Viridans group streptococci (VGS) are a common cause of subacute bacterial endocarditis (Roberts et al., 1979), and can cause sepsis in neutropenic patients (Bochud et al., 1994). They are also the leading cause of catheter- and neutropenia-related bloodstream infections (Uh et al., 2004). Several microorganisms cause infective endocarditis and the oral cavity is the most common source of these bacteria. In particular, VGS cause 30–40% of cases of infective endocarditis (Kaye, 1992). Previously, VGS were uniformly susceptible to several antimicrobial agents such as β-lactams, aminoglycosides, tetracyclines, and macrolides; however, the frequent use of these antibiotics to treat and prevent infections has led to an increase in drug resistance in many parts of the world (Diekema et al., 2001; Ioannidou et al., 2001; Teng et al., 1998; Uh et al., 2004).

Oral VGS are the commonest cause of infective endocarditis (Kaye, 1992; Knox and Hunter, 1991), and dental plaques are the major source of these bacteria. However, despite the important role played by this streptococcal group in the onset of infective endocarditis, information about its antibiotic resistance profile is lacking. Here, we examined both the incidence of antimicrobial resistance and the susceptibility patterns among 635 VGS isolates obtained from dental plaques.

Viridans group streptococci (VGS) are a common cause of subacute bacterial endocarditis, and dental plaque is the major source of these bacteria. The present study examined the antibiotic resistance of 635 VGS isolates obtained from dental plaques. Isolates from supragingival plaques were identified using the rapid ID 32 Strep and mini API reader (bioMérieux, France), and minimal inhibitory concentrations (MICs) were determined by a broth microdilution method. High rates of resistance to ampicillin and tetracycline were detected among the isolates. The most resistant species were *Streptococcus sanguinis* and *Streptococcus salivarius*. Among the 635 isolates, 9.1% were resistant to erythromycin, and 20.6% to tetracycline. All isolates were sensitive to vancomycin. Resistance to amoxicillin was observed in 0.2% of all isolates. In this study, we showed the incidence of antimicrobial resistance and the susceptibility patterns among 635 VGS isolates from dental plaque.

**Key words** : Dental plaque / Streptococci / Antibiotic / Resistance.
and Company). The hemolytic activity of these isolates was examined using blood agar plates (KOMED, Sungnam, South Korea). Further identification of the isolates was performed using the rapid ID 32 Strep system and mini API reader (bioMérieux, Marcy-l’Etoile, France).

Stock solutions of amoxicillin, ampicillin, cefotaxime, erythromycin, gentamicin, penicillin G, tetracycline and vancomycin (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were prepared to determine the minimal inhibitory concentration (MIC). MICs were determined in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood using the microdilution method recommended by the CLSI (Clinical and Laboratory Standards, 2018). Streptococcal colonies were picked from blood agar plates (KOMET) after aerobic culture at 37°C for 18 h, and a cell suspension equivalent to a 0.5 McFarland standard (approximately 1 x 10^5 CFU/ml) was prepared in cation-adjusted Mueller-Hinton broth. The bacterial suspensions were inoculated into serially-diluted antibiotic solutions in 96-well microtiter plates such that the final cell concentration was 5 x 10^5 CFU/ml. The range of concentrations tested for each antibiotic was 0.001 µg/ml to 1024 µg/ml. The microtiter plates were incubated in an ambient air incubator at 37°C for 24 h, and then read visually. The minimum concentration of antibiotic that produced no growth-related turbidity was recorded as the MIC. Antibiotic resistance was determined using interpretive standard concentrations from the CLSI guidelines (Clinical and Laboratory Standards, 2020). All tests were repeated at least twice.

We obtained 635 VGS isolates from dental plaques and identified 154 S. oralis, 136 S. mitis, 129 S. anginosus, 123 S. sanguinis, 33 S. salivarius, 27 S. mutans, 22 S. gordonii, and 11 S. constellatus species. The MIC ranges, the antimicrobial concentrations that inhibited 50% and 90% of the isolates, and the percentage of isolates tested are listed in Table 1. Almost all of the isolates were sensitive to amoxicillin (99.6%) and cefotaxime (99.4%) and all were sensitive to vancomycin; however, some S. constellatus (9.1%) and S. oralis (0.6%) isolates were resistant to amoxicillin. 

The VGS species isolated in the present study showed the greatest resistance to ampicillin (46.1%) and tetracycline (34.8%). Isolates of S. gordonii and S. mutans were sensitive to six of the antibiotics, but showed some resistance to ampicillin and tetracycline (Table 2). Overall, 293 isolates showed high and intermediate levels of resistance to ampicillin: 5.5% of all isolates showed high resistance to ampicillin (MICs ≥ 8 µg/ml) and 40.6% showed intermediate resistance (MICs = 0.5 – 4 µg/ml). S. mitis (10.3%) and S. salivarius (12.1%) showed the highest rates of resistance to ampicillin.

Overall, 2.0% and 9.1% of all isolates showed intermediate or high resistance, respectively, to erythromycin. The highest rates of erythromycin resistance were observed for S. salivarius (18.2%), S. oralis (14.3%), and S. mitis (11.8%). All isolates of S. constellatus, S. gordonii, and S. mutans were sensitive to erythromycin. Regarding tetracycline, 20.6% of isolates were highly resistant (MIC ≥ 8 µg/ml) whereas 14.2% showed intermediate resistance (MIC = 4 µg/ml). Two of the 27 S. mutans (7.4%) isolates were highly resistant to tetracycline and two showed intermediate resistance. S. gordonii (72.7%) and S. constellatus (36.4%) showed the highest rates of resistance to tetracycline. Isolates of S. mutans were most sensitive to the antimicrobial agents used in this study, and the most resistant were S. sanguinis (85.4%) and S. salivarius (78.8%), which were resistant to at least one of the antibiotics.

The present study examined the levels of antibiotic resistance among strains of VGS isolated from dental plaques. We found that 2.7% of the streptococcal isolates were resistant to penicillin G. VGS are generally susceptible to penicillin (Álcaide et al., 1995), however, strains resistant to this antibiotic (which were identified in the gingival flora of patients receiving penicillin prophylaxis for rheumatic fever) were first identified in 1962 (Naiman and Barrow, 1963). Penicillin-resistant VGS were also identified in the oropharyngeal flora of South African children in 1978. This coincided with the emergence of a Streptococcus pneumoniae strain that was also resistant to penicillin (Farber et al., 1983).

Since 1983, several studies have identified high rates of penicillin-resistance among VGS isolated from patients with clinically significant infections (Goldfarb et al., 1984; Gossling, 1988). Earlier reports indicated that 16.1% of S. mitis isolates in Spain showed a high level of resistance to penicillin (Álcaide et al., 1995), however, only 0.7% of the S. mitis isolates in the present study were highly resistant to penicillin G. 

In our study, 40.6% and 5.5% of all isolates showed intermediate or high resistance, respectively, to ampicillin. Pasquantonio et al. (2012) reported that 14% of the VGS isolated from dental plaque and gingival crevices in Italy showed the resistance to ampicillin and 13% to penicillin. It has been also reported that 36.2% of the VGS originated from clinical samples such as blood, pus, urine and peritoneal fluid in Korea was resistant to ampicillin (Chun et al., 2015). Recently, the rate of resistance and reduced sensitivity of the VGS from the gums and buccal mucosa against ampicillin in Turkey was reported as 55.1% (Suzuk et al., 2016). Although the resistance rate of streptococci to the penicillin was similar to the ampicillin in those studies, only 2.7% of streptococcal isolates was not susceptible to penicillin G in our study. Ampicillin has very similar structure with
TABLE 1. *In vitro* activity of antibiotics against 635 viridans group streptococcal isolates.

| Species   | Antimicrobial agent | MIC (µg/ml) Range | 50% | 90% | Percent Susceptible (%) |
|-----------|---------------------|-------------------|-----|-----|-------------------------|
| S. anginosus | Amoxicillin            | <0.001–0.25       | 0.008 | 0.06 | 100                     |
|           | Ampicillin             | <0.001–1          | 0.125 | 0.5  | 87.6                    |
|           | Erythromycin           | <0.001–16         | 0.002 | 0.125 | 98.4                    |
|           | Gentamicin             | 0.001–64          | 0.5   | 2    | 97.6                    |
|           | Penicillin G           | <0.001–1          | 0.004 | 0.015 | 99.2                    |
|           | Cefotaxime             | <0.001–2          | 0.015 | 0.125 | 99.2                    |
|           | Tetracycline           | <0.001–512        | 0.5   | 64   | 60.4                    |
|           | Vancomycin             | 0.001–0.5         | 0.125 | 0.25 | 100                     |
| S. constellatus | Amoxicillin           | <0.001–16         | 0.03  | 0.06 | 90.9                    |
|           | Ampicillin             | 0.008–128         | 0.25  | 1    | 63.6                    |
|           | Erythromycin           | 0.001–0.06        | 0.002 | 0.015 | 100                     |
|           | Gentamicin             | 0.015–1           | 0.5   | 1    | 100                     |
|           | Penicillin G           | <0.001–8          | 0.004 | 0.015 | 90.9                    |
|           | Cefotaxime             | <0.001–8          | 0.03  | 0.125 | 90.9                    |
|           | Tetracycline           | 0.03–32           | 2     | 16   | 63.6                    |
|           | Vancomycin             | 0.06–1            | 0.125 | 1    | 100                     |
| S. gordonii | Amoxicillin            | 0.002–0.5         | 0.015 | 0.125 | 100                    |
|           | Ampicillin             | 0.03–4            | 0.125 | 0.25 | 90.9                    |
|           | Erythromycin           | <0.001–0.125      | 0.002 | 0.03 | 100                     |
|           | Gentamicin             | 0.25–4            | 1     | 4    | 100                     |
|           | Penicillin G           | <0.001–0.015      | 0.002 | 0.015 | 100                     |
|           | Cefotaxime             | <0.001–1          | 0.008 | 0.03 | 100                     |
|           | Tetracycline           | 0.015–128         | 64    | 64   | 27.3                    |
|           | Vancomycin             | 0.002–1           | 0.25  | 0.5  | 100                     |
| S. mitis | Amoxicillin            | <0.001–0.5        | 0.03  | 0.025 | 100                    |
|           | Ampicillin             | 0.015–64          | 0.5   | 8    | 39.7                    |
|           | Erythromycin           | <0.001–1024       | 0.002 | 2    | 88.2                    |
|           | Gentamicin             | 0.03–16           | 1     | 4    | 99.3                    |
|           | Penicillin G           | <0.001–64         | 0.015 | 0.125 | 94.2                    |
|           | Cefotaxime             | <0.001–8          | 0.03  | 0.25 | 98.6                    |
|           | Tetracycline           | <0.001–0.5        | 0.25  | 64   | 71.3                    |
|           | Vancomycin             | <0.001–1          | 0.125 | 0.25 | 100                     |
| S. mutans | Amoxicillin            | <0.001–0.125      | 0.008 | 0.06 | 100                     |
|           | Ampicillin             | 0.004–1           | 0.125 | 0.25 | 92.6                    |
|           | Erythromycin           | <0.001–0.06       | 0.004 | 0.015 | 100                     |
|           | Gentamicin             | 0.015–4           | 0.125 | 1    | 100                     |
|           | Penicillin G           | <0.001–0.015      | 0.002 | 0.015 | 100                     |
|           | Cefotaxime             | <0.001–0.015      | 0.004 | 0.015 | 100                     |
|           | Tetracycline           | <0.001–16         | 0.125 | 4    | 85.2                    |
|           | Vancomycin             | 0.004–0.5         | 0.125 | 0.5  | 100                     |
| S. oralis | Amoxicillin            | <0.001–0.5        | 0.03  | 0.125 | 99.4                    |
|           | Ampicillin             | <0.001–16         | 0.25  | 4    | 52.6                    |
|           | Erythromycin           | <0.001–1024       | 0.008 | 4    | 79.2                    |
|           | Gentamicin             | <0.001–8          | 1     | 4    | 94.8                    |
|           | Penicillin G           | <0.001–0.25       | 0.015 | 0.06 | 98.8                    |
|           | Cefotaxime             | <0.001–0.5        | 0.06  | 0.25 | 100                     |
|           | Tetracycline           | <0.001–512        | 0.5   | 64   | 63                     |
|           | Vancomycin             | <0.001–1          | 0.125 | 0.25 | 100                     |
| S. salivarius | Amoxicillin            | 0.004–0.5         | 0.125 | 0.25 | 100                     |
|           | Ampicillin             | 0.06–256          | 1     | 8    | 33.4                    |
|           | Erythromycin           | <0.001–1024       | 0.015 | 8    | 81.8                    |
|           | Gentamicin             | 0.06–8            | 1     | 4    | 97                      |
|           | Penicillin G           | <0.001–0.5        | 0.03  | 0.125 | 99.9                    |
|           | Cefotaxime             | 0.001–16          | 0.03  | 0.125 | 100                     |
|           | Tetracycline           | 0.001–256         | 0.125 | 128  | 69.7                    |
|           | Vancomycin             | 0.001–1           | 0.06  | 0.5  | 100                     |
| S. sanguinis | Amoxicillin            | <0.001–2          | 0.06  | 0.25 | 100                     |
|           | Ampicillin             | 0.002–32          | 1     | 4    | 25.2                    |
|           | Erythromycin           | <0.001–1024       | 0.002 | 0.5  | 87.8                    |
|           | Gentamicin             | 0.002–256         | 1     | 4    | 99.2                    |
|           | Penicillin G           | <0.001–0.5        | 0.015 | 0.06 | 97.6                    |
|           | Cefotaxime             | <0.001–0.5        | 0.015 | 0.06 | 100                     |
|           | Tetracycline           | <0.001–256        | 0.125 | 64   | 67.5                    |
|           | Vancomycin             | 0.008–0.5         | 0.125 | 0.25 | 100                     |
**TABLE 2.** Percentage of resistant viridians group streptococcal isolates.

| Species       | No. of Isolates | Percentage of Resistant Isolates (No. of Isolates) |  |  |  |  |  |  |  |
|---------------|----------------|--------------------------------------------------|---|---|---|---|---|---|---|
|               |                | Amoxicillin I | Ampicillin I | Erythromycin | Gentamicin | Penicillin G | Cefotaxime | Tetracycline | Vancomycin |
| S. anginosus  | 129            | 0            | 0            | 12.4 (16)    | 0          | 1.6 (2)      | 0.8 (1)    | 0.8 (1)      | 0          | 34.9 (45)  | 4.7 (6) | 0          |
| S. constellatus | 11             | 0            | 9.1 (1)     | 27.3 (3)     | 9.1 (1)    | 0            | 0          | 0            | 0          | 9.1 (1)    | 0       | 36.4 (4)  |
| S. gordonii   | 22             | 0            | 0            | 9.1 (2)      | 0          | 0            | 0          | 0            | 0          | 0          | 0       | 72.7 (16) |
| S. mitis      | 136            | 0            | 0            | 50 (68)      | 10.3 (14)  | 0            | 11.8 (16)  | 0            | 0.7 (1)    | 5.1 (7)    | 0.7 (1) | 0.7 (1)   |
| S. mutans     | 27             | 0            | 0            | 7.4 (2)      | 0          | 0            | 0          | 0            | 0          | 0          | 0       | 7.4 (2)   |
| S. oralis     | 154            | 0.6 (1)      | 0            | 43.5 (67)    | 3.9 (6)    | 6.5 (10)     | 14.3 (22)  | 5.2 (8)      | 0          | 0.6 (1)    | 0.6 (1) | 0          |
| S. salivarius | 33             | 0            | 0            | 54.5 (18)    | 12.1 (4)   | 0            | 18.2 (6)   | 3.0 (1)      | 0          | 6.1 (2)    | 0       | 0          |
| S. sanguinis  | 123            | 0            | 0            | 66.7 (82)    | 8.1 (10)   | 2.4 (3)      | 9.8 (12)   | 0            | 0.8 (1)    | 2.4 (3)    | 0       | 2.4 (3)  |
| (n=635)       |                | 0.2 (1)      | 0.2 (1)     | 40.6 (256)   | 5.5 (35)   | 2.0 (13)     | 9.1 (58)   | 1.6 (10)     | 0.6 (4)    | 2.2 (14)   | 0.5 (3) | 0.3 (2)   |

I, intermediate resistant; R, resistant.
penicillin G. The reason why streptococci have resistance to ampicillin and not to penicillin in our study is not clear at this point. As opposed to our case, the spread of a penicillin-resistant but ampicillin-susceptible phenotype among Enterococcus faecalis isolates in several countries has been reported (Metzidie et al., 2005; Kim et al., 2019). They assumed that alterations in penicillin binding proteins in E. faecalis that specifically influence for these antibiotics might be responsible for that observation. Although other resistance mechanisms cannot be excluded, further studies on the alterations in penicillin binding proteins in our oral streptococcal strains might explain ampicillin-resistant but penicillin-susceptible phenotype.

Erythromycin is the recommended alternative treatment for patients who are allergic to penicillin, and it is also used to prevent bacterial endocarditis associated with dental procedures (Teng et al., 1998; Wu et al., 1997). In the past, most VGS were sensitive to erythromycin; however, the resistance of oral streptococci to this antibiotic is increasing (Gershon et al., 2002; Prabhu et al., 2004). A study of VGS isolates obtained between 1993 and 1994 in southern Taiwan reported the frequency of erythromycin resistance as 56.5% (Wu et al., 1997). In Spain, the rate of erythromycin resistance in VGS isolated from healthy children is reported to be 48.3% (Aracil et al., 2001). However, the present study found that overall, 11.1% of isolates from Korean samples showed high or intermediate levels of resistance to erythromycin. This figure is much lower than those reported in Taiwan and Spain. This discrepancy may be due to the different sources of streptococci used in these studies.

Tetracyclines are broad-spectrum agents that are active against a wide range of Gram-positive and Gram-negative bacteria, and atypical organisms such as chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. Tetracycline resistance was identified in all streptococcal species tested in the present study, with the highest resistance detected in S. gordonii (72.7%), S. constellatus (36.4%), and S. oralis (33.1%). These figures are in line with those reported in studies conducted in Spain and South Africa, in which 40% and 41%, respectively, of S. mitis isolates were resistant to tetracycline (Potgieter et al., 1992; Santini et al., 1988).

Although the antimicrobial susceptibility patterns of VGS have been reported previously (Alcaide et al., 1995; de Azavedo et al., 1999; Gomez-Garces et al., 1994; Potgieter et al., 1992; Tuohy and Washington, 1997; Uh et al., 2004), most of the VGS isolates in those studies were from blood samples. Recently, a couple of studies reported the antibacterial activity against VGS isolated from the oral cavity (Pasaquantonio et al., 2012; Suzuk et al., 2016). The time when samples were taken from one paper was 2015 (Suzuk et al., 2016), which was not much different from ours (2011-2013). Even though the other paper did not show the sampling period, the antibacterial activity patterns of both reports were similar to ours. All isolates were found to be susceptible to vancomycin and more than 30% of isolates were not susceptible to tetracycline. Resistance to ampicillin was also observed in those studies, but unlike our study, some penicillin-resistant strains were also observed.

In the present study, we showed the incidence of antimicrobial resistance and the susceptibility patterns among 635 VGS isolates from dental plaque. Here, we identified high rates of resistance to ampicillin and tetracycline among VGS isolated from dental plaques, and found that the most resistant species were S. sanguinis and S. salivarius.

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