-sectional study conducted at a tertiary referral hospital in Western Mexico. Determination of antimicrobial resistance of microorganisms isolated in blood or cerebrospinal fluid of patients with EONS or nosocomial LONS.

Results. Yeasts and bacteria were isolated from 235 cultures corresponding to 67 events of EONS and 166 events of LONS. Of all isolates, the most common bacteria were Enterobacteriaceae (51.5%), followed by Streptococcus spp. in EONS, and by Staphylococcus spp. in LONS. Of all nosocomial Enterobacteriaceae, 40% were extended spectrum beta-lactamase producing bacteria. Among Staphylococcus species, resistance to oxacillin was recorded in 65.5%. Among Enterobacteriaceae (n: 121), resistance to amikacin, piperacillin-tazobactam, and meropenem was below 3%. Non-fermenting bacteria did not show resistance to amikacin, ciprofloxacin or cefepime; however, the number of isolates was scarce.

Conclusions. The most commonly identified bacteria in EONS were Enterobacteriaceae (67.6%) and Streptococcus spp. (17.6%), and Enterobacteriaceae (44.9%) and Staphylococcus spp. (34.7%) in LONS. Forty percent of nosocomial Enterobacteriaceae were extended spectrum beta-lactamase producing bacteria, and 65.5% of Staphylococcus spp. showed resistance to oxacillin.

Key words: early neonatal sepsis, late neonatal sepsis, resistance to drugs.

INTRODUCTION

It is estimated that four million neonatal deaths occur worldwide every year, and approximately one third of these are caused by infections. Sepsis and bacterial meningitis continue to be one of the main causes of neonatal mortality, especially among very low birth weight newborn infants (NBIs).2 Early-onset neonatal sepsis (EONS) refers to the presence of a confirmed infection in the blood or cerebrospinal fluid (CSF) of patients younger than 72 hours of life, and late-onset neonatal sepsis (LONS) refers to the onset of such infection between 72 hours and 90 days old.4 Information on etiologic agents is heterogeneous. While in developed countries the most common cause of EONS is group B Streptococcus, its main cause in developing countries are Enterobacteriaceae.1,2,4-10

The most commonly isolated bacteria in nosocomial LONS correspond to Staphylococcus species or Enterobacteriaceae.6,10,11 In these bacteria, the hospital setting favors the acquisition and transmission of antimicrobial resistance genes due to the selective pressure caused by antibiotics.12,13 Resistance mechanisms, such as the production of extended spectrum beta-lactamases (ESBLs) in Klebsiella species or resistance to methicillin in Staphylococcus spp., may result in therapeutic failures.10,12,13

When an invasive bacterial infection is suspected in a hospitalized newborn infant, an empirical antimicrobial treatment is recommended until culture and antimicrobial susceptibility test results are available that would allow to establish a specific management; therefore, it is fundamental to know the epidemiology of neonatal sepsis and the resistance patterns of identified bacteria. The objective of this study was to describe etiologic
agents and their antimicrobial susceptibility among newborn infants with early-onset or late-onset neonatal sepsis at a Neonatal Intensive Care Unit.

MATERIAL AND METHODS

A cross-sectional study was conducted at Nuevo Hospital Civil de Guadalajara “Dr. Juan I. Menchaca” (HCGJIM) in the city of Guadalajara, Jalisco. The study period extended from March 7th, 2013 to July 4th, 2014. The project was approved by the hospital’s ethics and research committees.

HCGJIM is a tertiary referral hospital in Western Mexico that provides health services to an open, low-resource population. The Division of Neonatology is made up by a Neonatal Intensive Care Unit with 18 active cribs and 67 cribs that provide intermediate care. Gestational age was <37 weeks in 55% of hospitalized patients, and ≤32 weeks in 19%. If necessary during patient management, central venous lines, mechanical ventilation and total parenteral nutrition were provided. There is no antenatal maternal diagnostic program in place to detect group B beta-hemolytic *Streptococcus* infections.

Microbiological tests were conducted according to the neonatal sepsis diagnosis protocol established by HCGJIM. Blood and CSF samples for cultures were collected from newborn infants who had more than one clinical manifestation and/or abnormal lab test indicative of sepsis (fever, hypothermia, tachycardia, bradycardia, polypnea, leukocytosis, leukopenia, or C-reactive protein > 1.0 mg/dL) and those whose mothers had one or more of the following risk factors: active urinary tract infection, chorioamnionitis, fever, and premature rupture of membranes ≥18 hours.

Following culture collection, patients with clinical manifestations and/or lab tests indicative of sepsis were started on an empirical antimicrobial schedule. Newborn infants with risk factors but no clinical manifestation were kept under monitoring with no antimicrobial treatment until an infection was ruled out. The antimicrobial schedule included ampicillin and gentamicin in EONS, or vancomycin and amikacin or meropenem in LONS.

Inclusion and exclusion criteria

The diagnosis of EONS was established in hospitalized newborn infants who presented microbial growth in blood or CSF cultures collected before 72 hours of life, and the diagnosis of nosocomial LONS was established in hospitalized newborn infants who presented microbial growth in blood or CSF cultures collected at 72 hours of life or later.

Sample collection and processing

For the diagnosis of bloodstream infections, two or more blood samples were obtained by peripheral venipuncture at different sites using an aseptic technique and inoculated into blood culture bottles (BacT/ALERT PF Pediatric FAN®). Microbial growth was monitored using the Bact/ALERT® 3D automated system for seven days. Cultures with positive results were reseeded in blood and McConkey agar. Blood cultures were considered positive if they showed Gram-negative bacteria or yeast growth in one or more bottles. In the case of Gram-positive bacteria, cultures were positive if isolated in two or more bottles. If bacteria were isolated only in one bottle, they were classified as contaminating.

CSF samples were collected by performing a lumbar puncture using a sterile technique, then the sample was inoculated into enhanced media (BacT/ALERT PF Pediatric FAN®) and subjected to direct culture in blood agar. All bacterial growth was considered significant, except for coagulase-negative *Staphylococcus*. In these cases, the event was classified as infectious if the culture was positive and there was an abnormality in the number of leukocytes and glucose in the CSF cytochemistry.

Bacteriological identification

For each bacteria isolated in every event of EONS and LONS, bacterial species were identified and an antimicrobial susceptibility test was performed using the *MicroScan* autoSCAN-4 System® automated system. Gram-positive bacteria were inoculated into type 2 and 3 *MicroScan®* dehydrated panels, while Gram-negative bacteria were inoculated into type 40 *MicroScan®* dehydrated panels and incubated at 35 °C for 16-24 hours. Cut-off points for minimum inhibitory concentrations (MIC) to define antimicrobial resistance or susceptibility were in accordance with the criteria established by the Clinical and Laboratory Standards Institute (CLSI). Results were qualitative and expressed as susceptible, intermediate or resistant.

Panels used for Enterobacteriaceae and
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*Staphylococcus* also showed phenotypic test results indicative of ESBL production using the cefotaxime and ceftazidime susceptibility test, with and without clavulanic acid.

Isolated bacteria were classified into five groups: Enterobacteriaceae, non-fermenting bacteria, *Staphylococcus* spp., *Enterococcus* spp. and *Streptococcus* spp. The most representative microorganisms of the Enterobacteriaceae group were *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*, while *Pseudomonas* spp. and *Acinetobacter* spp. were more common in the non-fermenting group. Isolated yeasts were also recorded; however, no antifungal susceptibility test was performed.

**Statistical analysis**

Frequency and percentage values for antimicrobial resistance were estimated for each bacterial group. Antimicrobial resistance frequencies were compared based on the diagnosis (EONS or LONS). A $\chi^2$ or $\gamma^2$ test with Yates’ correction was performed to contrast the hypothesis. The IBM SPSS Statistics software, version 20, and the OpenEpi software, version 3.01, were used.

**RESULTS**

Between March 7th, 2013 and July 4th, 2014, 14 207 births were recorded; 1550 (9.2%) of all newborn infants were hospitalized; 602 patients were suspected of EONS, which was confirmed in 166 (incidence: 10.7%). In both EONS (44.9%) and LONS (34.7%), the most common bacteria in each group were Enterobacteriaceae (67.6%), followed by *Streptococcus* spp. (17.6%), while the most common ones in LONS were Enterobacteriaceae (44.9%) and *Staphylococcus* spp. (34.7%). In both events, the most common bacterial species was *Klebsiella pneumoniae* (n: 62) (Table 1).

Enterobacteriaceae were more likely to show antimicrobial resistance in strains isolated from events of LONS, compared to the bacteria in EONS, except for the following antibiotics: ticarcillin with clavulanic acid, imipenem, amikacin, piperacillin with tazobactam and meropenem. For the last three, the frequency of resistance was below 3% in early and nosocomial infections (Table 2).

One hundred seventy-five strains of coagulase-negative *Staphylococcus* were isolated; however, 77.1% (n: 135) were classified as contaminating because they were observed only in one blood culture bottle or because CSF cytochemistry was normal. Of all *Staphylococcus* spp., 95% were isolated in nosocomial infections. The rate of resistance to oxacillin was 65.5%. No vancomycin-resistant *Staphylococcus* species were recorded. Only one strain of *Staphylococcus epidermidis* showed resistance to linezolid and rifampicin. For the other antibiotics, the rate of resistance was over 26% (Table 3).

No resistance to amikacin, ciprofloxacin, cefepime and tobramycin was observed in non-fermenting bacteria; however, only three strains were identified in EONS and 15, in LONS; therefore, these findings are not conclusive. The rate of resistance to meropenem was 6/18; these bacteria corresponded to *Pseudomonas* species (Table 4).

Ten strains were isolated from the *Enterococcus* spp. group, four in EONS. No bacteria in this group showed resistance to ampicillin, penicillin,
vancomycin, ciprofloxacin or linezolid. One strain of Enterococcus faecalis showed resistance to gentamicin. Among Streptococcus species identified (n: 16), only nine were subjected to antibiogram analysis: Streptococcus bovis (n: 5), Streptococcus agalactiae (n: 3) and Streptococcus pyogenes (n: 1). One strain of Streptococcus bovis resistant to ampicillin, ceftriaxone and clindamycin and one of Streptococcus agalactiae resistant to clindamycin were identified.

Table 1. Total microbial isolations based on diagnosis and bacterial group

| Group and species | Early-onset neonatal sepsis (n: 68) | Late-onset neonatal sepsis (n: 167) | Total (n: 235) | P (χ²) |
|-------------------|--------------------------------------|-------------------------------------|----------------|--------|
| Enterobacteriaceae | 46 (67.6%)                           | 75 (44.9%)                          | 121            | 0.005  |
| Klebsiella pneumonia | 14                                   | 48                                  | 62             |        |
| Escherichia coli | 17                                   | 12                                  | 29             |        |
| Enterobacter cloacae | 5                                    | 10                                  | 15             |        |
| Citrobacter spp. | 2                                    | 2                                   | 4              |        |
| Otras* | 8                                    | 3                                   | 11             |        |
| Staphylococcus | 3 (4.4%)                             | 58 (34.7%)                          | 61             | < 0.001|
| Coagulase-negative Staphylococcus | 1                                    | 39                                  | 40             |        |
| Staphylococcus aureus | 2                                    | 19                                  | 21             |        |
| Non-fermenting bacteria | 3 (4.4%)                         | 15 (9.0%)                           | 18             | 0.2    |
| Pseudomonas spp. | 2                                    | 12                                  | 14             |        |
| Acinetobacter spp. | 1                                    | 3                                   | 4              |        |
| Enterococcus | 4 (5.9%)                             | 6 (3.6%)                            | 10             | 0.4    |
| Enterococcus faecalis | 3                                    | 6                                   | 9              |        |
| Enterococcus faecium | 1                                    | 0                                   | 1              |        |
| Streptococcus | 12 (17.6%)                           | 4 (2.4%)                            | 16             | 0.02   |
| Streptococcus agalactiae | 2                                   | 1                                   | 3              |        |
| Streptococcus bovis | 6                                    | 2                                   | 8              |        |
| Streptococcus pneumoniae | 3                                   | 1                                   | 4              |        |
| Streptococcus pyogenes | 1                                    | 0                                   | 1              |        |
| Yeasts | 0                                    | 9 (5.4%)                            | 9              |        |
| Candida parapsilosis | 0                                    | 5                                   | 5              |        |
| Candida albicans | 0                                    | 4                                   | 4              |        |

* Other Enterobacteriaceae include Proteus mirabilis, Serratia spp. and Yersinia spp.
The number of microbial isolates exceeds that of neonatal sepsis events by two polymicrobial cultures.

Table 2. Frequency of antimicrobial resistance in Enterobacteriaceae isolated from patients with early-onset neonatal sepsis or nosocomial late-onset neonatal sepsis with a p value (χ²)

| Enterobacteriaceae: % of resistance (resistant strains/analyzed strains) | Strains isolated in early-onset neonatal sepsis (n: 46) | Strains isolated in nosocomial late-onset neonatal sepsis (n: 75) | p (χ²) |
|-------------------------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------------------|--------|
| Amikacina | Amikacina | 2.3 (1/44) | 1.4 (1/74) | 0.70 |
| Gentamicina | Gentamicina | 15.9 (7/44) | 41.9 (31/74) | 0.003 |
| Ampicillin | Ampicillin | 57.1 (24/42) | 89.7 (61/68) | < 0.001 |
| Ampicillin/sulbactam | Ampicillin/sulbactam | 19.3 (8/41) | 54.4 (37/68) | 0.001 |
| Piperacillin/tazobactam | Piperacillin/tazobactam | 0 (0/45) | 2.8 (2/71) | 0.42 |
| Ticarcillin/ac. clavulánico | Ticarcillin/ac. clavulánico | 0 (0/36) | 7.5 (4/53) | 0.15 |
| Aztreonam | Aztreonam | 17.4 (8/46) | 45.3 (34/75) | 0.002 |
| Ceftriaxona | Ceftriaxona | 13.0 (6/46) | 45.3 (34/75) | < 0.001 |
| Cefazidima | Cefazidima | 13.5 (5/37) | 43.3 (26/60) | 0.001 |
| Cefotaxima | Cefotaxima | 11.1 (4/36) | 49.2 (29/59) | < 0.001 |
| Cefepime | Cefepime | 13.0 (6/46) | 45.3 (34/75) | < 0.001 |
| Ciprofloxacin | Ciprofloxacin | 6.7 (3/45) | 20 (15/75) | 0.05 |
| Meropenem | Meropenem | 2.2 (1/46) | 1.3 (1/75) | 0.72 |
| Imipenem | Imipenem | 0 (0/35) | 3.5 (2/57) | 0.43 |
| Trimethoprim-sulfamethoxazole | Trimethoprim-sulfamethoxazole | 20.5 (9/44) | 49.3 (35/71) | 0.002 |
| ESBL | ESBL | 6.5 (3/46) | 40.0 (30/75) | < 0.001 |

ESBL: extended spectrum beta-lactamase.
All yeasts were isolated in patients with LONS (n: 9); five corresponded to Candida parapsilosis and four, to Candida albicans. Four isolates were observed in cerebrospinal fluid.

**DISCUSSION**

Similarly to what has been reported in studies conducted in developing countries, the most commonly identified bacteria was *Klebsiella pneumoniae* (n: 62). In developed countries, the predominant bacteria in EONS is group B *Streptococcus*; in our study; it was isolated in three occasions.

Conditions that define the etiology of infections in newborn infants may be related to invasive therapies or antimicrobial prophylaxis. Such interventions may be of little access or non-existent in low-resource countries, thus favoring a different epidemiological scene.

Viswanathan, et al. identified that 71.7% of bacteria causative of neonatal sepsis corresponded to Gram-negative bacilli, and *Klebsiella pneumoniae* was the most-commonly isolated bacteria. They showed a high percentage of resistance to first- and second-line antibiotics: ampicillin (98.5%), gentamicin (84.4%), amikacin (65.6%), and cefotaxime (83.3%). In our study, Enterobacteriaceae isolated from our patients with EONS showed resistance to ampicillin (57.1%), gentamicin (15.9%), amikacin (2.3%), and cefotaxime (11.1%). Among nosocomial bacteria, resistance increased significantly, except for amikacin.

Saritha Kamath, et al., from the Department of Microbiology of the Medical Association of Kasturba, India, isolated 205 bacteria from nosocomial infections seen at the Neonatal Intensive Care Unit (NICU). Of those, 83.1% were isolated from the bloodstream or the central nervous system. And 71.8% corresponded to

### Table 3. Frequency of antimicrobial resistance in *Staphylococcus* species isolated in patients with early-onset neonatal sepsis or nosocomial late-onset neonatal sepsis with a p value ($\chi^2$)

| Staphylococcus spp. | Strains isolated in early-onset neonatal sepsis (n: 3) | Strains isolated in nosocomial late-onset sepsis (n: 58) | p ($\chi^2$) |
|---------------------|--------------------------------------------------------|--------------------------------------------------------|-------------|
| Penicillin          | (3/3)                                                   | 89.7 (52/58)                                           | 0.46        |
| Amoxicillin/clavulanic acid | (1/3)                                                 | 65.5 (38/58)                                           | 0.61        |
| Oxacillin           | (1/3)                                                   | 65.5 (38/58)                                           | 0.61        |
| Ceftriaxone         | (1/3)                                                   | 62.1 (36/58)                                           | 0.69        |
| Clindamycin         | (3/3)                                                   | 62.1 (36/58)                                           | 0.62        |
| Ciprofloxacin       | (0/3)                                                   | 44.8 (26/58)                                           | 0.12        |
| Mexofloxacin        | (0/3)                                                   | 29.8 (17/57)                                           | 0.87        |
| Trimethoprim-sulfamethoxazole | (0/3)                                           | 33.3 (19/57)                                           | 0.76        |
| Cefoxitin           | (0/2)                                                   | 26.1 (6/23)                                            | 0.67        |
| Rifampicin          | (0/3)                                                   | 1.7 (1/58)                                             | 0.70        |
| Vancomycin          | (0/3)                                                   | 0 (0/56)                                               | 0.78        |
| Linezolid           | (0/3)                                                   | 1.7 (1/58)                                             | 0.70        |
| ESBL                | (3/3)                                                   | 89.7 (52/58)                                           | 0.81        |

ESBL: extended spectrum beta-lactamase.

### Table 4. Frequency of antimicrobial resistance in non-fermenting bacteria isolated from patients with early-onset neonatal sepsis or nosocomial late-onset neonatal sepsis with a p value ($\chi^2$)

| Non-fermenting bacteria | Strains isolated in early-onset neonatal sepsis (n: 3) | Strains isolated in nosocomial late-onset sepsis (n: 15) | p ($\chi^2$) |
|-------------------------|--------------------------------------------------------|--------------------------------------------------------|-------------|
| Amikacin                | 0/2                                                    | 0/15                                                   | 0.66        |
| Piperacillin/tazobactam | 0/2                                                    | 1/13                                                   | 0.48        |
| Ticarcillin/clavulanic acid | 1/3                                                 | 4/12                                                   | 0.49        |
| Cefazidime              | 0/3                                                    | 1/12                                                   | 0.40        |
| Ciprofloxacin           | 0/2                                                    | 0/15                                                   | 0.66        |
| Cefepime                | 0/2                                                    | 0/15                                                   | 0.66        |
| Meropenem               | 1/3                                                    | 5/15                                                   | 0.50        |
| Tobramycin              | 0/2                                                    | 0/15                                                   | 0.66        |
| Trimethoprim-sulfamethoxazole | 0/2                                                 | 4/11                                                   | 0.99        |
Gram-negative bacteria, while 81.8% were ESBL producers. In our study, 40% of Enterobacteriaceae isolated from LONS cases were ESBL producers (n: 30), while only 6.5% of Enterobacteriaceae isolated from EONS corresponded to this type (p < 0.001). The bacterial species that most commonly showed to be a producer of ESBL was *Klebsiella pneumoniae* (54.8%).

It is known that the presence of ESBL is related to the exposure of broad-spectrum antibiotics, such as cefotaxime, due to the induction of chromosomal beta-lactamases. At the NICU of Khomeini, Iran, Hassan Aletayeb et al. identified rates of resistance to ampicillin and gentamicin in 100% of isolated *Klebsiella pneumoniae* (n: 153) and to cefotaxime in 95.8%.

It has been observed that, at NICUs were cefotaxine of choice include third-generation cephalosporins, it has been possible to reduce the rates of resistance to different antimicrobials by limiting the use of cefotaxime. Jyoti Bagla, et al. observed that, following the use of cefotaxime at the NICU, the rate of resistance to amikacin reduced by 28% and to ceftriaxone, by 19%. The reason why restricting the use of cephalosporins modifies the rates of resistance to different antibiotics is that resistance mechanisms may be transferred by plasmids carrying more than one resistance gene.

For the Enterobacteriaceae identified at our NICU, the rates of resistance to amikacin, piperacillin with tazobactam and meropenem were below 3% in early and late infections. Such percentages suggest that empirical therapeutic schedules should include at least one of these antibiotics. Bambala Puthattayil Zakariya, et al. observed that most strains of *Klebsiella pneumoniae* isolated from patients with neonatal sepsis did not show resistance to amikacin and meropenem. Given amikacin’s low rate of resistance and the fact that it does not induce chromosomal beta-lactamases, it should be used instead of cephalosporins as empirical management of neonatal sepsis, and treatment should be modified based on antibiogram results.

Of all nosocomial LONS, 34.7% were caused by *Staphylococcus spp.* The conditions that favor these infections include prematurity, invasive procedures, such as central venous lines or mechanical ventilation, and an immature immune system. In this group of bacteria, resistance to oxacillin was identified in 65.5% of cases, therefore suggesting the limited use of beta-lactams.

The number of non-fermenting bacteria, *Enterococcus spp.* and *Streptococcus spp.* was not enough to make conclusive comparisons on the resistance patterns of bacteria isolated in EONS and LONS. It should be noted that every hospital unit may have different patterns of antimicrobial resistance, so these findings should be analyzed and compared at each unit before making decisions in terms of management.

This study allows to know the epidemiology of LONS; however, given that there is not enough information on the factors associated to infection, it is not possible to define what subgroups are at a higher risk, which is one of this study’s limitations.

**CONCLUSIONS**

The most commonly identified bacteria in EONS were Enterobacteriaceae and *Streptococcus spp.*, while Enterobacteriaceae and *Staphylococcus spp.* were predominant in LONS. Considering all events, the most common bacteria species was *Klebsiella pneumoniae*.

Among Enterobacteriaceae isolated from LONS, there was a higher rate of ESBL producers (40%), compared to those isolated from EONS (6.5%) (p < 0.001).

Among *Staphylococcus* species, resistance to oxacillin was recorded in 65.5% of cases.

**REFERENCES**

1. Ganatra HA, Stoll BJ, Zaidi AKM. International perspective on early-onset neonatal sepsis. *Clin Perinatol* 2010;37(2):501-23.
2. Puopolo KM. Epidemiology of neonatal early-onset sepsis. *Neo Rev* 2008;9(12):571-9.
3. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? *Lancet* 2005;365(9462):891-900.
4. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary care hospital in South India: bacteriological profile and antibiotic sensitivity pattern. *Indian J Pediatr* 2011;78(4):413-7.
5. Hofer N, Müller W, Resch B. Neonates presenting with temperature symptoms: role in the diagnosis of early onset sepsis. *Pediatr Int* 2012;54(4):486-90.
6. Aletayeb SMH, Khosravi AD, Dehdashian M, Kompani F, et al. Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a tertiary hospital. *Afr J Microbiol Res* 2011;5(5):528-31.
7. Bhat YR, Lewis LES, Vandana K. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. *Ital J Pediatr* 2011;37:32.
8. Li Z, Xiao Z, Li Z, Zhong Q, Zhang Y, Xu F. 116 cases of neonatal early-onset or late-onset sepsis: A single center retrospective analysis on pathogenic bacteria species distribution and antimicrobial susceptibility. *Int J Clin Exp Med* 2013;6(8):693-9.
9. Marchant EA, Boyce GK, Sadarangani M, Lavioe PM. Neonatal sepsis due to coagulase-negative staphylococci. *Clin Dev Immunol* 2013;2013:586076.
10. Kamath S, Mallaya S, Shenoy S. Nosocomial infections in neonatal intensive care units: profile, risk factor assessment and antibiogram. *Indian J Pediatr* 2010;77(1):37-9.

11. Dudeck MA, Horan TC, Peterson KD, Allen-Bridson K, et al. Department of Health and Human Services. National healthcare safety network (NHSN) Report, data summary for 2011, Device-associated Module; 2013. [Accessed on: May 2013]. Available at: http://www.cdc.gov/nhsn/pdfs/dataset/nhsn-report-2011-data-summary.pdf.

12. Opal S, Pop-Vicas A. Molecular mechanisms of antibiotic resistance in bacteria. In: Mandell G, Bennett J, Dolin R, eds. *Mandell, Douglas, and Bennett’s Principles and Practice of Infectious Diseases*. 7th ed. Philadelphia: Elsevier; 2010. Págs. 279-96.

13. Miller M, Gilligan P. Mechanisms and detection of antimicrobial resistance. In Long S, Pickering L, Prober C, eds. *Principles and practice of pediatric infectious diseases*. 4th ed. Philadelphia: Elsevier; 2012. Págs. 1421-32.

14. Polin RA, Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. *Pediatrics* 2012;129(5):1006-15.

15. Hall KK, Lyman JA. Updated review of blood culture contamination. *Clin Microbiol Rev* 2006;19(4):788-802.

16. Buttery JP. Blood cultures in newborns and children: optimizing an everyday test. *Arch Dis Child Fetal Neonatal Ed* 2002;87(1):F25-8.

17. Beekmann SE, Diekema DJ, Doern GV. Determining the clinical significance of coagulase-negative staphylococci isolated from blood cultures. *Infect Control Hosp Epidemiol* 2005;26(6):559-66.

18. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. Document M100-S22. Wayne, PA, 2012;32(3). [Accessed on: March 2013]. Available at: http://antimicrobียนos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf.

19. Shrestha S, Shrestha N, Dongol Singh S, Shrestha R, et al. Bacterial Isolates and its Antibiotic Susceptibility Pattern in NICU. *Kathmandu Univ Med J (KUMJ)* 2013;11(41):66-70.

20. Sheth KV, Patel TK, Tripathi CB. Antibiotic sensitivity pattern in neonatal intensive care unit of a tertiary care hospital of India. *Asian J Pharm Clin Res* 2012;5(3):46-50.

21. West BA, Peterside O. Sensitivity pattern among bacterial isolates in neonatal sepsis in Port Harcourt. *Ann Clin Microbiol Antimicrob* 2012;11:7.

22. Viswanathan R, Singh AK, Mukherjee S, Mukherjee R, et al. Aetiology and antimicrobial resistance of neonatal sepsis at a tertiary care centre in eastern India: a 3 year study. *Indian J Pediatr* 2011;78(4):409-412.

23. Bagla J, Ghosh V, Ramji S, Gothi D. Antimicrobial susceptibility patterns following change in antibiotic policy in NICU. *Pediatr Infect Dis* 2013;32(2):59-63.