Novel Identification of Group B *Streptococcus* and *Enterobacter sakazakii* in Infant Suspected to Septicemia

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Abstract

Group B *Streptococcus* (GBS) is a major pathogen in neonates and pregnant women. Infant infections are significantly associated with maternal vaginal colonization with GBS during pregnancy. *Enterobacter sakazakii* is potentially a harmful bacterium, widely found in nature, transmitted from infected milk powder to infants causes various diseases. One of the biggest problems of these bacteria is the development of septicemia in newborns, causes fever, increased heart rate, respiration, and confusion. In this study, 200 blood samples were collected from infant suspected to septicemia. The bacterial genome content was then extracted by Fermehtas kits. By using the Multiplex-real time PCR technique, Group B **Streptococcus** & **Enterobacter sakazakii** were identified and their levels were determined. A total of 16 neonates (8%) with *Enterobacter sakazakii* infection and 10 infants (5%) with GBS infection were observed. Revealing the negative result of blood cultures in the current study, molecular level studies also suggested by involving other parts of the infants' body.

Introduction:

Group B *Streptococcus* (GBS) was introduced as a significant pathogen in neonates and pregnant women, a major contributor to neonatal infections (Palmeiro et al., 2010; Madzivhandila et al., 2011). Colonization of the genital tract occurs in 10-30% of pregnant women and is usually asymptomatic. However, it can cause urinary tract infection, septicemia, chorioamnionitis, endometritis and infectious abortion (Ulett, 2009; Turner et al., 2012). Infant's infections are significantly correlated with maternal vaginal colonization with GBS during pregnancy (Palmeiro et al., 2010). The acquisition of bacteria by the baby leads to colonization of the skin or mucous membranes that occurs in 15-50% of infants born to infected mothers (Madzivhandila et al., 2011). Among the infected infants, the disease progresses in 1 to 3%, and the onset of infection almost starts from the first 24 hours of birth (Convert et al., 2005). Infection with GBS in infants occurs in 2 forms; early-onset disease (in infants less than 7 days) and late-onset disease (in infants one week to 3 months and rarely in older babies) (Dhanoa et al., 2010; Valkenburg-van den Berg et al., 2006). Premature infant infections are associated with colonization during pregnancy and the transmission of bacteria to the baby occurs through ascites into the amniotic fluid or when passing through the delivery tube (Dhanoa et al., 2010). GBS can cause infections like septicemia, meningitis, cellulitis, adenitis, conjunctivitis, pneumonia, and otitis in newborns, including sepsis and meningitis, and high mortality despite antibiotic therapy (Amirmozafari et al., 2006). According to McGregor (2000), all pregnant women should perform early diagnosis of bacterial vaginosis in the first weeks of pregnancy. Leitch et al. (2003) showed that bacterial vaginosis is a risk factor for early labor and abortion. The prevalence of bacterial vaginosis varies widely among ethnic groups and geographical locations (Campbell et al., 2000; Tolosa et al., 2006) so it is suggested that their relationship with preterm labor must be investigated separately for each country (Goffinet et al., 2003).

A prevalence of GBS was reported in some countries belonging to different geographical regions, with 22% in the Middle East and North Africa, 19% in Asia and the Pacific, 19% in Sub-Saharan Africa, 14% in the Americas and 12% in India and Pakistan (Stoll & Schuchat, 1998). Epidemiologic studies also showed that the colonization of
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GBS among Iranian women ranged from 1.9% to 26.7% (Fatemi et al., 2010; Rabiee et al., 2015).

*Enterobacter sakazakii* is a gram negative, negative oxidase, positive catalase, anaerobic, spores free from Entero-bacteriaceae family, which is widely found on the nature and intestinal tract of humans and animals (Farmer et al., 1980). It is a potentially harmful bacterium that is transmitted from infected milk to infants and due to various diseases, including meningitis, brain abscesses, necrotizing enterocolitis, encephalitis, meningococcal necrosis encephalitis, bacteremia and sepsis in newborns, especially premature newborns. Babies with suppressed immune systems, as well as low birth-weight infants, have been reported by Dumen (2010). Although there is no evidence of an epidemiological association with the infectious dose of pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli* O157, and *Neisseria meningitides* (Baumgartner et al., 2009; Iverse et al., 2003). As stated, this pathogenic bacterium is an opportunistic and potentially pathogenic, especially in infants, the most commonly reported illnesses to occur in infants below the age of 12 months, especially preterm infants, birth deficient infants and suppressed immune neonates (MRAS-6, 2004). It is because this group of individuals is more susceptible to bacterial sepsis due to gram-negative and endotoxin bacteria associated with necrotizing enterocolitis (Beck-Sague et al., 1994; Stoll et al., 2004). In many cases, the importance of diseases caused by this organism is ignored due to the lack of proper sampling and the lack of advanced diagnostic facilities in clinical laboratories and the persistence of infection by *Enterobacter sakazakii* by physicians. Furthermore, in our country there are no clinical laboratories in hospitals available for automatic culture systems, and the blood culture is carried out only through the traditional culture method. Thus the chances for proper identification and isolation of this organism are greatly reduced (CDCP, 2002). One of the biggest dangers of these bacteria is the development of septicemia in newborns, which itself create problems like fever, increased heart rate, increased breathing, confusion. There are also other symptoms that are associated with certain infections such as coughing with pneumonia or urinating with pain and kidney infection.

**Methodology:**

For this study, blood samples were collected from 200 infants below the age group of 12 months who were suspected of septicemia, admitted in hospitals of Shiraz and Jahrom for one year. We isolated the blood samples using 3000 rpm, then, using a sterile loop, we cultivate the whole blood on MacConkey Agar medium and incubated for 24 hour in 37°C. Further, using the Fermentans kit, the genome content of the bacteria grown on the medium was extracted following the kit protocol. As a positive control of DNA, the purified genomes of GBS and *E.sakazakii* were prepared from the reference laboratory. Then, using the real-time method, the bacterial presence was determined in the specimens.

**Extraction of DNA from blood:** 200 µL of whole blood was extracted by the Fermentas DNA blood mini kit within a week of sample collection. Extracted DNA was frozen at -80°C until it underwent Real time PCR.

**Primer design:** based on the GBS sequences in GenBank, one pairs of primers were designed with PRI-MER3.0 (http://frodo.wi.mit.edu/primer3), Primer Explorer (http://primerexplorer.jp/e/) and NCBI primer-Blast tools (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Primers were synthesized commercially at Geneary Co. Ltd. (China). The primer 16S-23S rRNA inter-genics spacer region (ISR), within the GBS genome (Table 1).

**Table 1. primers: Gene bank: FJ555494**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Forward</th>
<th>Primers</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS</td>
<td>IGS-1, GGAAACCTG</td>
<td>IGS-2, AATCTATTTCT</td>
<td>AGATCGTGGAAT</td>
</tr>
<tr>
<td></td>
<td>CCATTGGCTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E.sakazakii</em></td>
<td>ESOMP5-F: 5'</td>
<td>ESOMP5-R: 5'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GGGAAGGATTATA</td>
<td>GGCCTCTGTATCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACCGTGAACCTT-3</td>
<td>TCCAAA-3</td>
<td></td>
</tr>
</tbody>
</table>

**Real time PCR:** the PCR mixture (20 µL) contained 10 µL of 2X SYBR Green Real-time PCR master mix (Ampliquon, Denmark), 0.5 µM of each specific primer and 1 µL of template DNA. The PCR was performed in an AB real-time RCR thermal cycler with the following program: 94°C for 1 min, 40 cycles of amplification (94°C for 30 s, 55°C for 30 s and 72°C for 20 s), followed by a final 10-min extension at 72°C. The qPCR run was done in triplicate for each assay.

**Results:**

In our study, Novel identification was done by using multiplex-realtime PCR on 200 suspected septicemia neonates, using primers GBS:

"IGS-1 GGAAACCTGGAATCTTAGATCGTGGAAT" &
"IGS-2 AATCTATTTCTAGATCGTGGAAT"

*Entrobacter sakazakii:*

ESOMP5-F: 5'GGTGAAGGATTTAACCGTGAACTT-3 &
ESOMP5-R: 5'-GGCCTCTGTATCAATC-3

Total 16 infants from 200 (8%) with *Enterobacter sakazakii* infection and 10 infants from 200 (5%) with GBS infection were observed.

**Discussion:**

As mentioned earlier, *Enterobacter sakazakii* and GBS are two significant types of opportunistic pathogens for neonatal infections and can also cause various diseases in infants and sometimes cause death (Farmer et al., 1980;
pregnant women were positive. PCR assay, the colonization of GBS 24 out of 250 (9.6%) and rectal cultures of 21 women out of 250 (8.4%) and in the pregnant women, Kazem (2011) reported that vaginal hemolytic GBS, colonized in the reproductive system of various bacteria and 13 of them (10.5%) were some of them had multiple infections. There were 128 diagnosed with septicemia, found positive cultures, and conducted on 114 children of below the age of 28 days, 1.3 and 3 per 1,000 live births. In a study by Behjati (1998) that the incidence of this infection in neonates was between newborn babies in American and British hospitals reported in varietys of ready-to-eat foods other than infant formula. Int J Food Microbiol, 136(2):189-192.


