Purpose: Intra-amniotic infection (IAI) is often polymicrobial, and the 16S rDNA PCR assay has a major limitation that its interpretation is difficult in the presence of multiple 16S rDNAs. Denaturing gradient gel electrophoresis (DGGE) can overcome this limitation by separating PCR products based on sequence. We performed the DGGE analysis to investigate bacterial prevalence and diversity in amniotic fluids from pregnant women with preterm births and gastric fluids from their newborns.

Methods: DNA was extracted from bacterial cells in amniotic fluid (AF) and gastric fluid (GF) and was amplified with universal 16S rDNA primers. For DGGE analysis, the PCR products were loaded onto polyacrylamide gels that were made with denaturing gradients.

Results: Bacterial 16S rDNA was detected by PCR from all AF and GF samples. The bacterial species in AF samples were the following: *Lactobacillus reuteri* (87.0%), uncultured *Enterococcus* species (65.2%), *Ureaplasma urealyticum* (13.0%), and *Enterococcus faecalis* (4.3%). The bacterial species in GF samples were the following: *Lactobacillus reuteri* (95.2%), uncultured *Enterococcus* species (42.9%), and *Ureaplasma urealyticum* (4.8%). Two or more species were identified from 69.6% of AF and 47.6% of GF samples.

Conclusion: We suggest that DGGE analysis allows improved understanding of microbial diversity and community in AF and GF.

Key Words: Preterm infant, Amniotic fluid, Gastric fluid, Intra-amniotic, Infection, Denaturing gradient gel electrophoresis, Bacterial diversity
Intra-amniotic infection (IAI) is an important mechanism that might account for 25–40% of preterm birth and has been associated with maternal and neonatal morbidity and mortality. Although microbial culture of amniotic fluid (AF) is recognized as the gold standard for the detection of IAI, the traditional culture method of AF has been of limited clinical value due to a low yield of bacteria, owing to the bacteriostatic properties of AF and the difficulty in growing certain bacterial species in culture. In terms of culture-independent methods, various molecular diagnostic techniques, such as PCR, have been developed to help the diagnosis of bacterial infection by detecting bacterial genetic material. Of these, sequence analysis of the 16S rRNA gene (rDNA) has emerged as a preferred genetic technique for definite species identification. Bacterial 16S rDNA consists of highly conserved nucleotide sequences that are shared by all bacterial species. By using PCR primers that are targeted at the conserved regions of the bacterial 16S rDNA, it is possible to design broad-range PCRs capable of detecting DNA from almost any bacterial species. However, IAI is often polymicrobial, and the major limitation of the 16S rDNA PCR assay is that its interpretation is difficult with the presence of heterogeneous 16S rDNAs from multiple species. PCR using DNAs from polymicrobial clinical samples can generate templates containing different DNA sequences that represent many of the microbial organisms. These PCR products from a given reaction are of similar size (bp), and conventional separation by agarose gel electrophoresis results in a single DNA band that is largely non-descriptive.

Denaturing gradient gel electrophoresis (DGGE), a widely used fingerprinting method for detection of the bacterial population and diversity, can overcome this limitation. The principle of DGGE is the separation of DNA fragments with differences in the base sequence. During DGGE, PCR products encounter increasingly higher concentrations of chemical denaturant as they migrate through a polyacrylamide gel, and DNA fragments of the same length but different base pair sequences can be separated. Therefore, using this advantage of DGGE analysis, we have combined 16S rDNA PCR and DGGE and investigated the microbial community and diversity in amniotic fluids of women who delivered preterm infants. There have been several studies on the identification of microorganisms in AF using a 16S rDNA PCR assay. To our knowledge, however, DGGE analysis has seldomly been performed on AF that may contain more than one microorganism, and it will be helpful to expand our understanding of the possible microorganisms causing IAI.

**MATERIALS AND METHODS**

1. Study population

Between April 2009 and December 2009, pregnant women at risk of preterm delivery, admitted at Pusan National University Hospital, were approached to participate in this prospective study. All their newborns were <35 weeks gestation and born by Cesarean sections. The premature newborn-mother dyads were excluded if either the AF or gastric fluid (GF) sample could not be obtained due to certain reasons, such as a sample contaminated with blood, insufficient amount of collection, and high viscosity of fluid. Our study protocol was approved by the Institutional Review Board, and informed consent was obtained from the patients before study.

2. Sample collection and genomic DNA extraction

AF samples were collected using aseptic techniques during Cesarean section procedure. After a small transverse incision in the lower uterine segment with a scalpel, just before an opening is made in the amniotic sac, a 30 mL syringe with 18 gauge needle was cautiously inserted through the amniotic membrane and 20–30 mL of the AF was aspirated in each patient. GF samples in premature newborns were collected immediately after birth using 5F orogastric feeding tubes. No additional supplement, such as 0.9% saline, was instilled to prevent any chance of dilution or contamination. Collected AF and GF samples were centrifuged at 20,000 \( \times \) g for 10 minutes at 4 \( ^\circ \)C temperature. After centrifugation, the supernatants were removed and the pellets were stored at -70 \( ^\circ \)C until they were used for DNA extracts. Genomic DNA was extracted from the collected AF and GF samples using the Wizard genomic DNA purification kit (Promega, USA), according to the manufacturer’s instructions. The final dilution volume was 100 \( \mu \)L. For each PCR analysis, we used 1 \( \mu \)L of DNA extracts.

3. 16S rDNA Polymerase Chain Reaction (PCR)

PCR amplification of 16S rDNA was performed using primers 91E (5’-GGAATTCAAAACGAAATTGAGGGG-3’) and 13B (5’-CGGGATCCCCGGGGGGGAACGTTAC-3’). Briefly, the amplification was conducted with 20 pmol of primers 91E and 13B with an initial denaturation at 94 \( ^\circ \)C (5 minutes) and cycled...
as follows: 94 °C, 60 °C, and 72 °C (1 minute each) for 30 cycles. For positive controls, 100 ng of *E. coli* DNA was used, and a mixture of all reagents used for DNA extraction was processed as the negative control. Each PCR analysis was performed in duplicate. Only those PCR runs delivering correct results for both controls were considered valid and reported. Amplification products were detected by electrophoresis on 1.5% agarose gels, with 10 μg/L ethidium bromide staining and UV illumination. These primers produce a 475-bp product containing species-variable rDNA sequences. For DGGE analysis of the PCR product, a GC-rich sequence (GC-clamp) was attached to the 5’ end of primer Bact-f(GC) (5’-CGCCCGGCGCGCGGGCGGGGGGGGAGAGTTTGTATCGTGCTCAG-3’), and then a combination of primers Bact-f(GC) and Bact-0536r (5’-GWATACCGCGGCKGCTG-3’) was used to amplify the 16S rDNA regions in different bacterial species. PCR amplification was performed with a MyCycler thermal cycler (Bio-Rad, UK) as follows: after preheating to 95 °C for 10 minutes, 20 cycles were carried out at 95 °C, 55 °C, and 72 °C for 30 seconds each. The annealing temperature was initially set at 55 °C and was then increased by 0.5 °C every cycle until it was 72 °C. In the post-extension process, 72 °C was used for 7 minutes after additional 15 cycles at 95 °C, 55 °C, and 72 °C for 30 seconds each. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and then by ethidium bromide staining.

4. Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed with a DCode denaturing gel electrophoresis system (Bio-Rad, USA). PCR products were loaded onto 8% (wt/vol) polyacrylamide gels in 1x buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA at pH 8.0). The 8% (wt/vol) polyacrylamide gels (acylamide/bisacrylamide ratio, 37.5:1) were made with denaturing gradients ranging from 30-45% for 16S rDNA fragments. Denaturant (100%) contained 7 M urea and 40% formamide. The electrophoresis was run at 65 °C for 16 h at 60 V. After electrophoresis, the gel was stained with ethidium bromide (10 μg/mL in distilled water [DW]) solution for 30 minutes, washed for 10 minutes with DW, and observed by UV illumination.

5. Sequencing of DGGE fragments

Bands in the DGGE gel of 16S rDNA fragments were excised with a sterile blade. The excised bands were mixed with 50 μL of diffusion buffer [0.5 M ammonium acetate; 10 mM magnesium acetate; 1 mM EDTA, pH 8.0; 0.1% sodium dodecyl sulfate (SDS)] and incubated for 30 minutes at 50 °C. DNA was extracted from DGGE bands using a QIAEX II kit (QIAgen, Canada) according to manufacturer’s instructions. The DNA was reamplified with primers Bact-0008-f (5’-AGAGTTTGATCTGGCTCAG-3’) and Bact-0536r (5’-GWATACCGCGGCKGCTG-3’) as follows: after preheating to 95 °C for 5 minutes, 30 cycles were carried out at 95 °C, 62 °C, and 72 °C for 40 seconds each. Amplified PCR products were purified with a gel extraction kit (Qiagen, USA) and sent to Genotech Co. (Korea) for sequence analysis.

6. Sequence Alignment and Phylogenetic Analysis

The sequencing data were compared with the GenBank sequence database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) and Ribosomal Database Project II tool (RDP). The sequence showed 97-100% homology was assigned as a species. The determined sequences were aligned and compared with their related sequences using a multiple sequence alignment system of CLUSTAL W (EBI, UK) for preparing phylogenetic trees.

7. Detection of Ureaplasma species by PCR

Additionally, PCR was performed for detection of *Ureaplasma urealyticum* and *Ureaplasma parvum*, common pathogens known to cause IAI. The genomic DNA extracts used were identical to those for PCR amplification of 16S rDNA. The PCR conditions were as follows: an initial denaturation at 95 °C for 5 minutes; 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 1 minute; and an additional extension at 72 °C for 5 minutes. Strain-specific primers U5 (5’-CAATCTGCTGTAAGTATTAC-3’) and U4 (5’-ACGACGTCATCAGAATC-3’) for *Ureaplasma urealyticum* and UMS125 (5’-GTATTGCAATCTTTATATGTCTTC-3’) and UMA226 (5’-AGCGATGATGGAAGCAGCATTAAATTC-3’) for *Ureaplasma parvum* were used for gene amplification. The PCR products were analyzed by 1.5% agarose gel electrophoresis followed by staining with ethidium bromide.

**RESULTS**

1. Characteristics of study population

Of the 59 pregnant women eligible for this study, 21 who delivered premature newborns <35 weeks by Cesarean sections were finally recruited for the study. The remaining women were excluded because of refusal to participate in the study (n=12),
failure to obtain either the AF or GF sample \( n=17 \), contamination of the sample with blood \( n=6 \), and failure in separation of high-viscosity fluids \( n=3 \). Of the 21 recruited, two delivered dizygotic twins. Two GF samples from singletons were omitted due to lack of amounts. Thus, 23 AF and 21 GF samples, respectively, were collected for this study. The main reasons for preterm delivery include preterm premature rupture of membranes \( n=6 \), preterm labor \( n=14 \), and pregnancy-induced hypertension \( n=3 \). Of the 6 women with premature rupture of membranes, 3 had prolonged rupture of membranes more than 24 hours prior to delivery. The mean body weight and gestational length of the study participants’ newborns were 1,907.4±522.7 g and 32.9±2.1 weeks, respectively (Table 1).

2. PCR amplification and DGGE

By using 16S rDNA PCR analysis, bacteria were detected in all AF and GF samples. The amplified products were shown as single bands with a 475-bp product size under the UV lights. For DGGE analysis of the PCR product, a GC-rich sequence (GC-clamp) was attached to the 5’ end of primer Bact-f(GC), and a combination of primers Bact-f(GC) and Bact-0536r was used for the second round of PCR amplification. DGGE was performed with DCode

Table 1. Clinical Characteristics of the 23 Enrolled Newborns

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>32.9±2.1</td>
<td>27-34</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1,907.4±522.7</td>
<td>710-2,720</td>
</tr>
<tr>
<td>Apgar score at 1 minute</td>
<td>5±2</td>
<td>2-8</td>
</tr>
<tr>
<td>Apgar score at 5 minutes</td>
<td>8±1</td>
<td>5-9</td>
</tr>
<tr>
<td>Male (%)</td>
<td>16 (69.6)</td>
<td>-</td>
</tr>
<tr>
<td>Twin (%)</td>
<td>4 (17.4)</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

Table 2. Primers Used for PCR Amplification and Sequencing

<table>
<thead>
<tr>
<th>Primer</th>
<th>Direction</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>91E</td>
<td>Forward</td>
<td>5’-GGAATTCAACAGAATTGACGCGGCGG-3’</td>
<td>Hitti et al. 8)</td>
</tr>
<tr>
<td>13B</td>
<td>Backward</td>
<td>5’-CGGGATCCAGGCCGCCGCGCGCGCGGAGAGTTTGATCCTGGCTCAG-3’</td>
<td>Hitti et al. 8)</td>
</tr>
<tr>
<td>Bact_f(GC)</td>
<td>Forward</td>
<td>5’-CGCCCGCGCGCCGCCGCCGCCGCGCGGCAGAGTTTGATCCTGGCTCAG-3’</td>
<td>Wimpenny et al. 17)</td>
</tr>
<tr>
<td>Bact-0536r</td>
<td>Backward</td>
<td>5’-GWATTACCGCGGCGCGCCTG-3’</td>
<td>Wimpenny et al. 17)</td>
</tr>
<tr>
<td>Bact-0008-f</td>
<td>Forward</td>
<td>5’-AGAGTTTGATCCTGGCTCAG-3’</td>
<td>Wimpenny et al. 17)</td>
</tr>
<tr>
<td>U5</td>
<td>Forward</td>
<td>5’-CAATCTGCTCGTAAGTATTAC-3’</td>
<td>Kong et al. 21)</td>
</tr>
<tr>
<td>U4</td>
<td>Backward</td>
<td>5’-AGCAGCTCAAAAGCACT-3’</td>
<td>Kong et al. 21)</td>
</tr>
<tr>
<td>UMS125</td>
<td>Forward</td>
<td>5’-GTATTGGAATTCTTTAATGTTTCG-3’</td>
<td>Blanchard et al. 22)</td>
</tr>
<tr>
<td>UMA226</td>
<td>Backward</td>
<td>5’-AGCTGATGTAAGTGACATTAAATTC-3’</td>
<td>Blanchard et al. 22)</td>
</tr>
</tbody>
</table>
denaturing gel electrophoresis system (Bio-Rad, USA), and the PCR products from the second round of amplification emerged as two or even more bands in the DGGE gel (Fig. 1, 2). The sequences and sources of primers that used for this study are shown in Table 2.

3. Sequencing the DGGE fragments and microbial identification

The target bands separated by DGGE were excised with a sterile blade, and 16S rDNA fragments were extracted from the DGGE bands using the gel extraction kit. The extracted DNA was reamplified with primers Bact-0008-f and Bact-0536r, and purified with gel extraction kit. The purified DNA was sent for sequence analysis. The resulting profiles were inspected and identified as bacterial species by a BLAST search. The sequences were deposited in the GenBank database. A total of 5 different species were identified in AF and GF samples, respectively. The bacterial species identified were as follows: *Lactobacillus reuteri* (20/23), uncultured *Enterococcus sp.* (15/23), *Ureaplasma urealyticum* (3/23), uncultured *Enterococcus sp.* (1/23), and *Enterococcus faecalis* (1/23) in AF samples (Table 3); *Lactobacillus reuteri* (20/21), uncultured *Enterococcus sp.* (9/21), *Ureaplasma urealyticum* (1/21), uncultured *Enterococcus sp.* (1/21), and uncultured low G+C Gram-positive bacterium (1/21) in GF samples (Table 4). Mixed infections, in which more than one strain of microorganism was isolated, were identified in 69.6% (16/23) of AF and 47.6% (10/21) GF samples.

The correspondence rate between AF and GF samples was 100% for uncultured *Enterococcus sp.* (DQ975475), 95.2% (20/21) for *Enterococcus faecalis*, 90.5% (19/21) for *Lactobacillus reuteri*, 90.5% (19/21) for *Ureaplasma urealyticum*, and 42.9% (9/21) for uncultured *Enterococcus species* (DQ975475), respectively (Table 5).

Phylogenetic analysis of 16S rDNA sequences amplified from AF and GF samples was performed to confirm the identities of the species (Fig. 3, 4).

4. PCR for *Ureaplasma* species

Additionally, we performed PCR with strain-specific primers for *Ureaplasma urealyticum* and *Ureaplasma parvum* to verify if the result of sequence analysis using the selected band in the DGGE gel actually represented the specific microorganism in the AF or GF sample. The genomic DNA extracts used were the same as the ones for the 16S rDNA PCR analysis. Using 1.5% agarose gel electrophoresis, bands for *Ureaplasma urealyticum* appeared in three AF and one GF samples (Fig. 5), while a band for *Ureaplasma parvum* was in only one GF sample (Fig. 6). These samples for PCR analysis were the same as the ones that were identified as *Ureaplasma urealyticum* by DGGE and sequence analysis. The results of PCR analysis using the specific primers corresponded well with the results of the DGGE and sequence analysis.

| Table 3. Identification of Microbes in Amniotic Fluids by DGGE Analysis (n=23) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Fragment*       | Nearest neighbor strain       | Frequency (%)   | Similarity (%)  | Accession no.   |
| 1               | uncultured *Enterococcus sp.* | 15 (65.2)       | 100             | DQ975475        |
| 2               | *Lactobacillus reuteri*       | 20 (87.0)       | 97.9            | AJ878034        |
| 3               | *Ureaplasma urealyticum*      | 3 (13.0)        | 98.0            | U06095          |
| 4               | uncultured *Enterococcus sp.* | 1 (4.3)         | 98.1            | DQ975479        |
| 5               | *Enterococcus faecalis*       | 1 (4.3)         | 97.6            | AI692453        |

*Fragment refers to the identification given in Fig. 2.

| Table 4. Identification of Microbes in Gastric Fluids by DGGE Analysis (n=21)* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Fragment†       | Nearest neighbor strain       | Frequency (%)   | Similarity (%)  | Accession no.   |
| 1               | uncultured *Enterococcus sp.* | 9 (42.9)       | 100             | DQ975475        |
| 2               | *Lactobacillus reuteri*       | 20 (95.2)      | 97.2            | AJ878034        |
| 3               | *Ureaplasma urealyticum*      | 1 (4.8)        | 99.1            | U06095          |
| 4               | uncultured *Enterococcus sp.* | 1 (4.8)        | 97.2            | DQ975479        |
| 5               | uncultured low G+C Gram-positive bacterium | 1 (4.8) | 97.4 | DQ170970 |

*Two gastric fluid samples from singletons were omitted due to lack of amounts.
†Fragment refers to the identification given in Fig. 3.

100% for uncultured *Enterococcus sp.* (DQ975479), 95.2% (20/21) for *Enterococcus faecalis*, 90.5% (19/21) for *Lactobacillus reuteri*, 90.5% (19/21) for *Ureaplasma urealyticum*, and 42.9% (9/21) for uncultured *Enterococcus species* (DQ975475), respectively (Table 5).
By employing DGGE followed by sequence analysis, we determined the identity of bacteria that presented in amniotic and gastric fluids. Although the diversity of bacteria in AF was not as wide as we expected, a substantial number of women with preterm delivery possessed at least one microorganism in their amniotic fluid, suggesting that multiple organisms might be concurrently involved in the development of IAI. A single band on the agarose gel electrophoresis was separated into several bands on the polyacrylamide gel of DGGE; the bands were thus distinguishable from each other visually. Based on the results of our study, it is suggested that DGGE is a useful screening tool for identification of bacterial 16S rDNA profiles in AF samples.

The results of our study are different from other previous studies in several aspects. First, to overcome the limitation of 16S rDNA PCR analysis, we used DGGE as a tool for identification and sequencing of bacterial rRNA genes in AF and GF samples, which possibly contained multiple species. As a result, PCR products that were run through a DGGE gel was separated into several fragments according to their mobility, and easily cloned and sequenced for identification of rDNA. Intraamniotic infection is often polymicrobial, involving both facultative and anaerobic...
bacteria. We also demonstrated that multiple microbial species were identified from a significant number of AF and GF samples. In previous reports, researchers used bacterial 16S rDNA for PCR analyses as a culture-independent method to identify the microorganism causing IA in previous reports, researchers used bacterial 16S rDNA for PCR analyses as a culture-independent method to identify the microorganism causing IAI. Although bacterial 16S rDNA PCR analysis is a very useful method capable of detecting DNA from almost any bacterial species, it has major limitation in that it cannot identify the specific microorganism in mixed bacterial infections, making sequencing ladders difficult to interpret when trying to compare the sequence with known sequences located in GenBank or other databases. For example, Hitti et al. reported that bacterial rDNA was detected by PCR in 30.4% of women in premature labor whose membranes were intact and 94% of patients with positive AF cultures. However, they also found that some intra-amniotic infections were polymicrobial and suggested the development of a new sequencing technique or customized probes as an alternative approach. Second, in a large portion of the samples, an identified bacterial gene in AF was also found in GF of her newborn. From the second trimester to near term, fetuses in utero can swallow up to 500-1,000 mL AF a day, which assists in normal development of the gastrointestinal system. Our previous study has demonstrated that the concentration of gastric interleukin-6 immediately after birth was associated with funisitis, the histological hallmark of fetal inflammatory response syndrome. Miralles et al. also reported that all newborns from pregnancies with histologic chorioamnionitis whose gastric fluid was available had 16S rRNA identified in the GF. Therefore, our findings based on 16S rDNA PCR and DGGE analysis suggest that the presence or absence of a microorganism in AF can be studied postnataally in GF of the newborn, and GF may be used as a source
of information about neonatal exposure to bacteria in utero when amniocentesis has not been performed.

This study has some limitations. First, although molecular techniques, such as PCR, provide high sensitivity and specificity for detecting the microorganism, they are prone to contamination with exogenous DNA. We took extra precautions to prevent contamination by using positive and negative controls during the PCRs. It is unclear whether the detected microorganisms are new human pathogens causing IAI or whether they have been agents of IAI that were previously misclassified by conventional methods.

Second, traditional bacteria culture was not used in our study. Considering the low efficiency of bacterial culture methods in the study of AF samples, we estimated that the culture method was not as necessary as shown in previous studies. For this reason, however, we could not compare the results of PCR analysis with culture method. Third, the percentage of positive samples seems to be higher than what others have reported, but these positive results don’t correlate with clinical characteristics. That might be due to several factors such as lack of a sufficient number of study participants, partly performed histopathologic examination of the placenta, inhomogeneous group of subjects that included women with premature preterm rupture of membranes, preterm labor with intact membrane, and pregnancy-induced hypertension. Further studies are warranted with a larger number of patients to define the microbiological profile of pathogenic bacteria in AF and to clearly determine the relationship between the presence of bacteria in AF and clinical impact on maternal and neonatal health.

In summary, 16S rDNA PCR and DGGE analysis were performed to survey the bacterial prevalence and diversity in amniotic fluids of women with preterm deliveries and gastric fluids of her newborns. We demonstrated that several species of bacteria were present in AF and GF, and some of them were polymicrobial. DGGE is highly preferred and could be one of the few techniques that allow a fast and reproducible microbial analysis of bacterial communities. To obtain the best possible results, DGGE and sequence analysis followed by PCR with species-specific primers might be a reasonable method to confirm the identification of microbial species causing IAI.

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175:937-44.

Denaturing Gradient Gel Electrophoresis를 이용한 양수 및 신생아 위액 내 미생물 동정 및 다양성 연구
부산대학교 의학전문대학원 미생물학교실, 제주대학교 의학전문대학원 소아청소년학교실*, 부산대학교 의학전문대학원 산부인과학학교실†
김영돈*·유선녕·김승철†·안순철

목적: 조기 분만을 경험한 산모의 양수와 그 산모로부터 출생한 미숙아의 위액에서 16S rDNA PCR과 denaturing gradient gel electrophoresis (DGGE)를 이용하여 세균의 분포와 그 다양성을 조사하고자 하였다.

방법: 2009년 4월부터 12월까지 제왕절개술을 통하여 출생한 재태연령 35주 미만의 미숙아와 그 산모를 대상으로 하였다. 출생 직후 미숙아의 위액과 산모의 양수를 채집하여 DNA를 분리하고 이를 16S rDNA 시동체를 이용하여 PCR을 통하여 증폭하였다. PCR 산물은 변성 기울기를 가지는 polyacrylamide 겔 상에서 DGGE를 시행하였다.

결과: 양수에서는 Lactobacillus reuteri (85.7%), Ureaplasma urealyticum (19.0%), uncultured enterococcus species (66.7%), and Enterococcus faecalis (4.8%)가 검출되었고, 미숙아의 위액에서는 Lactobacillus reuteri (82.6%), uncultured enterococcus species (30.4%), and Ureaplasma urealyticum (4.3%)가 검출되었다. 두 개 혹은 그 이상의 균주가 검출된 시료는 양수의 69.6%, 위액의 47.6%였다.

결론: 16S rDNA PCR 및 DGGE 분석은 미숙아를 분만하는 산모의 양수 내 미생물의 종류와 다양성을 이해하는 도움을 줄 수 있다. 향후 보다 많은 검사자 수를 확보하여 이를 균주가 조기분만 및 자궁 내 감염 등에 미치는 임상적 의의에 대하여 조사해보는 것이 필요하다고 판단된다.