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Original Article

Prevalence of Anaerobic and Aerobic Bacteria in Early Onset Neonatal Sepsis

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Abstract

Background: To determine prospectively the prevalence of anaerobic and aerobic infection in early onset (during 72 hours of age) neonatal sepsis, in Tehran Vali-e-Asr Hospital.

Methods: Among all the live birth, neonates suspecting of having septicemia were investigated for isolation of micro-organisms. Culture bottle containing enriched tryptic soy broth was used for standard blood culture system to detect aerobes and an ANAEROBIC/F bottle was inoculated using BACTEC 9120 continuous monitoring blood culture system to determine the growth of anaerobic bacteria. Among 1724 live births, 402 consecutive neonates suspecting of having septicemia were investigated for isolation of micro organism.

Results: A total of 27 episodes of early onset neonatal sepsis occurred with an incidence of 15.66 (11.6 aerobe + 4.0 anaerobe) per 1000 live births. Aerobic bacteria were the major etiological agents, accounting for 20 cases. 7 (26%) cases had positive blood cultures with anaerobic bacteria. Propionibacterium and Peptostreptococccus (amongst anaerobic) and coagulase-negative staphylococci and staphylococcus aureus (amongst aerobic) were the most commonly isolated organisms. Comparison of clinical findings and demographic characteristics between aerobic and anaerobic infection did not have a significant statistical difference.

Conclusion: Our impression is that while anaerobic bacteremia in the newborn infants can occasionally cause severe morbidity and mortality, majority of cases experience a self limited illness with transient bacteremia.

Keywords: *Neonatal sepsis, Anaerobe, Aerobe, Bacteria*

Introduction

Anaerobic bacteria colonize the newborn after delivery and have been recovered in several types of neonatal infections such as cellulites at the site of fetal monitoring, neonatal aspiration pneumonia, bacteremia, conjunctivitis, omphalitis, and infant botulism. The lack of directing adequate therapy against these organisms may lead to clinical failure (1). Although well established as an important cause of sepsis in the adult population, the incidence and significance of these organisms in neonatal septicemia have not been adequately documented (2). In a study (3) the incidence occurred twice as frequently in newborn infants as in older children and accounted for 12% of all cases of neonatal bacteremia. This percentage is not surprising because after delivery, neonates are colonized with maternal vaginal flora that contains both aerobic and anaerobic bacteria (3).

Gram positive anaerobes associated with clinical disease in patients of any age include *Peptococcus, Peptostreptococcus, microaerophilic streptococci, Closteridium* spp*, Propionibacterium,* and *Eubacterium*. Clinically significant gram negative anaerobes include *Bacteroid* spp, in particular *Bacteroid fragilis*, *Fusobacterium, Veillonella, and Prevotella melaninogenicus* (4).

Bacteroids and *Clostridium* species followed by *Peptostreptococci, Propionibacterium acnes, Veillonella, Fusobacterium and Eubacterium* species were responsible for neonatal sepsis (5). Obstetric, fetal and neonatal factors including prolonged rupture of membrane, maternal chorioamnninitis, prematurity, fetal and respiratory distress

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are associated with increased risk of infections with anaerobic bacteria (4).
Mortality rate associated with anaerobic bacteremia was reported to be from 4 to 38% (3).
Isolation of anaerobic bacteria requires appropriate methods of collection, transportation, and cultivation of specimens (1). The cost of anaerobic bacteriological techniques is high and usually warranted in selected tertiary care centers (4). Treatment of anaerobic infection is complicated by the slow growth of these organisms and due to the growing resistance to antimicrobials (1).
The purpose of this study was to determine the prevalence, importance and type of anaerobic infection in early onset neonatal septicemia because organisms concurring neonatal infection are different depending upon the geographic area (2).

Materials and Methods

This study was carried out at Vali-e-Asr maternity Hospital in neonatal intermediate and intensive care units from April 2005 to April 2006.
Early onset sepsis was defined by a positive culture of blood drawn within 72 h after birth. Cultures that tested positive for coagulase-negative staphylococci were reviewed to distinguish definitive or possible coagulase negative staphylococcal infections from the effects of contaminant. Definite infection was defined by two positive cultures of blood specimens drawn within two days of each other or one positive culture and a blood C- reactive protein level greater than 1 mg per deciliter within two days after the blood culture. Possible infection was defined by one positive culture and treatment for at least five days with vancomycin or another drug to which the organism was susceptible. For all other pathogens, sepsis was defined by the presence of the organism in cultured blood. Infants with definitive or possible coagulase-negative staphylococcal sepsis were included in the analysis. Hyperbilirubinemia was defined as a total serum bilirubin concentration of ≥15 mg/dl. Blood cultures were obtained within 72 h of birth because of clinical suspicion of sepsis. Management strategy for asymptomatic neonates at risk for sepsis was tailored according to Gerdes recommendations (6). After sterile acquisition by venipuncture, two samples of blood were collected for isolation of aerobes and anaerobes. A culture bottle containing enriched tryptic soy broth was used for standard blood culture system; in addition an ANAEROBIC/F bottle was inoculated using a BACTEC 9120 continuous monitoring blood culture system (Becton Dickinson, Sparks, MD 21152) to determine the growth of anaerobic bacteria. All cultures were incubated at 37º C for at least 14 d. Identification of anaerobic bacterial isolates was based on a method described earlier (7).
Antimicrobial susceptibility was performed by agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS), (8) (now Clinical Laboratory Standards Institute). Antimicrobial agents were obtained from the following manufacturers: penicillin from Merck, clindamycin, metronidazole and imipenem from Mast Diagnostics.
The minimum inhibitory concentration (MIC) break point value considered for resistant isolates including penicillin ≥2μg/mL, clindamycin ≥8μg/mL, metronidazole ≥ 32μg/mL and imipenem ≥16 μg/mL.
Statistical analysis was done using Odds Ratio or Chi-square and Fisher's exact t-test as applicable. The study was approved by the institutional review board and informed- consent procedures were followed as required.

Results

Among 1724 live births, 402 consecutive neonates suspecting of having septicemia were investigated for isolation of micro organism.
A total of 27 episodes of early onset neonatal sepsis occurred with an incidence of 15.66 (11.6 aerobe+4.0 anaerobe) per 1000 live births.
Aerobic bacteria were the major etiological agents, accounting for 74.1% of the cases (n= 20). Seven cases (25.9%) had positive blood cultures for anaerobic bacteria.

Propionibacterium (amongst anaerobic) and *Staphylococcus coagulase* negative (amongst aerobic) were the predominant organisms isolated (Table 1). The mean gestational ages and birth weights in infants infected with anaerobic were 32.1±4.4 w & 1838±885 g and in aerobic group were 33.2±3.9 w & 1950±990 g, respectively.

Comparison of clinical findings and demographic characteristics between aerobic and anaerobic infection did not have a significant statistical difference but frequency of respiratory distress, pneumonia, hyperbilirubinemia and hypoglycemia were much more in anaerobic bacteremia (Table 2).

Characteristics of patients with anaerobic bacteremia have been demonstrated in Table 3. The minimum inhibitory concentrations (MIC) for anaerobic bacteria, using agar dilution method are being summarized in Table 4. It is notable that most of the isolates were resistant to the majority of antibiotics specified for anaerobic bacteria.

All the patients with aerobic infection were premature except three cases that required cardiopulmonary resuscitation at birth.

Table 1: Frequency of microorganisms isolated from blood cultures and mortality rate

Aerobes			Anaerobes		
Organisms	**n (%)**	**Mortality (%)**	**Organisms**	**n (%)**	**Mortality (%)**
Coagulase negative staphylococci	10(50)	2	*Bacteroides fragilis*	1 (14)	1
Staphylococuus aureus	3(15)	0	*Eubacterium* sp	1 (14)	0
Klebsiela	2(10)	0	*Peptostreptococcus* sp	2 (29)	0
E.Coli	1(5)	1	*Propionibacterium* sp	3 (43)	1
Acinetobacter baumani	1(5)	0			
Acintobacter lowffii	1(5)	0			
Alcaligens	1(5)	0			
Enterobacter aeroginosa	1(5)	1			
Total	20(100)	4 (20)		7	2 (28.5)

Table 2: Comparison of clinical sign, demographic characteristics and laboratory tests between aerobic and anaerobic infection

Variables	Anaerobes	Aerobes	Odds ratio	*P*-value	CI 95%
Pneumonia	4	4	5.33	0.14	0.8-34
Abdominal distention	1	2	1.5	0.75	0.1-19
Respiratory distress	6	10	6.00	0.18	0.6-59
Cardiorespiratory resuscitation	4	10	1.3	1.00	0.2-7.5
Apgar 5 min≤ 6	1	12	0.1	0.07	0.03-2.7
Fetal distress	2	6	0.9	1.00	0.14 -6
Premature rupture of membranes	3	5	2.25	0.63	0.3-13
Prolonged rupture of membrane> 24 hr	2	2	3.6	0.26	0.4-32
Small for gestational age	2	6	0.93	1.00	0.1-6.
Male sex	3	12	0.5	0.6	0.0.8-2.8
Maternal urinary tract infection	1	2	1.5	1.00	0.20-33.24
Hypoglycemia	3	3	4.25	0.29	0.6-29
Hyperbilirubinemia	2	1	7.6	0.15	0.5-101
Vaginal delivery	2	8	0.6	0.67	0.09-3
Twin	2	2	3.6	0.26	0.4-32

Table 3: Characteristics of patients with associated anaerobic bacteremia

Organism	Gestational age (weeks)	Duration of rupture of membrane & perinatal events	Apgar 1, 5 min &, ‡CPR	Positive clinical sign & used antibiotics	Positive laboratory test
Eubacterium Spp.	35	- Twins	7,6 +	Grunting, R. distress AMP+AG	Hypoglycemia
Peptostreptococcus Spp.	35	- -	9,10 -	Mottling AMP+AG	Hypoglycemia
Propionibacterium Spp.	33	3 d -	9,10 -	Pneumonia R. distress GI bleeding AMP+AG VAN+CEPH	Hypoglycemia Hyperbilirubinemia
Peptostreptococcus Spp.	30	48 h -	8,9 +	R. distress Abdominal distention, AMP+AG VAN+CEPH	C-Reactive protein +
Propionibacterum Spp.	38	- Maternal †UTI	9/10	Pneumonia, R. distress AMP+AG VAN+CEPH	C-Reactive protein + Hyperbilirubinemia
Bacteroid Fragilis Group	25, *SGA	- Olygohydramnious Twins	6/10, +	Pneumonia, §RDS R. distress **Dead** AMP+AG VAN+CEPH	Hyperglycemia
Propionibacterium Spp.	29, SGA	- Abruptio Placenta	5/8 +	Fetal distress, Pneumonia, R. distress RDS ‖P.hemorrhage, **Dead** AMP+AG VAN+CEPH	Disseminated intravascular coagulation

*= Small for gestational age, †= Urinary tract infection, ‡= Cardiopulmonary resuscitation, §= Respiratory distress syndrome, ¶= Respiratory, ‖= Pulmonary. AMP= Ampicillin, AG= Aminoglycoside, CEPH= 3rd or 4th generation cephalosporines, VAN= Vancomycin

Table 4: The minimum inhibitory concentrations [MIC (µg/mL)] for anaerobic bacteria isolated from neonatal bacteremia, using agar dilution method.

Organism	Penicillin	Imipenem	Metronidazole	Clindamycin
Propionibacterium	≥4	≥32	≥32	≥8
Propionibacterium	≥4	≥8	≥32	< 1
Propionibacterium	1	≥32	≥32	4
Bacteroides fragilis	≥4	8	≥32	≥8
Peptostreptococcus	≥4	8	16	≥8
Peptostreptococcus	<0.25	16	16	4
Eubacterium	0.5	16	≥32	2

Discussion

In our study the prevalence of anaerobic bacteria was 4 per 1000 live birth. The true incidence of neonatal anaerobic bacteremia is difficult to ascertain because anaerobes are not widely recognized as neonatal pathogens, and blood culture is rarely obtained (4). In one study the incidence of recovery of anaerobes in neonatal sepsis was 1.8 per 1000 live birth (9). Surveys in the 1960s and 1970s suggested that anaerobic bacteria were the causative agents in up to one fourth of all neonatal bacteremias (4). In one study from India, 6.6% of bacteremia was due to anaerobes (10). The importance of anaerobic bacteria in early onset neonatal sepsis was previously reported by Mitra et al in India (11). Higher rate was also reported from San Francisco (23%) and Torrance (26%) (2). Wilson and colleagues identified anaerobes accounting for as much 27% of positive blood cultures (12). Twenty six percent of isolates in our cases was due to anaerobic bacteria which is higher than many other reports (2.9).

Anaerobic cultures in our cases belonged to normal flora of human and obstetric, fetal and neonatal factors including prolonged rupture of membrane, maternal chorioamnionitis, prematuritry, fetal and respiratory distress, hyperbilirubinemia and hypoglycemia were the most commonly associated conditions with these bacteria as also shown in other studies (2,4).

In our study, *Propionibacterium, Eubacterium* and *Peptostreptococcus* (gram positive anaerobes) as well as, *Bacteroid fragilis* (gram-negative anaerobe) were associated with clinical disease however the clinical manifestations of neonatal bacteremia caused by these bacteria were not distinguishable from other causes of neonatal sepsis. Twenty three infants with anaerobic bacteremia were observed in a 3½ yr period at Harbor General Hospital in Torrance California. In this study the clinical presentation of anaerobic sepsis was similar to that of other types of sepsis in newborn (9). Similar findings have already been reported by others (4).

It should be noted that the epidemiology of antimicrobial resistance among anaerobes is evolving rapidly and varies by locale. In recent years, many anaerobic bacteria have demonstrated increased resistance to antimicrobial agents commonly used, thus complicating empiric therapy for infections with these bacteria. This resistance is especially true among *Bacillus fragilis* group, for which several studies have shown increasing resistance to β-lactam antibiotics and to clindamycin (3). Our case with *Bacteroid fragilis* was resistant to clindamycin, metronidazole and had intermediate activity to imipenem. Mortality in 2 of 7 (28%) cases with *Bacteroid* and *Propionibacterium*, showed that anaerobic pathogens could be a potential cause of severe neonatal infections and serious perinatal morbidity, although both of these cases had other risk factors for neonatal mortality. These two cases died before the availability of antibiotic susceptibility test because of the slow growth of these organisms. The mortality rate of sepsis caused by anaerobic bacteria was low in Torrance (9) (1 of 23 cases) and in the small series from Baltimore (none of 4 cases) (13), but it was high in the series reported from St. Louis (three of eight infant died) (14). Also two deaths occurred in three infants with *Bacteroids* sepsis in Santa Clara, California (15). Other studies reported different rates of mortality from 4-38% (2, 9).

Two cases of anaerobic bacteremia (*Eubacterium & Peptostreptococcus*) treated with ampicillin and amikacin and discharged from hospital without prescription of further antibiotics. While anaerobic bacteremia in the newborn infants can occasionally cause severe morbidity and mortality, some cases probably experience a self-limited illness with transient bacteremia (9) as might have happened in these two cases.

Although the most common microorganism responsible for early onset neonatal sepsis are predominantly group B *Streptococcus* species and *E. coli* in Western countries (4) but the most common microorganism of neonatal sepsis in our unit was coagulase negative staphylococci in a very

recent study (16) so 5 out 7 of our patients received vancomycin, according to clinical unresponsiveness to empiric antibiotics. We did not consider vancomycin in testing antibiotics' susceptibility for anaerobic infection because if anaerobic organisms are known or suspected to be responsible for infection drugs such as chloramphenicle or clindamycin must be added to the penicillin plus aminoglycoside regimen (2). It is probable that these patients responded to vancomycin which has an activity against gram positive anaerobes (3).

The bacterial etiology of neonatal sepsis varies from one community to another. These differences probably reflect characteristics of the population served, including unique cultural features and sexual practices, local obstetric and nursery practices and patterns of use of antimicrobial agents. The bacteriology of neonatal sepsis in Western Europe and Jamaica is in general similar to that in the United States. In tropical areas, a different pattern is evident (2). The predominance of CONS infections, particularly in relation to late onset sepsis was reported previously (17). In Norway, coagulase-negative staphylococcus was predominantly isolated from very early (61%), early (91%) and late onset (78%) neonatal sepsis and anaerobes comprised of 17% of very early onset cultures (18). This report is close to our findings. It should also be noted that demographics, pathogens, and outcome associated with neonatal sepsis continue to change. Statistical analysis revealed a significant increase in the number of early onset cases of infection with commensal species (19).

Our study showed that microbiological pattern of neonatal sepsis was not compatible with most European and North American reports and anaerobic bacteria consisted of respectable component of neonatal sepsis although their importance needs more investigations. Our impression is that while anaerobic bacteremia in the newborn infants can occasionally cause severe morbidity and mortality, the majority of cases probably experience a self limited illness with transient bacteremia as shown by Chow et al (9).

In severely ill infants especially with risk factors for anaerobic infections, the possibility of some of the bacteremias being due to anaerobes should be kept in mind and cultures employing special media should be considered particularly when aerobic cultures have been negative. Increasing antimicrobial resistance among anaerobes highlights the importance of conducting periodic hospital surveys of antimicrobial susceptibility patterns, so that adequate antimicrobial coverage can be provided for anaerobic infections when facing negative aerobic culture with/ or clinical unresponsiveness to empiric antibiotics against aerobic bacteria even before the availability of antibiotic susceptibility tests.

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The authors declare that they have no Conflict of Interests.

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