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In-vitro susceptibility, tolerance and glycocalyx production in *Streptococcus mutans*

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We studied the presence of high-level resistance to aminoglycosides, penicillin tolerance and glycocalyx production in 160 isolates of *Streptococcus mutans*. Susceptibility to amoxycillin, cefazolin, imipenem, erythromycin, clindamycin, vancomycin and teicoplanin was also investigated. Of the isolates analysed, 58.8% produced glycocalyx *in vitro* and 2.5% were penicillin-tolerant. High-level resistance to streptomycin was found in 16.3% of the isolates, but all were sensitive to all other antibiotics tested. We found no significant relationship between glycocalyx production and high-level streptomycin resistance, penicillin tolerance or antibiotic susceptibility, except for a greater susceptibility to clindamycin and vancomycin in isolates that produced glycocalyx. Although our findings reflect the clinically favourable pattern of susceptibility currently found in this species, the appearance in some isolates of resistance, tolerance and glycocalyx production should be investigated because of the risks involved in endocarditis caused by *S. mutans*.

Introduction

Of the varied flora in the oral cavity, *Streptococcus mutans* has particular relevance because of its association with local infections such as dental caries, and with systemic processes such as endocarditis, sepsis and abscesses.[1–3] Growth on the heart valves is usually a result of bacteraemia after odontological procedures. Although bacteraemia is normally transient and has no clinical repercussions, endocarditis may develop in individuals with underlying valvular disease or who undergo invasive procedures.[1]

The initiation and course of this process are influenced by several characteristics of the microorganism involved, such as its capacity to produce glycocalyx, susceptibility to antibiotics, tolerance to β-lactams (MBC/MIC ratio $\geqslant 32$) and high-level resistance to aminoglycosides.[4–6] Although isolates with high-level resistance to aminoglycosides appear less frequently among viridans streptococci than in other species such as *Enterococcus* spp., the phenomenon none the less requires further research to ensure the success of antimicrobial treatments for endocarditis.[4,7] This study was designed to evaluate glycocalyx production, the incidence of high-level resistance to aminoglycosides, penicillin tolerance and susceptibility to amoxycillin, cefazolin, imipenem, erythromycin, clindamycin, vancomycin and teicoplanin in oral isolates of *S. mutans*.

Materials and methods

A total of 160 isolates of *S. mutans* were isolated from human saliva or supragingival plaque, and were identified in accordance with the criteria of Maiden *et al.*[8]

Susceptibility studies

Antibiotic susceptibilities were determined using a solid medium dilution method in accordance with NCCLS recommendations for streptococci.[9] Amoxycillin, cefazolin, erythromycin, clindamycin and vancomycin were from Sigma Chemical Co. (St Louis, MO, USA), imipenem from Merck Sharp & Dohme (Madrid, Spain) and teicoplanin from Merrell Dow (Madrid, Spain). Antibiotics were used at concentrations of 0.003–1 mg/L, except for vancomycin and teicoplanin, which were used at concentrations of 0.007–2.0 mg/L.

Detection of high-level resistance to aminoglycosides

High-level resistance to aminoglycosides was tested by the solid medium macrodilution method. The antibiotics assayed were gentamicin, streptomycin, tobramycin, kanamycin and amikacin (Sigma), each of which was tested at concentrations of 500, 1000 and 2000 mg/L.

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Penicillin tolerance

Tolerance to penicillin was detected by the liquid medium macrodilution method in Todd–Hewitt broth (Oxoid, Basingstoke, UK). We tested ten concentrations of penicillin (Sigma) ranging from 0.003 to 2 mg/L. The inoculum consisted of approximately 5×10^5 cfu/mL from a suspension scoring 0.5 on the McFarland scale, in accordance with the recommendations of the NCCLS. Briefly, 1 mL of penicillin solution at each concentration plus 1 mL of bacterial inoculum were incubated at 36 ± 1°C for 24 h, and the MIC was determined. The MBC was then found by the method of James.[10] A 0.1 mL volume from each tube showing no evident growth was transferred on to 1% horse blood agar plates (frozen–thawed laked erythrocytes) and 0.5 U/mL β-lactamase (Sigma). The plates were incubated for 24 h at 36 ± 1°C and the MBC was determined.

Glycocalyx production

Qualitative assays were used with all 160 isolates of *S. mutans* to detect the production of glycocalyx *in vitro*, according to the method of Molisch.[11] Bacteria from Wilkins–Chalgren agar (Oxoid) were inoculated in 4 mL of fetal bovine serum (Bio Whittaker, Heidelberg, Germany), incubated for 48 h at 36 ± 1°C and then harvested by centrifugation at 950**g** for 10 min. The pellet was resuspended in 4 mL of 0.9% saline solution and gently sonicated for 30 s. After centrifugation, two drops of α-naphthol (Sigma) diluted to 10% with absolute ethanol were added to 0.5 mL of supernatant and 0.5 mL of sulphuric acid (Panreac, Barcelona, Spain). Tests were considered positive when a purple–red interface appeared between the two solutions. As a negative control we used a strain of *Proteus mirabilis* known to be a non-producer of glycocalyx. *S. mutans* strain OMZ176 (now *Streptococcus sobrinus*) was used as a positive control.

Statistical analyses

The results were compared using the chi-squared test and analysis of variance, by means of the R-Sigma statistical program (Horus Hardware, Madrid, Spain). All assays were repeated several times to check the consistency of the results.

Results

The MIC_{50}, MIC_{90} and mean MIC values for the antibiotics tested are shown in the Table for the 160 isolates of *S. mutans*. In all isolates, the MICs were lower than the breakpoint for resistance. Only ten isolates showed reduced sensitivity to amoxycillin (four with a MIC of 0.25 mg/L and six with a MIC of 0.5 mg/L). Two isolates showed reduced sensitivity to erythromycin and clindamycin (MIC = 1 mg/L).

We found no high-level resistance to gentamicin, tobramycin, kanamycin or amikacin at any of the concentrations tested. However, 26 isolates (16.3%) were resistant to streptomycin at 500 mg/L, 16 (10%) at 1000 mg/L and eight (5%) at 2000 mg/L.

Four isolates of *S. mutans* (2.5%) were penicillin-tolerant. Two of these had an MIC of 0.03 mg/L and an MBC of ⩾1 mg/L; in the other two, the MIC was 0.25 mg/L and the MBC ⩾8.0 mg/L.

Glycocalyx production was found in 94 of the 160 isolates (58.8%). Analysis of variance failed to substantiate a significant relationship between glycocalyx production and MIC for most antibiotics, except for increased susceptibility to clindamycin and vancomycin ($P < 0.001$ in both cases). Likewise, chi-squared tests failed to detect any significant relationships between the results for the different parameters.

Discussion

The initiation and maintenance of subacute endocarditis is influenced by several characteristics of the microorganism involved, such as its capacity to produce glycocalyx, susceptibility to antibiotics, high-level resistance to aminoglycosides and penicillin tolerance. This makes it necessary to identify these features in *S. mutans*, a species often involved in such infections. As *S. mutans* is the main pathogen found in connection with dental caries and is present in the oral cavity in most humans, the risk of bacteraemia caused by this microorganism after dental procedures is high.[3]

Aminoglycosides, especially streptomycin and gentamicin, are frequently used to treat endocarditis caused by viridans streptococci. These antibiotics are often used in conjunction with penicillin because of the synergic effect found both *in vitro* and *in vivo*.[4] Other aminoglycosides, such as tobramycin or kanamycin, are rarely used to treat endocarditis, because they show no greater synergic effects when combined with penicillin.[12]

Different studies have used different breakpoints for

Table. MIC (mg/L) of several antibiotics for 160 isolates of *S. mutans*

	Range	Mean	MIC_{50}	MIC_{90}
Amoxycillin	0.003–0.5	0.06	0.02	0.06
Cefazolin	0.007–1	0.08	0.03	0.07
Erythromycin	0.007–1	0.08	0.03	0.12
Clindamycin	0.007–1	0.07	0.03	0.06
Imipenem	0.003–1	0.09	0.01	0.17
Vancomycin	0.03–1	0.46	0.34	0.73
Teicoplanin	0.06–1	0.56	0.38	0.85

high-level resistance, namely ⩾500 mg/L,[13] ⩾1000 mg/L or even ⩾2000 mg/L.[4,7] Although it is difficult to define the concentration at which high-level resistance to a particular antibiotic appears, it is advisable to use as a breakpoint the MIC at which synergy between penicillin and the aminoglycoside disappears and therapeutic effectiveness is lost.[4]

The appearance of high-level resistance to aminoglycosides is usually mediated by acetyl-, adenyl- or phosphotransferase enzymes that modify the antibiotic.[7,14] Although this mechanism of resistance is the most widespread, a ribosomal mechanism of streptomycin resistance has also been described and found to depend on a single aberrant protein (S12) of the smaller ribosomal subunit.[15,16]

The rates of high-level resistance to streptomycin we found in *S. mutans* did not differ significantly from those in earlier reports of viridans streptococci, in which resistance was detected in 2–7.9% of the isolates tested.[4,17] The absence of cross-resistance to other aminoglycosides among the isolates we tested suggests that resistance may have arisen via the adenyltransferase pathway, which inactivates only streptomycin, as reported in *Enterococcus* spp.[7]

The term 'tolerance' is used when the MBC is significantly higher than the MIC (MBC/MIC ratio ⩾ 32).[18,19] Tolerant bacteria are killed more slowly than non-tolerant cells with the same MIC.[18] Tolerance to β-lactams is related to a deficit in murein hydrolase, an enzyme involved in processes of bacterial lysis that take place after the β-lactam antibiotic has bound to penicillin-binding proteins on the bacterial wall.[5,18,20,21] This phenomenon can be studied *in vitro*, although it is important to develop standardized methods to ensure reproducibility of the results, and to facilitate comparisons between studies.[9,21]

Tolerance is especially widespread among streptococci from gingival plaque and in blood cultures of samples obtained after dental extractions.[22] Tolerant strains have also been reported in Spain among bacteria that cause endocarditis.[18] The percentages of tolerant strains are largest in *Streptococcus sanguis*, especially in type I,[23] *Streptococcus mitior* (currently designated *Streptococcus mitis*) and *S. mutans*, which are the species most frequently involved in the development of endocarditis.[19] Although the appearance of tolerance was initially thought to have no clinical repercussions,[24] it may be associated with the failure of penicillin treatment in diseases such as endocarditis;[9,20] tolerant isolates show weaker responses to penicillin, even when it is administered in conjunction with streptomycin.[25,26] Penicillin tolerance is also a factor in the failure of prophylaxis with a single dose of amoxycillin in patients at risk of developing endocarditis.[5,19,26]

Of the 160 isolates we studied, only four (2.5%) had an MBC/MIC ratio of >32; this proportion is lower than the figures given in earlier studies.[5,22,27]

Most streptococci involved in subacute endocarditis are sensitive to antibiotics commonly used both for prophylaxis and for treatment.[28–30] β-Lactams have classically constituted the basis of therapy against this infection, as the causal organisms continue to show excellent levels of susceptibility to these antibiotics.[17,28] Another group of drugs also found to be effective against subacute endocarditis comprises bacteriostatic agents such as erythromycin and clindamycin, which are used in prophylaxis against bacteraemia after dental extraction.[31,32] In contrast with drugs that lead to bacterial lysis, bacteriostatic agents are thought to prevent bacteraemia by inhibiting the adherence of streptococci to the heart valves.[33,34] Vancomycin and teicoplanin, used in people who are allergic to penicillin, are useful in both the prophylaxis and treatment of endocarditis.[35,36] Imipenem and other carbapenems are active both alone and in combination with aminoglycosides in the treatment of streptococcal endocarditis and may therefore constitute an alternative in patients who are allergic to penicillin.[36–39] These drugs may also be useful in hospitalized patients with endocarditis.

S. mutans is usually one of the most sensitive of all oral streptococci to antibiotics, both in isolates from dental plaque and in patients with infectious endocarditis.[28,40,41] The results of our susceptibility tests were similar to those found by other authors for oral streptococci. Our data were also in agreement with the findings of earlier studies in our setting, which showed that clindamycin was more effective than erythromycin against *S. mutans*, and support the choice of the former antibiotic in the prophylaxis against streptococcal endocarditis.[29,30]

A crucial aspect of the pathogenesis of endocarditis caused by viridans streptococci is the interaction between the bacteria and the damaged heart valve. The ability of some members of this group, notably *S. mutans*, *S. sanguis* and *S. mitis*, to adhere to the extracellular matrix of the cardiac endothelium partly accounts for the high incidence of endocarditis these species cause.[6,42] This trait is associated with high levels of glycocalyx production.[42] Streptococci able to form large amounts of glycocalyx form larger vegetations than strains without this ability;[43] this may be a direct consequence of the larger numbers of bacterial cells adhering to the valvular surface, or to increased platelet and fibrin deposition.[44] Within the vegetation there are two populations of bacteria: resting cells, located more deeply, and growing cells, located superficially. Only the latter population is susceptible to treatment with β-lactams and aminoglycosides; hence, the thicker the vegetation, the more difficult it is to eliminate with antibiotic treatment.[45] Moreover, the formation of large cardiac vegetations makes it difficult for the antibiotic to penetrate them fully and also interferes with the host's defence mechanisms (e.g., complement, antibodies and phagocytic cells). This makes the lesion more likely to persist and makes cure more difficult.[9,43,45,46]

The in-vitro production of glycocalyx reflects the ability of a strain to produce this component in the living heart and detection of this ability is thus a potentially valuable

predictor of pathogenicity.[42] Strains that cause endocarditis vary in the amount of exopolysaccharide they produce;[43] moreover, qualitative as well as quantitative differences in the glycocalyx have been found between streptococci that cause the disease and species not associated with this infection.[47] We detected glycocalyx formation in 58.8% of the isolates tested. However, because the glycocalyx may be lost in the course of subculture, the in-vivo figure may be higher. Statistical analyses confirmed earlier findings that suggested no difference in susceptibility *in vitro* (except for clindamycin and vancomycin) between isolates that produce exopolysaccharide and those that do not.[43] Failure of treatment was not related to changes in MIC, but rather to lower accessibility of the vegetation to the antibiotic in the cardiac focus of infection. The apparent association of glycocalyx production with susceptibility to clindamycin and vancomycin is hard to explain, although it may have been due to chance.

In conclusion, we found that *S. mutans* was susceptible to antibiotics commonly used in the prophylaxis and treatment of endocarditis. The frequencies of penicillin tolerance and high-level resistance to aminoglycosides among the isolates we investigated were low. However, the large percentage of isolates able to produce glycocalyx *in vitro* may make this microorganism difficult to eradicate in infectious endocarditis.

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References

1. Ullman, R. F., Miller, S. J., Strampfer, M. J. & Cunha, B. A. (1988). *Streptococcus mutans* endocarditis: report of three cases and review of the literature. *Heart and Lung* **17**, 209–12.

2. Ullman, R. F., Strampfer, M. J. & Cunha, B. A. (1988). *Streptococcus mutans* vertebral osteomyelitis. *Heart and Lung* **17**, 319–21.

3. Lu, J. R. & Wu, H. C. (1992). Morphologic and biochemical characteristics of *viridans* streptococci isolated from dental plaque. *Chung Hua Min Kuo Wei Sheng Wu Chi Mien I Hsueh Tsa Chih* **25**, 91–100.

4. Enzler, M. J., Rouse, M. S., Henry, N. K., Geraci, J. E. & Wilson, W. R. (1987). *In vitro* and *in vivo* studies of streptomycin-resistant, penicillin-susceptible streptococci from patients with infective endocarditis. *Journal of Infectious Diseases* **155**, 954–8.

5. Holbrook, W. P., Olafsdottir, D., Magnusson, H. B. & Benediktsdottir, E. (1988). Penicillin tolerance among oral streptococci. *Journal of Medical Microbiology* **27**, 17–22.

6. Tart, R. C. & Van de Rijn, I. (1991). Analysis of adherence of *Streptococcus defectivus* and endocarditis associated streptococci to extracellular matrix. *Infection and Immunology* **59**, 857–62.

7. Chen, H. Y. & Williams, J. D. (1985). Transferable resistance and aminoglycoside-modifying enzymes in enterococci. *Journal of Medical Microbiology* **20**, 187–96.

8. Maiden, M. F. J., Lai, C. H. & Tanner, A. (1992). Characteristics of oral Gram-positive bacteria. In *Contemporary Oral Microbiology and Immunology* (Slots, J. & Taubman, M. A., Eds), pp. 342–72. Mosby Year Book, St Louis, MO.

9. National Committee for Clinical Laboratory Standards. (1993). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. *Third edition, Approved Standard M7–A3.* NCCLS, Villanova, PA.

10. James, P. A. (1990). Comparison of four methods for the determination of MIC and MCB of penicillin for *viridans* streptococci and the implications for penicillin tolerance. *Journal of Antimicrobial Chemotherapy* **25**, 209–16.

11. Dall, L. & Herndon, B. (1989). Quantitative assay of glycocalyx produced by *viridans* group streptococci that cause endocarditis. *Journal of Clinical Microbiology* **27**, 2039–41.

12. Watanakunakorn, C. & Glotzbecker, C. (1977). Synergism with aminoglycosides of penicillin, ampicillin and vancomycin against non-enterococcal group D streptococci and *viridans* streptococci. *Journal of Medical Microbiology* **10**, 133–8.

13. Potgieter, E., Carmichael, M., Koornhof, H. J. & Chalkley, L. J. (1992). *In vitro* antimicrobial susceptibility of *viridans* streptococci isolated from blood cultures. *European Journal of Clinical Microbiology & Infectious Diseases* **11**, 543–6.

14. Collatz, E., Carlier, C. & Courvalin, P. (1984). Characterisation of high level aminoglycoside resistance in a strain of *Streptococcus pneumoniae*. *Journal of General Microbiology* **130**, 1665–71.

15. Hummel, H., Piepersberg, W. & Bock, A. (1980). 30S subunit mutations relieving restriction of ribosomal misreading caused by L6 mutations. *Molecular and General Genetics* **179**, 147–53.

16. Lietman, P. S. (1991). Aminoglucósidos y espectinomicina; aminociclitoles. In *Enfermedades Infecciosas: Principio y Práctica* (Mandell, G., Douglas, R. & Bennett, J., Eds), pp. 281–95. Panamericana, Buenos Aires.

17. Etienne, J., Gruer, L. D. & Fleurette, J. (1984). Antibiotic susceptibility of streptococcal strains associated with infective endocarditis. *European Heart Journal* **5**, *Suppl. C*, 33–7.

18. Pujadas, R. & Escrivá, E. (1987). Profilaxis de la endocarditis estreptocócica: ¿nuevos criterios en base a la tolerancia antibiótica? *Enfermedades Infecciosas y Microbiología Clínica* **5**, 261–4.

19. Powley, L., Meeson, J. & Greenwood, D. (1989). Tolerance to penicillin in streptococci of *viridans* group. *Journal of Clinical Pathology* **42**, 77–80.

20. Slater, G. J. & Greenwood, D. (1983). Detection of penicillin tolerance in streptococci. *Journal of Clinical Pathology* **36**, 1353–6.

21. López, R. & García, E. (1989). Tolerancia antibiótica. *Revista Española de Quimioterapia* **11**, 119–24.

22. Holloway, Y., Dankert, J. & Hess, J. (1980). Penicillin tolerance and bacterial endocarditis. *Lancet i*, 589.

23. James, P. A., Young, S. E. & White, D. G. (1991). Incidence of penicillin tolerance among blood culture isolates of *Streptococcus sanguis*, 1987–88. *Journal of Clinical Pathology* **44**, 160–3.

24. Anderson, A. W. & Cruickshank, J. G. (1982). Endocarditis due to viridans-type streptococci tolerant to beta-lactam antibiotics: therapeutic problems. *British Medical Journal* **285**, 854.

25. Wilson, W. R., Zak, O. & Sande, M. A. (1985). Penicillin therapy for treatment of experimental endocarditis caused by *viridans* streptococci in animals. *Journal of Infectious Diseases* **151**, 1028–33.

26. Meeson, J., McColm, A. A., Acred, P. & Greenwood, D. (1990). Differential response to benzylpenicillin *in vivo* of tolerant and non-tolerant variants of *Streptococcus sanguis* II. *Journal of Antimicrobial Chemotherapy* **25**, 103–9.

27. van der Meer, J. T., van Vianen, W., Hu, E., van Leeuwen, W. B., Valkenburg, H. A., Thompson, J. *et al.* (1991). Distribution, antibiotic susceptibility and tolerance of bacterial isolates in culture-positive cases of endocarditis in The Netherlands. *European Journal of Clinical Microbiology & Infectious Diseases* **10**, 728–34.

28. Bourgault, A. M., Wilson, W. R. & Washington, J. A. (1979). Antimicrobial susceptibilities of species of *viridans* streptococci. *Journal of Infectious Diseases* **140**, 316–21.

29. Liébana, J., Castillo, A., Peis, J., Baca, P. & Piédrola, G. (1991). Antimicrobial susceptibility of 1042 strains of *Streptococcus mutans* and *Streptococcus sobrinus*: comparison from 1985 to 1989. *Oral Microbiology and Immunology* **6**, 146–50.

30. Liébana, J., Parejo, E., Castillo, A., Gutiérrez, J., García-Mendoza, A. & Piédrola, G. (1993). *In vitro* activity of macrolides and lincosamides against oral streptococci: a therapeutic alternative in prophylaxis for infective endocarditis. *International Journal of Antimicrobial Agents* **2**, 255–61.

31. Glauser, M. P. & Francioli, P. (1982). Successful prophylaxis against experimental streptococcal endocarditis with bacteriostatic antibiotics. *Journal of Infectious Diseases* **146**, 806–10.

32. Shanson, D. C., Akash, S., Harris, M. & Tadayon, M. (1985). Erythromycin stearate, 1.5 mg, for the oral prophylaxis of streptococcal bacteraemia in patients undergoing dental extraction: efficacy and tolerance. *Journal of Antimicrobial Chemotherapy* **15**, 83–90.

33. Etienne, J., Coulet, M., Brun, Y., Blanchon, J. F., Demoux, F. & Fleurette, J. (1988). Susceptibilities of streptococcal strains associated with infective endocarditis to nine antibiotics. *Chemotherapy* **34**, 113–6.

34. Dall, L., Keilhofner, M., Herndon, B., Barnes, W. & Lane, J. (1990). Clindamycin effect on glycocalyx production in experimental *viridans* streptococcal endocarditis. *Journal of Infectious Diseases* **161**, 1221–4.

35. Shanson, D. C., Shehata, A., Tadayon, M. & Harris, M. (1987). Comparison of intravenous teicoplanin with intramuscular amoxycillin for the prophylaxis of streptococcal bacteraemia in dental patients. *Journal of Antimicrobial Chemotherapy* **20**, 85–93.

36. Venditti, M., Gelfusa, V., Serra, P., Brandimarte, C., Micozzi, A. & Martino, P. (1992). 4 week treatment of streptococcal native valve endocarditis with high-dose teicoplanin. *Antimicrobial Agents & Chemotherapy* **36**, 723–6.

37. Horstkotte, D. & Rosin, H. (1984). Therapy and prevention of infectious endocarditis. *Schweitzerische Medizinische Wochenschrift* **114**, 1575–86.

38. Dickinson, G., Rodríguez, K., Arcey, S., Alea, A. & Greenman, R. (1985). Efficacy of imipenem/cilastatin in endocarditis. *American Journal of Medicine* **78**, 117–21.

39. Dornbusch, K., Henning, C. & Linden, E. (1989). *In vitro* activity of the new penems FCE 22101 and FCE 24362 alone or in combination with aminoglycosides against streptococci isolated from patients with endocarditis. *Journal of Antimicrobial Chemotherapy* **23**, *Suppl. C*, 109–17.

40. Ferretti, J. J. & Ward, M. (1976). Susceptibility of *Streptococcus mutans* to antimicrobial agents. *Antimicrobial Agents & Chemotherapy* **10**, 274–6.

41. Baker, C. N. & Thornsberry, C. (1974). Antimicrobial susceptibility of *Streptococcus mutans* isolated from patients with endocarditis. *Antimicrobial Agents and Chemotherapy* **5**, 268–71.

42. Dall, L. H. & Herndon, B. L. (1990). Association of cell-adherent glycocalyx and endocarditis production by *viridans* group streptococci. *Journal of Clinical Microbiology* **28**, 1698–700.

43. Pulliam, L., Dall, L., Inokuchi, S., Wilson, W., Hadley, W. K. & Mills, J. (1985). Effects of exopolysaccharide production by *viridans* streptococci on penicillin therapy of experimental endocarditis. *Journal of Infectious Diseases* **151**, 153–6.

44. Hook, E. W. & Sande, M. A. (1974). Role of the vegetation in experimental *Streptococcus viridans* endocarditis. *Infection and Immunology* **10**, 1433–8.

45. Dall, L., Barnes, W. G., Lanes, J. W. & Mills J. (1987). Enzymatic modification of glycocalyx in the treatment of experimental endocarditis due to *viridans* streptococci. *Journal of Infectious Diseases* **156**, 736–40.

46. Yersin, B. R., Glauser, M. P. & Freedman, L. R. (1982). Effect of nitrogen mustard on natural history of right-sided streptococcal endocarditis in rabbits: role for cellular host defences. *Infection and Immunology* **35**, 320–5.

47. Dall, L. H., Herndon, B. L. & Smith, R. (1993). Reactivity of the glycocalyx of endocarditis-producing *viridans* group streptococci. *Diagnostic Microbiology and Infectious Diseases* **17**, 259–64.

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