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Original Article

Bacterial Etiology and Antibiotic Sensitivity Patterns of Early-Late Onset Neonatal Sepsis among Newborns in Shiraz, Iran 2004-2007

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Abstract

Background: Neonatal sepsis is a major cause of mortality and morbidity, especially in developing countries. The goal of the present study was to determine the bacterial etiology and antibiotic sensitivity patterns of neonatal sepsis.

Methods: This cross sectional study was conducted on 208 neonates admitted with clinically suspected sepsis over a period of 30 months. Sepsis was divided into early onset sepsis (EOS, ≤5 days of age) and late onset sepsis (LOS, >5 days of age). The two groups were further divided into proven (culture positive ± abnormal markers) and probable (culture negative + abnormal markers) subgroups. Blood culture was performed using Bactec.

Results: Of 208 cases, 90 had neonatal sepsis consisting of 38 (26 proven) presented as EOS and 52 (42 proven) as LOS. In the EOS, *Escherichia coli (E. coli)* was the most common organism followed by *klebsiella spp, Staphylococcus aureus* (*S. aureus*). As for LOS, *Coagulase-negative staphylococci* (CONS) were the most common organism followed by *Enterococcus spp, S. aureus*. The antibiogram on the isolated *E. coli* and *klebsiella spp* revealed a greater combined sensitivity to cefotaxime. *Coagulase-negative staphylococci* and *S. aureus* had 100% and *Enterococcus spp* 90% sensitivity to vancomycin.

Conclusion: *Escherichia coli* and *CONS* were the most common organisms causing EOS and LOS, respectively. Since the antibiotic sensitivity patterns of these organisms are changed, it seems necessary to conduct bacterial etiology studies and to determine antibiotic sensitivity patterns periodically in order to promote the empirical therapy.

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Keywords ● Sepsis ● neonate ● microorganism ● antibiotic

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Introduction

A newborn is very vulnerable to infectious diseases because of the immaturity of his immune system. The bacterial infections are still an important cause of neonatal morbidity and mortality.[1] Mortality from neonatal infection is approximately 25% despite the use of potent antibacterial agents and supportive care.[2]

Neonatal sepsis occurs in two distinct patterns based on the postnatal age at onset, early onset sepsis (EOS) and late onset sepsis (LOS).[3] Currently, the criteria for neonatal sepsis

usually include the documentation of infection in a neonate with a serious systemic illness in which noninfectious explanations for the abnormal pathophysiologic state are excluded or unlikely.[4] However, some babies with clinical sings of infection and abnormal septic markers may have negative cultures.[5]

The bacterial pathogens responsible for neonatal sepsis vary with geographical areas, and tend to change over time.[6] *Group B streptococci* (GBS) and *E. coli* predominate in the USA and Europe, whereas *staphylococci* and *Gram- negative bacilli* are much more common in developing countries.[7,8] The lack of a recent study in the region on the etiology of the pathogens and their susceptibility patterns to the antibiotics conventionally used in the neonatal wards served as an impetus for the present study.

Material and Methods

The study was a cross sectional one conducted on 208 outborn and inborn neonates (age 0-28 days) with clinically suspected sepsis, who were admitted to Nemazee and Hafez hospitals, Shiraz, Fars, Iran, over a 30-month period from October 2004 to March 2007.

None of the recruited neonates received antibiotic therapy before sepsis work up. They were with at least three of the clinical signs and symptoms including poor feeding and lethargy, and respiratory problems such as respiratory rate > 60 /min, apnea, grunting, cyanosis and retraction, temperature instability for longer than 1 h such as hyperthermia (axillary temperature: >37.5), hypothermia (axillary temperature: <36.5), gastrointestinal problems including vomiting, abdominal distension, diarrhea and abnormal gastric residual, and central nervous system symptoms such as convulsion, hypotonia and irritability.

The neonates with sepsis were divided into two groups of EOS (≤5 days of age) and LOS (>5 days of age). Each group was further classified into proven and probable sepsis groups. Proven sepsis was defined as growth from normally sterile sites (blood, cerebrospinal fluid or urine) of either pathogenic organisms, or *Coagulase-negative staphylococci (CONS)* and other low-pathogenic organisms with abnormal septic markers including high or low total WBC and neutrorphil counts, and a CRP of ≥10 mg/L at the onset or the next day. Probable sepsis was defined as neonates with negative cultures, but with clinical signs of infection and abnormal septic markers.[5]

Demographic data, clinical manifestations and outcomes for both EOS and LOS were identified and recorded. The evaluation of sepsis was performed for all the cases using blood culture, C-reactive protein (CRP), full white blood cell (WBC) count and differential, cerebrospinal fluid (CSF) analysis and culture, and urine culture. All cultures were analyzed at Professor Alborzi Clinical Microbiology Research Center at Nemazee hospital, Shiraz, Iran.

Blood culture volumes were intended to be at least 0.5 ml. Blood for culture was introduced into a single aerobic blood culture bottle (ped plus aerobic). The Bactec 9240 system (Becton Dinckinson USA) was used to incubate and detect bacterial growth. For CSF, 2 ml were obtained after lumbar puncture. A Gram-stained smear was examined and the sheep blood agar and chocolate agar were inoculated. Cultures were incubated at 37˚C during 24 and 48 hours. Also, CSF specimens were analyzed for cell count, and protein and sugar concentrations.

The sensitivity of bacteria to different antibiotics including ampicillin, amikacin, gentamicin, cefotaxime, ceftazidime, ciprofloxacin, imipenem, vancomycin and clindamycin was investigated according to the standard disk diffusion (Kirby-Bauer) method using Mast Co (Merseyside, UK) or Difco (BBL, USA) disks.

Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 11.0. Continuous data were described as mean±SD. Univariate analyses included independent t and Chi-square tests for the comparison of the means and qualitative variables, respectively. A P value of ≤0.05 was considered to be statistically significant.

Results

Two hundred and eight neonates (115 males and 93 females) with clinically suspected sepsis were enrolled in the study. One hundred and forty (67.3%) had birth weights heavier than 1499 g and 68 (32.7%) had very low birth weights (VLBW; birth weight <1500 g). The median gestational age (GA) for all neonates were 36.80±3.22 weeks. Of the total 208 cases, 90 (43.3%) had neonatal sepsis out of which 38 (42.2%) presented as EOS and 52 (57.8%) as LOS. The sex ratio was 1/1.2 (17 males, 21 females) for EOS, and 2.4/1 (37 males, 15 females) for LOS. The median gestational age in EOS and LOS were 37.19±2.93 and 36.62±3.49 weeks, respectively. There was significant difference in neonate maturity between the two groups. In EOS, 14 (36.8%) and in LOS, 29 (55.8%) neonates were delivered by cesarean section.

The most common clinical signs and symptoms in the cases with EOS were poor feeding and lethargy in 29 (76.3%), respiratory distress in 17 (44.7%), jaundice in 16 (42%), convulsion in 11 (29%) and fever in 11 (29%) neonates. The most common clinical signs and symptoms in the LOS group were poor feeding and lethargy in 36 (69%), respiratory distress in 14 (27%), convulsion in 10 (19%), jaundice in 8 (15%) and fever in 16 (31%) neonates.

There were 26 (28.9%) and 42 (46.7%) neonates with proven sepsis in the EOS and LOS groups, respectively. The corresponding isolated organisms are illustrated in table 1. The most common isolated microorganisms in the EOS were *E. coli* (23%), *klebsiella spp* and *S. aureus* (15.4%) and *Enterococcus spp* (11.5%). All the 26 had septicemia, and no organism was isolated from the urine or CSF.

In the proven LOS group, *CONS* (28.5%) was the commonest organism followed by *Enterococcus spp*, *S. aureus* and *Enterobacter* (table 1). In 4 of the 42 neonates with septicemia, an organism was also isolated from either the urine or CSF. Three neonates with *klebsiella spp, Acinobacter spp and CONS* had the same pathogens isolated in the urine as in their bloods and another neonate with group A streptococci had the same bacterium isolated in CSF and blood.

Antibiotic sensitivity patterns of microorganisms isolated from blood cultures are shown in table 2. The antibiogram on the isolated *E. coli* and *klebsiella spp* revealed a sensitivity to ciprofloxacin (85%, 50%), amikacin (66%, 50%), imipenem (66.6 %, 33 %), cefotaxime (50 %, 100%) and gentamicin (33.3%, 83%), respectively. *Coagulase-negative staphylococci* and *S. aureus* had 100% and *Enterococcus spp* 90% sensitivity to vancomycin, and a high degree of resistance to ampicillin.

Of the EOS group, 25 (65.8%) cases had CRP≥10mg/l of which 14 were with positive blood culture. Moreover, there were 40 (77%) infants with CRP≥10 mg/L of which 30 had culture-proven sepsis. These was no significant difference between proven EOS and proven LOS with positive CRP (P=0.111).

Table 1: The number and frequency of bacteria causing proven early and late neonatal sepsis

Bacteria Causing Early-Onset Sepsis	**Blood (n)**	**CSF* (n)**	**Urine (n)**	**Isolates (%)**
Escherichia coli	6	0	0	23
Staphylococcus aureus	4	0	0	15.4
Klebsiella spp	4	0	0	15.4
Enterococcus spp	3	0	0	11.5
Group B streptococci	2	0	0	7.7
Coagulase-negative staphylococci	2	0	0	7.7
Streptococcus viridians	2	0	0	7.7
Enterobacter	2	0	0	7.7
Proteus	1	0	0	3.8
Total	26	0	0	100
Bacteria Causing late-Onset Sepsis				
Coagulase-negative staphylococci	12	0	1[a]	28.6
Enterococcus spp	7	0	0	16.7
Staphylococcus aureus	6	0	0	14.3
Enterobacter	6	0	0	14.3
Acinobacter spp	4	0	1[a]	9.5
Escherichia coli	3	0	0	7.1
Klebsiella spp	2	0	1[a]	4.7
Group A streptococci	1	1[a]	0	2.4
Streptococcus viridance	1	0	0	2.4
Total	42	1	3	100

* CSF: cerebrospinal fluid, [a] The same bacterium was isolated from the blood culture.

Table 2: The antibiotic sensitivity patterns (%) of the isolated bacteria to ampicilin (AP), amikacin (AK), gentamicin (GM), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), imipenem (IMI), vancomycin (VA), clindamycin (CD) and cefalotin (KF).

Organism	**AP**	**AK**	**GM**	**CTX**	**CAZ**	**CIP**	**IMI**	**VA**	**CD**	**KF**
Gram-positive bacteria										
Coagulase-negative staphylococci	35.7	78.5	50	85.7	21.5	93	78.5	100	64	57
Staphylococcus aureus	20	90	60	70	10	80	70	100	100	100
Enterococcus spp	30	0	10	20	0	20	20	90	10	20
Gram-negative bacteria										
Escherichia coli	0	66.6	33.3	50	50	83	66.6	0	0	33.3
Klebseilla	33.3	50	83	100	83	50	33	0	16.6	100
Enterobacter	0	100	37.5	50	25	87.5	100	0	12.5	12.5
Acinetobacter spp	25	50	0	100	75	100	100	25	25	50

In the EOS, 28 (73.7%) patients were discharged, 2 (5.3%) left the hospital without the consent of the medical staff, and 8 (21%) died. Of the neonates with LOS, 44 (84.6%) were discharged, 6 (11.4%) left the hospital and 2 (4%) died.

Discussion

Septicemia is one the major causes of morbidity and mortality in the neonatal period, and it often has a rapid and fulminant course. The incidence and major pathogens of the infection vary with geographical regions and among nurseries, and within the same nursery at different times. Identification of the pathogens is important since it can induce a change in policy management.[3]

The incidence of neonatal sepsis is estimated to be about 1 to 8 cases per 1000 live births.[9,10] In our study, of the total 208 cases admitted with clinically suspected sepsis, 68 (32.7%) were with proven and 22 (10.6%) with probable sepsis. As we included both inborn and outborn neonates, it was not possible to calculate a rate of neonatal sepsis per 1000 live births in the present study, therefore, comparison with other studies was not possible. However, there have been two similar types of studies in developing countries that reported the rate of proven culture sepsis in neonates screened for sepsis. In Nigeria, the rate of proven sepsis was 23.9%,[11] but in Pakistan it was much higher than that in the present study (54% vs. 32.7%).[12]

In EOS and LOS, the most prominent presenting clinical findings were respiratory distress, fever and jaundice, which are consistent with other reports.[11,13] Eight (21%) neonates with EOS and 18 (34.6%) in LOS group were premature (less than 37 weeks gestation). In contrast with other studies in which the mean gestational age was higher in neonates with EOS than those with LOS,[5,14] there was no significant difference in maturity between the two groups in the present study (P value=0.424). The mortality rate in EOS group was 5 times that of LOS (21% vs. 4%).

In this study *E. coli* was the commonest pathogen causing EOS, followed by *klebsiella spp, S.aureus* and *Enterococcus spp.* The predominant presence of *E.coli* in neonatal sepsis has been also reported in other regions.[15] There are the reports from Nigeria, Iraq and Pakistan that showed *E. coli*, *Klebsiella* and *S. aureus* as the commonest pathogens.[11,16,17] In Israel and Panama, *E. coli* and *Klebsiella spp* were reported as the most important causes of neonatal sepsis.[18,19] These reports showed that *S. aureus* and *Gram-negative bacilli* are more common than other pathogens in developing countries. However, in Europe and the United States the predominance of *GBS* has been reported.[14,20-22]

In Singapore, out of a total of 4636 live births in 2 years, there was one infant with culture-proven *GBS* septicemia.[23] However, Daoud and colleagues,[13] showed no cases of infection with *GBS* in Jordan. In a similar vein, only two neonates with *GBS* sepsis of proven EOS were detected in the present study. With the implementation of intrapartum *GBS* screening and antibiotic administration, the international incidence of early-onset *GBS* disease has declined from an estimated 1.8 cases per 1000 live birth in 1990 to 0.32 cases per 1000 live births in 2003,[24] but the reasons for continued disease in developed countries are still unclear. It is worth mentioning that for some unknown reason, no case of infection with *Listeria monocytogens* was detected in this study. There have been no reports of such infection from many other regions either.

Present study showed that *CONS* was the most common cause of culture-proven LOS, followed by *Enterococcus spp*, *S. aureus* and *Enterobacter.* By early 1990s, *CONS* had become the major cause of neonatal LOS in Australia.[5,14] Moreover, *it* was identified as the most common organism of proven LOS in the United States, Saudi Arabia and Taiwan.[15,25,26] To the best of our knowledge, there have been a few studies on neonatal sepsis addressing the most common microorganisms including *Klebsiella, S. aureus, and CONS* in Iran.[27,28]

The patterns of antibiotic sensitivity of the most common pathogens isolated from culture-proven sepsis were evaluated (table 2). As routinely practiced, in empirical therapy antibiotics are given most often to the patients before the specific microorganism causing an infection is identified. Empirical antibiotics are typically broad-spectrum, that is, they are effective against a wide variety of possible microorganisms. According to the results of the present study, it seems that cefotaxime could be used as more effective agent against both *E. coli* and *Klebsiella* and vacomycine against *S. aureus* and *Enterococcus spp*, the most common pathogens in EOS. Also, *CONS*, *Enterococcus spp* and *S. aureus*, as the most common Gram-positive, and *Enterobacter* and *Acinetobacter spp,* as the most commont Gram-negative pathogens causing LOS, had the highest sensitivity to vacomycin and imipenem, respectively.

Conclusion

Taking the findings of the present study into account, it is suggested that the existing conventional empirical therapy using ampicillin and gentamicin should be replaced with vancomycin and cefotaxime for critically ill patients in EOS. As for LOS, it is advisable to introduce vancomycin and imipenem as empirical therapy. As antimicrobial resistance in microorganisms associated with both EOS and LOS is an increasing problem in neonatal intensive care unit patients, there is a need to conduct studies to determine the bacterial etiology of neonatal sepsis and determine its antibiotic sensitivity pattern once every few years in order to make the empirical therapy more effective and efficient. Meanwhile, to consolidate and generalize the present findings, it is necessary to conduct multicenter investigations on the efficacy of the suggested therapies.

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