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.

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INTRODUCTION

It is estimated that four million neonatal deaths occur worldwide every year, and approximately one third of these are caused by infections.[1-3] 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.[2,4] 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;[1,14] 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 study included blood and CSF cultures with microorganism growth from newborn infants who had EONS or nosocomial LONS. Bacteria isolated from patients who had not been born at HCGJIM or rehospitalized following discharge were not analyzed.

The diagnosis of EONS was established in hospitalized newborn infant patients 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 infant patients 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[15] 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.[15-17]

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).[18]Results were qualitative and expressed as susceptible, intermediate or resistant.

Panels used for Enterobacteriaceae and

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 $\div_2$ or $\div^2$ 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 67 (incidence: 4.7 events per 1000 newborn infants). Nosocomial LONS was suspected in 24.9% (n: 386) of hospitalized patients, which was confirmed in 166 (incidence: 10.7%).

The average gestational age of all newborn infants was 38.5 weeks (maximum: 42; minimum: 22; SD: 2.11). A statistically significant difference was observed in the gestational age of hospitalized patients (35.8 weeks) and non-hospitalized patients (38.8 weeks) ($p < 0.001$), and between patients with EONS (35.6 weeks) and LONS (34.6 weeks) (p= 0.03).

A gestational age younger than 37 weeks showed an association with EONS (OR: 10.8; 95% CI: 6.6–17.6; $p < 0.001$) and LONS (OR: 1.5; 95% CI: 1.1-2.1; p 0.01). In this subgroup of patients, those with a weight of ≤1500 g (n: 345) were most likely to have EONS (OR: 2.9; 95% CI: 1.6-5.4; $p < 0.001$) and LONS (OR: 2.6; 95% CI: 1.8-3.9; $p < 0.001$).

The average weight of hospitalized patients (2263.6 g) was significantly lower than that of non-hospitalized patients (3101.7 g) ($p < 0.001$);

however, the weight of patients with EONS (2155.9 g) was not significantly different from that of patients with LONS (1894.8 g) (p 0.056). A weight of ≤2500 g showed an association with EONS (OR: 11.4; 95% CI: 6.9-19; $p < 0.001$) and with LONS (OR: 1.77; 95% CI: 1.2-2.5; $p < 0.001$).

Infection sites of patients with EONS and LONS were the bloodstream in 63 (94.03%) and 147 (88.5%), the central nervous system in 1 (1.49%) and 6 (3.6%), and both sites in 3 (4.48%) and 13 (7.9%), respectively. Fourteen patients had EONS and subsequently developed nosocomial LONS.

Bacteria or yeasts were isolated from 235 cultures, 28.9% (n: 68) corresponded to patients with EONS and the rest, to patients with LONS (n: 167). The most common bacteria in EONS 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 agalactie (n: 3) and *Streptococcus pyogenes* (n: 1). One strain of *Streptococcus bovis* resistant to ampicillin, ceftriaxone and clindamycin and one of *Streptococcus agalactie* resistant to clindamycin were identified.

TABLE 1. *Total microbial isolations based on diagnosis and bacterial group*

Group and species	Early-onset neonatal (n: 68)	Late-onset neonatal sepsis (n: 167)	Total (n: 235)	P (χ2)
Enterobacteriaceae	**46 (67.6%)**	**75 (44.9%)**	**121**	**0.005**
Klebsiella pneumoniae	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 agalactie	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)		
Antibiotic	**Strains isolated in early-onset neonatal sepsis (n: 46)**	**Strains isolated in nosocomial late-onset neonatal sepsis (n: 75)**	**p (χ2)**
Amikacina	2.3 (1/44)	1.4 (1/74)	**0.70**
Gentamicina	15.9 (7/44)	41.9 (31/74)	0.003
Ampicillin	57.1 (24/42)	89.7 (61/68)	< 0.001
Ampicillin/sulbactam	19.5 (8/41)	54.4 (37/68)	0.001
Piperacillin/tazobactam	0 (0/45)	2.8 (2/71)	**0.42**
Ticarcillin/ác. clavulánico	0 (0/36)	7.5 (4/53)	**0.15**
Aztreonam	17.4 (8/46)	45.3 (34/75)	0.002
Ceftriaxona	13.0 (6/46)	45.3 (34/75)	< 0.001
Ceftazidima	13.5 (5/37)	43.3 (26/60	0.001
Cefotaxima	11.1 (4/36)	49.2 (29/59)	< 0.001
Cefepime	13.0 (6/46)	45.3 (34/75)	< 0.001
Ciprofloxacino	6.7 (3/45)	20 (15/75)	0.05
Meropenem	2.2 (1/46)	1.3 (1/75)	**0.72**
Imipenem	0 (0/35)	3.5 (2/57)	**0.43**
Trimethoprim-sulfamethoxazole	20.5 (9/44)	49.3 (35/71)	0.002
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).[4,19,22] In developed countries, the predominant bacteria in EONS is group B *Streptococcus*;[2] 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.[4,8,9]

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%).[22] 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 (χ2)*

	% of resistance (resistant strains/analyzed strains)		
Staphylococcus spp.	Strains isolated in early-onset neonatal sepsis (n: 3)	Strains isolated in nosocomial late-onset sepsis (n: 58)	p (χ2)
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
Moxifloxacin	(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 (χ2)*

	Resistant strains/analyzed strain		
Non-fermenting bacteria	Strains isolated in early-onset neonatal sepsis (n: 3)	Strains isolated innosocomial late-onset sepsis (n: 15)	p (χ2)
Amikacin	0/2	0/15	0.66
Piperacillin/tazobactam	0/2	1/13	0.48
Ticarcillin/clavulanic acid	1/3	4/12	0.49
Ceftazidime	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.[10] 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.[23] 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%.[6]

It has been observed that, at NICUs were antibiotics 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.[23]

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. also 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.[4]

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.[9] 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. ∎

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