Comparison between the Rapid ID 32 Strep test system and 16S rRNA gene sequencing for the identification of α -hemolytic streptococci

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Oral viridans streptococcus is the most common bacterial species present in human dental plaque. The Rapid ID 32 Strep (bioMérieux, Marcy-l'Etoile, France) test system allows for the identification of most streptococci within 4 hours. The aim of this study was to evaluate the reliability of this test in identifying isolates of α -hemolytic oral streptococci in human dental plaque and compare it with that of the 16S rRNA sequencing method. A total of eighty-five α -hemolytic streptococcal isolates were identified using 16S rRNA gene sequencing and Rapid ID 32 Strep. The Rapid ID 32 Strep method correctly identified 80% of the species and 87% of the streptococcal groups but failed to accurately identify five isolates (three *S. cristatus*, one *S. australis*, and one *S. tigurinus*).

Key Words: Identification; Sequencing; Streptococci

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Introduction

Oral viridans streptococci are the major bacterial species present in human dental plaque, and most oral streptococci are α -hemolytic streptococci [1,2]. As the gold standard, 16S rRNA gene sequencing is used [3-6] for bacterial identification, with a sequence homology of 99% or greater with published species sequences considered to denote the same species, in accordance with Clinical & Laboratory Standard Institute (CLSI) guidelines [7].

The API 20 Strep strip contains twenty microtubes with dehydrated substrates that are necessary to detect enzymatic activity and fermentation of sugars. The results are obtained within 24-48 hours, and the identification is

based on numerical analysis after a visual interpretation of color changes (bioMérieux, Marcy-l'Etoile, France) [8]. Recently, it has been reported that agreement of α -hemolytic streptococci identification by API 20 Strep and 16S rRNA gene sequencing was only 26% by species and 63% by streptococcal group [9].

The Rapid ID 32 Strep system is a more recent system, which consists of thirty two cupules and allows the identification of most streptococci and members of related genera encountered in medical and veterinary bacteriology in just 4 hours [10]. The aim of this study was to evaluate the reliability of Rapid ID 32 Strep in identifying isolates of α -hemolytic streptococci from human dental plaque and to compare its identification capabilities at the species and

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group levels.

Materials and Methods

Bacterial isolates

Eighty-five α -hemolytic streptococci were isolated from the supragingival plaque of healthy persons. Supragingival plaque was transferred into a glass tube containing phosphate buffered saline (PBS) and glass beads (diameter 1 mm). Dispersed plaque samples were diluted with PBS and plated on Mitis Salivarius agar plates (BD Bioscience, Sparks, MD, USA). After incubating at 37°C for 72 hours in aerobic conditions supplemented with 5% CO₂, one bacterial colony from each plaque sample was isolated randomly and grown in Brain Heart Infusion broth (Becton, Dickinson, and Company). Hemolysis of these isolates was examined on blood agar plates (KOMED, Seongnam, Korea). All volunteers who wanted to donate their plaques for this study were informed about the procedure in depth, and written consent from all was obtained for inclusion in the study. This study was approved by the Institutional Review Board of the Gangneung-Wonju National University Dental Hospital (IRB2011-2).

Rapid ID 32 Strep identification

Rapid ID 32 Strep (bioMérieux) testing was carried out according to the manufacturers' instructions, using the PCM-4862 BIOS software for API codes. After growth on sheep blood agar medium, a bacterial suspension was prepared in 2 mL of sterile water and adjusted to a 4 McFarland standard turbidity. Fifty-five microliters of the suspension were deposited into each well. After 4 hours of incubation at 37°C, a reading was taken with a Mini API reader (bioMérieux). The Rapid ID 32 Strep database version used was V 2.30.

16S rRNA gene amplification and sequencing

The 16S rRNA gene was amplified using the bacterium-specific universal primers 27F (5'-AGAGTTTGATCMTG-GCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTAC-

GACTT-3') to amplify a 1.5-kb fragment [11,12]. PCR amplification was performed using a DNA thermal Cycler (GeneAMP PCR System 9700; Perkin Elmer, Waltham, MA, USA) under the following conditions: initial incubation at 95°C for 2 minutes, 34 cycles at 94°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute, followed by final incubation at 72°C for 10 minute. Primers and excess nucleotides were removed using a PCR purification kit (Bioneer, Deajeon, Korea). DNA sequencing was performed by Macrogen (Seoul, Korea) using the PCR primers. The 16S rRNA gene sequences were subjected to BLAST analysis against the NCBI nucleotide database.

Results

The 16S rRNA gene sequencing results of eighty-five isolates showed $\geq 99\%$ similarity to published species in NCBI BLAST data. The species identification of the eighty-five clinical isolates was accurately carried out by molecular methods, and the identification comparison results are shown in Table 1, along with the relative performance of

Table 1. Comparison of Rapid ID 32 Strep identification with 16S rRNA gene sequencing identification of clinical α -hemolytic streptococci at the group and species levels

Species by 16S rRNA sequencing	No. of isolates	Agreement by Rapid ID32 Strep, n (%)
Mitis group	10	7 (70) ^a
<i>S. mitis</i>	6	3 (50) ^b
<i>S. oralis</i>	2	2 (100)
<i>S. australis</i>	1	0 (0)
Sanguinis group	23	21 (91)
<i>S. sanguinis</i>	7	7 (100)
<i>S. gordonii</i>	13	13 (100)
<i>S. cristatus</i>	3	0 (0)
Anginosus group	28	23 (82)
<i>S. anginosus</i>	24	16 (67)
<i>S. constellatus</i>	4	4 (100)
Salivarius group	17	17 (100)
<i>S. salivarius</i>	17	17 (100)
Mutans group	7	6 (86)
<i>S. mutans</i>	7	6 (86)
Total	85	
Total agreement by group		74 (87)
Total agreement by species		68 (80)

^aPercentage of isolates assigned to correct group by a test. ^bPercentage of isolates assigned to correct species by a test.

the Rapid ID 32 Strep test method. Rapid ID 32 Strep correctly identified 80% of species and 87% of streptococcal groups. This signified higher identification success rates at the group level than at the species level. Rapid ID 32 Strep failed to correctly identify any of the three *S. cristatus*, one *S. australis*, and one *S. tigurinus* isolates.

Discussion

Gene sequencing analysis remains by far, the most reliable method for the laboratory identification of α -hemolytic streptococci. Davies et al. [9] reported that fifty-six α -hemolytic streptococcal isolates were identified using API 20 Strep (bioMérieux). When compared with the 16S rRNA gene sequencing identification, 26% of isolates were correctly identified by API 20 Strep at the species level. Davies et al. [9] used API 20 Strep instead of Rapid ID 32 Strep in their study for comparison of identification methods. In addition, blood was the main source of α -hemolytic streptococci used in this study. Different streptococci isolation sources might also cause discrepancies in identification ability.

In 1984, API 20 Strep for species identification of streptococci associated with bovine mastitis was compared with conventional biochemical methods [13]. Using profile indexes and table data issued by the manufacturer, the API 20 Strep system identified 71.4% of strains. Hinnebusch et al. [14] previously reported the comparison between different bacterial identification methods for viridans group streptococci. Viridans group streptococci (36 stock strains and 167 single patient blood culture isolates) were assessed using API 20 Strep. API 20 Strep results corresponded with those of conventional biochemical identification in 74% of cases; using the recommended supplemental test, agreement was augmented in 9% of the isolates. Disagreement with conventional biochemical methods occurred in 14% of the commercial techniques.

The Rapid ID 32 Strep system is a suitable alternative for rapid identification of members of the *Streptococcus* genus and related genera. The Rapid ID 32 Strep system is easy to use in routine work and gives rapid results (within 4 hours). The main advantage of this system is its extensive database, which allows correct identification of most newly

described species of medical or veterinary interest. Freney et al. [15] reported that the performance of the Rapid ID 32 Strep system in oral streptococci identification was good. However, additional tests were generally needed for complete identification of *Streptococcus sanguis* (7 of 8 strains tested), *Streptococcus oralis* (14 of 22), *Streptococcus mitis* (11 of 17), and *Streptococcus pneumoniae* (10 of 29). Most of the other streptococci (*S. bovis*, *S. suis*, and *S. ubenis*) were correctly identified without extra tests. For the other gram-positive species tested, results were in good agreement with the reference identification.

In our study, eighty-five α -hemolytic streptococcal isolates were identified using 16S rRNA gene sequencing and Rapid ID 32 Strep. Rapid ID 32 Strep correctly identified 80% of isolates at the species level. This system generally displayed excellent identification ability in our study. Rapid ID 32 Strep failed to correctly identify *S. cristatus*, *S. australis*, and *S. tigurinus* isolates, because the database used in our study did not contain the identification data for these species. *S. australis* and *S. tigurinus* are novel, recently identified oral streptococci [16–18]. Further database development may help improve its performance; in particular, an improvement in its ability to identify *S. anginosus*, *S. cristatus*, *S. australis*, and *S. tigurinus* would greatly enhance its reliability.

In our study, we showed that the Rapid ID 32 Strep correctly identified 80% of species and 87% of streptococcal groups.

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Conflicts of Interest

The author declares no competing interests.

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