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# Identification of *Streptococcus agalactiae* isolated from pregnant women by 16srRNA gene

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ABSTRACT

The main goal of the current study is to isolate and diagnose of *Streptococcus agalactiae* by using the diagnostic 16 SrRNA gene. *S.agalactiae* was isolated from 850 samples including (425) vaginal swabs, (425) rectal swabs and identified by studying the morphological characteristics of colonies on culture, microscopic characteristics of bacterial cells, biochemical methods, Vitek 2 system, API-20 Strep, and then confirmed the identification by detection of 16SrRNA gene by Polymerase Chain reaction (PCR) followed by DNA Sequence analysis for this gene. A total of 16 isolates of *S.agalactiae* were isolated from 12 (2.82%) vaginal swabs, 4 (0.99%) rectal swabs, and 16SrRNA was detected in 100% of the *S.agalactiae* isolates.

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## INTRODUCTION

Group B *Streptococcus*, or *Streptococcus agalactiae*, is gram-positive, catalase negative, anaerobic, spherical or oval, and less than two µm in diameter, usually a β-haemolytic blood analyzer, Reliable through its production of antigen B (Collin *et al.*, 2018; Whiley and Hardie, 2009).

*Streptococcus agalactiae* can colonize the intestinal tract and genital-urinary tract of healthy people without causing any disease (Ana *et al.*, 2013). However; these bacteria can cause life-threatening invasive diseases in pregnant women, newborns or adult non-pregnant women. Injury to women during pregnancy or postpartum period, usually symptomatic, but GBS may cause Bacteremia, Urinary tract infections, Endometritis, Puerperal sepsis, Meningitis, Septic thrombophlebitis (Fujita *et al.*, 2015; Furfaro *et al.*, 2018).

16S rRNA is an important diagnostic tool that contains unique proteins that facilitate the diagnosis of bacterial strains because they give information or signals about any bacteria they contain. This is the most widely used gene in diagnosis

The use of 16S rRNA in the diagnosis of bacterial compatibility rate has been shown to be about 87.5% at the species level and 96% at the genus level, and this indicates a high sensitivity in diagnosis (Srinivasan *et al.,* 2015).

## Study Design

### Samples Collection

In this study, 850 clinical samples were collected from pregnant women at (35-37 weeks) of gestation from Maternity and Children Teaching hospital, General Afak Hospital and women's clinics in Al-Diwaniyah city during the period from July 2018 to April 2019. The samples included 425 vaginal swabs and 425 enemas taken from women. The samples were collected using sterile cotton swabs containing a carrier medium to maintain the samples. Vaginal samples were collected by gently rotating swabs in the walls of the vagina. The anal samples were taken by sterile swabs 1-2.5 cm behind the anal muscle and gently rotated. Then the collected samples transferred to the Microbiology lab in the Faculty of Science of Al-

Qadisiyah university to conduct the necessary tests.

### Diagnosis of bacteria

The clinical samples were planted in the brain heart broth, then incubated with an anaerobic condition at 37°C for 24 h. After incubation, they were re-cultivated in plate contain blood agar, Granada and Chromogenic agar. All the plate was incubated anaerobically to obtain pure bacterial colonies. The bacterial isolates identified by biochemical test, Oxidase, Catalase, CAMP, API-20 Strip and Vitek System (Cowan and Steal, 2010; Trunat, 2016).

### DAN Primers

The primers needed for a PCR scan that used to diagnosis *S.agalactiae* by using 16SrRNA gene in this study and then DNA sequence analysis has been done using the NCBI-Genbank site to obtain the sequence of nitrogen bases for each gene and the Primer3 plus primer design program.

The primers designed by Korea's Bioneer (Table 1).

### Preparation of PCR master mix

The polymerase chain reaction mixture was present using the AccuPower® PCR PreMix kit supplied by the Korean Bioneer Company and according to the company's instructions (Table 2).

The components of the polymerase chain reaction mixture were placed in the PCR tubes fitted with the kit and container on the rest of the PCR reaction components. The tubes were then carefully mixed with the Vortex for 5 seconds, then transferred to the PCR Thermocycler.

### Programs of PCR Thermocycler

The polymerase chain reaction was investigated using a PCR thermocycler, using the Monoplex PCR technique, Table 3.

## RESULTS AND DISCUSSION

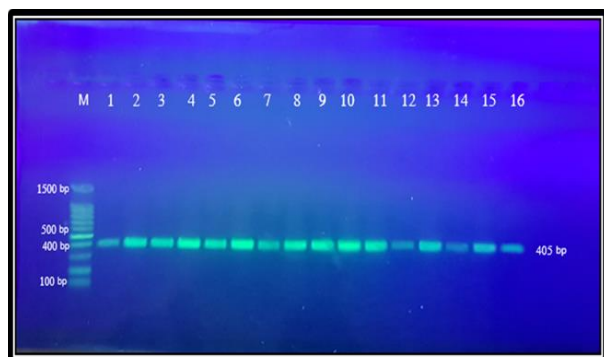


**Figure 1: Agarose gel electrophoresis of PCR assay show 16S rRNA gene analysis of *S.agalactiae*. Using a 100 volt an hour.** M (DNA Marker) and path (1-16) represent the *S.agalactiae* isolates and 405bp is the amplicon size

The diagnosis of *S.agalactiae* by 16SrRNA using Monoplex PCR technique was used to confirm that the 16 isolates belong to *S. agalactiae*. Results are shown in Fig. (1), where all the isolates found to have 16SrRNA gene with a product size of (405 bp) which considered a diagnostic hereditary of these bacteria. This result came close to many studies that have been conducted before using this type of prefixes (Abd El-Razik et al., 2010; Kerdsin et al., 2017).

**Figure 2: Multiple sequence alignment analysis of 16S ribosomal RNA gene in local Streptococcus agalactiae isolates and NCBI-Genbank Streptococcus sp isolates. The multiple alignment analysis was constructed using Clustal W alignment tool in (MEGA 6.0 version). That show the nucleotide alignment similarity as (*) with substitution mutations in 16S ribosomal RNA gene**

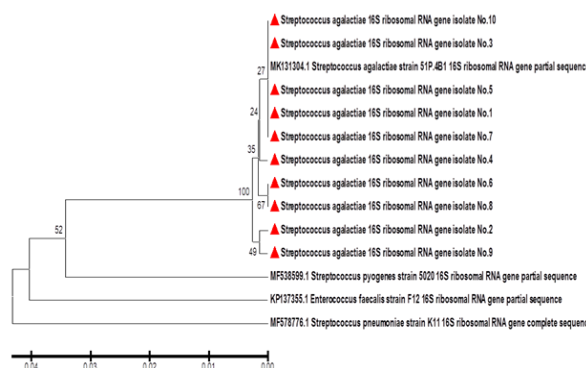


**Figure 3: Phylogenetic tree analysis based on 16S rRNA gene partial sequence in local *Streptococcus agalactiae* isolates that used for confirmative identification and genetic analysis.** The phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Streptococcus agalactiae* isolate No.1 No.10 were showed closed related to NCBI-BLAST *Streptococcus agalactiae* isolate (MK131304.1). Whereas the *Streptococcus* sp isolates were showed different at total genetic changes (0.01-0.04%).

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**Table 1: Primers of 16SrRNA gene with the product size(bp)**

| Reference | Amplicon size (bP) | Primer Sequences | | Primer |
|---|---|---|---|---|
| Yousefi *et al*. (2014) | 405 | CGCTGAGGTTTGGTGTTTACA | F | 16s rRNA |
| | | CACTCCTACCAACGTTCTTC | R | |

F: Forward R: Reverse

**Table 2: Components and sizes of polymerase chain reaction mixture**

| Volume (μL) | PCR Master mix |
|---|---|
| 2 | DNA template |
| 1 | Forward primer |
| 1 | Reveres primer |
| 16 | PCR water |
| 20 | Total volume |

**Table 3: Conditions of Thermocouples Used for PCR Examination**

| Cycles Number | Temperature (c)/time Cycling conditions | | | | | Gene Name |
|---|---|---|---|---|---|---|
| | Final Extension | Extension | Annealing | Denaturation | Initial Denaturation | |
| 29 | 72/5 min | 72/50sec | 57.5/30sec | 95/30sec | 95/5min | 16SrRNA |

**Table 4: The NCBI-BLAST Homology Sequence identity (%) between local Streptococcus agalactiae and NCBI-BLAST submitted Streptococcus sp isolates**

| *S. agalactiae* isolate No. | Gene bank Accession number | NCBI-BLAST Homology Sequence identity (%) | | |
|---|---|---|---|---|
| | | Identical *Streptococcus* sp | Accession number | Identity (%) |
| Isolate No.1 | MK680043.1 | *Streptococcus agalactiae* | MK131304.1 | 100% |
| Isolate No.2 | MK680044.1 | *Streptococcus agalactiae* | MK131304.1 | 99.72% |
| Isolate No.3 | MK680045.1 | *Streptococcus agalactiae* | MK131304.1 | 100% |
| Isolate No.4 | MK680046.1 | *Streptococcus agalactiae* | MK131304.1 | 99.72% |
| Isolate No.5 | MK680047.1 | *Streptococcus agalactiae* | MK131304.1 | 100% |
| Isolate No.6 | MK680048.1 | *Streptococcus agalactiae* | MK131304.1 | 99.72% |
| Isolate No.7 | MK680049.1 | *Streptococcus agalactiae* | MK131304.1 | 100% |
| Isolate No.8 | MK680050.1 | *Streptococcus agalactiae* | MK131304.1 | 99.72% |
| Isolate No.9 | MK680051.1 | *Streptococcus agalactiae* | MK131304.1 | 99.44% |
| Isolate No.10 | MK680052.1 | *Streptococcus agalactiae* | MK131304.1 | 100% |

The 16SrRNA gene is used as a diagnostic tool for bacteria because it is found in all bacteria, even utants. 16SrRNA is composed of highly conserved nucleotide sequences interspersed with variable regions of bacterial species and species.

PCRs target protected areas of rRNA. The nucleotides of the PCR product are followed by a comparison of this sequence with well-known sequences stored in the database (Claire *et al*., 2012).

DNA sequences analysis of 16SrRNA gene of 10 isolates belongs to *S. agalactiae* performed by using DNA Sequencer and the MEGA 6 program with the use of the UPGMA tree (Un weight Pair Group Method with Arithmetic Mean). The similarities of nitrogen bases sequences of the local isolates of *S.agalactiae* with that globally registered isolates in the gene bank Fig. (2).

The results of the analysis showed a significant convergence between the local isolates of *S. agalactiae* compared to other global isolates in the analysis of the genetic tree, as shown in figure (3).

The results also showed a clear genetic match of the local *S. agalactiae* isolates with the global isolates recorded in the gene bank. Table 4 shows this correlation.

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