Varidans Streptococci in the Oral Flora of the Patients at Risk for Infective Endocarditis : Species and Penicillin Susceptibilities

PANTIP CHAYAKUL, M.D.*, TADA YIPINTSOI, M.B.*,
RATRI HORTIWAKUL, M.Sc.*, NATNICHA INGVIYA, M.Sc.**

Abstract

This study examined viridans streptococci (SV) in oral flora of patients at risk of infective endocarditis (IE) by obtaining 57 isolates from gum-tooth margin swabs of 3 groups of patients. Penicillin minimal inhibitory concentrations (MICs) were determined by E test. Group 1 was from 30 patients with prosthetic heart valves who did not receive antimicrobial agents within 3 months prior to recruitment. Group 2 consisted of 21 patients with known rheumatic heart diseases who regularly received penicillin prophylaxis. Group 3 was 2 patients with IE caused by SV in whom dental swabs were performed before and on the third day of treatment. *Streptococcus mitis* was found most frequently (49% among 51 oral isolates of SV from patients in group 1 and 2) and in both blood cultures of IE patients. Ninety-four per cent of the isolates were penicillin-susceptible and the rest were intermediate-resistant (IR) equally distributed in both groups 1 and 2. In 2 patients with IE, oral SV obtained after 3 days of penicillin therapy had MICs rising 3 and 5 folds of the baselines. It is suggested that surveillance of susceptibilities of oral SV in patients at risk for IE should be kept up since this will affect the dose and type of antimicrobial agents in IE prophylaxis.

Key word : Viridans Streptococci, Oral Flora, Infective Endocarditis

**CHAYAKUL P, YIPINTSOI T,
HORTIWAKUL R, INGVIYA N
J Med Assoc Thai 2002; 85: 825-830**

* Department of Medicine
** Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand.

Community-acquired infective endocarditis (IE) remains a common disease and may cause serious complications with a mortality rate as high as 33 per cent[1]. The incidence of IE at Songklanagarind Hospital averaged about 20 cases per 20-30 thousand hospital admissions per year which puts this at the lower end of previous reported ranges of 0.8 to 5.4 cases per 1,000 admissions in 10 large surveys[2]. Community-acquired IE is most commonly associated with viridans streptococci in oral flora[2]. The principal species comprising these streptococci are *S. mutans*, *S. salivarius*, *S. mitis*, "*S. milleri*" (which encompass *S. anginosus*, *S. constellatus*, and *S. intermedius*) and *S. sanguis*[3]. If these are highly penicillin-sensitive then decision in treatment[4] and prophylaxis[5] should not be a problem. However, resistant strains have been reported among those on penicillin prophylaxis against acute rheumatic fever[6-9]. and also from healthy and diseased children[10-13]. Thailand is one of the developing countries where over-the-counter antimicrobial agent usage is rife, hence the behaviour of the oral flora to antimicrobial agents may be altered. This study looked at subsets of a population with regard to bacterial species in the oral flora that may give rise to community-acquired IE.

MATERIAL AND METHOD

Patients and test organisms

This study was conducted between March and August 2000 at Songklanagarind Hospital (1,500-beds tertiary care hospital), Prince of Songkla University and was approved by the Faculty's Ethics Committee. There were three groups of subjects. Group 1 were adult out-patients with prosthetic heart valves and did not knowingly receive antimicrobial agents within 3 months prior to recruitment. Group 2 consisted of paediatric patients with known rheumatic heart diseases who regularly received penicillin prophylaxis. Group 3 were 2 hospitalized patients with infective endocarditis caused by viridans streptococci, in whom the authors were able to obtain swabs of the oral cavity before and on the third day of treatment. In every subject, the specimen was obtained by rubbing the gum-tooth margin with a sterile swab. The specimen was kept in Stuart transport media and was sent to the laboratory for culture on blood agar and chocolate agar. From the blood agar plate, the authors selected one colony with characteristic morphology of viridans streptococcus to identify the species by using API 20 Strep® kit (bioMerieux, Mercy-l'Etoile, France)[14].

Antimicrobial susceptibility testing

Penicillin MICs were determined by using E test strips (AB Biodisk®, Sweden). E test uses a strip impregnated with a gradient of antimicrobial agent[15]. The inocula were prepared by emulsification of colonies from an overnight agar plate in a Mueller-Hinton broth to achieve a 0.5 McFarland turbidity standard. These were then inoculated onto Mueller-Hinton agar. Strips were applied on the surface. Plates were incubated at 35°C for 18-24 hours. Isolates were classified as penicillin G susceptibility according to the NCCLS criteria[16] : susceptible, MIC≤0.12 mg/L; intermediate-resistant, MIC 0.25 to 2 mg/L; and high level resistant, MIC≥4 mg/L. Daily test controls were performed by using *Streptococcus pneumoniae* ATCC 49619.

RESULTS

Of the 53 patients in the study, there were 30 adults in group 1 of whom 12 (40%) were males. The median age was 41 years with a range of 21-66 years. Group 2 consisted of 21 adolescents with a median age of 17 years, ranging from 5-27 years. Eighteen patients were on intramuscular benzathine penicillin monthly for durations of 2 months to 13 years (median 5.2 years), and the other 3 were on oral penicillin V 500 mg twice daily for 2, 7 and 8 years. In group 3, the two males with viridans streptococcal endocarditis were 30 and 13 years-old.

Among the 51 isolates from dental swabs of patients in groups 1 and 2, the following species predominate: 25 (49.2%) *Streptococcus mitis*, 14 (27.5%) *Streptococcus sanguis*, and 7 (13.7%) *Gemella morbillorum*, (Table 1). Penicillin MICs of each group are also shown. There were none with high-level resistance, i.e. penicillin MIC of 4 mg/L. There was only one intermediate–resistant isolate in group 2 and this was *S. mutans* showing the highest penicillin MIC (0.75 mg/L) among the oral isolates in both groups. This was from a 7 year-old boy who had received benzathine penicillin for 2 years. Among the 3 patients who were on oral penicillin V daily, the oral isolates were *S. mitis* in 2 patients (MICs of 0.016 and 0.064 mg/L) and *S. sanguis* (MIC of 0.032 mg/L) in another patient. In two patients with IE, the blood and initial

Table 1. Species and penicillin susceptibilities of oral viridans streptococci.

Organism	Group I (30)			Group II (21)		
	No.	S	IR	No.	S	IR
S. mitis	13	13	0	12	12	0
S. sanguis	8	7	1	6	6	0
S. salivarius	4	3	1	0	0	0
S. mutans	0	0	0	1	0	1
G. morbillorum	5	5	0	2	2	0
Total	30	28	2	21	20	1

No = Number of isolates, S = Susceptible, IR = Intermediate-resistant,
S. = Streptococcus, G. = Gemella

Table 2. Comparison of the *in vitro* susceptibilities of viridans streptococci to penicillin among the 2 groups.

Source of isolates	No. of Isolates				MIC		
	S	%	IR	%	50%	90%	range
Group I	28	92.3	2	6.7	0.047	0.125	0.008 - 0.5
Group II	20	95.2	1	4.8	0.032	0.047	0.002 - 0.75

S = susceptible (MIC≤0.12 mg/L), IR = intermediate resistant (MIC 0.25 - 2 mg/L)

dental swab cultures grew *S. mitis* with penicillin MICs of 0.016 and 0.012 mg/L in the first patient and 0.125 mg/L in both specimens in the second patient. On the third day of penicillin therapy, dental swabs grew *S. salivarius* with penicillin MIC of 0.5 mg/L in the first patient and *S. mitis* with MIC of 1 mg/L in the second. These 2 isolates from the oral flora post treatment were classified as showing intermediate-resistance to penicillin. QC strain yielded penicillin MIC range of 0.016-0.064 mg/L.

DISCUSSION

The most common strain from the oral flora

S. mitis and *S. sanguis* occurred frequently. In the present series these were seen in 49 and 28 per cent, respectively. These numbers were not dissimilar to the 51 and 29 per cent reported by Mogi et al[10] from oral swabs of Japanese children with upper respiratory tract infections and from those with oncohematologic diseases. In terms of blood culture, both the IE patients in the present study had *S. mitis*. *S. mitis* was present in 30-100 per cent of viridans streptococcal bacteremia in neutropenic patients[17-19], and was 37 per cent of 46 patients with viridans streptococcal endocarditis[20]. However, the dominance of *S. mitis* was not so in every study. A report on 49 patients with streptococcal IE found that *S. sanguis* and *S. oralis* were most common[21]. The interest in *S. sanguis* is that it could produce extracellular dextran from sucrose[22], and dextran has been shown to promote adhesion of bacteria to thrombus in endocarditis in rabbits[23]. *S. sanguis* also could aggregate human platelets *in vitro* more effectively than other streptococcal species[24].

Oral isolates and penicillin resistance

Several reports showed that the prevalence of viridans streptococci with intermediate or high-level resistance to penicillin can be 40-62.5 per cent in healthy children[10,11], healthy adults[12] and in children at risk for IE due to cardiac diseases[13]. Among the presented patients, the prevalence of oral isolates resistant to penicillin were low. There are very few reports supporting such a low prevalence (≤ 5% in adult leukaemic patients[11] and 0% in normal children[9]). The authors were not able to find fault with the present technique. The method of MIC determination using the E test is accepted as a standard method and has been proven to be compatable with broth microdilution test[25].

Oral isolates among those on chronic penicillin prophylaxis

There has been much discussion on whether penicillin-resistant viridans streptococci are more common among those on penicillin prophylaxis against rheumatic fever[6-9]. Children who had been on oral penicillin prophylaxis for rheumatic fever had been reported to acquire resistant viridans streptococci[6-9]. The authors have only 1 with intermediate resistance. Perhaps the route of administration of prophylaxis played a role in producing resistance. Sprunt et al[9] showed that 75 per cent of the pharynx of 72 patients on oral penicillin prophylaxis grew alpha-streptococci resistant to at least 1 unit /ml of penicillin, while only 15 per cent were so among 47 patients receiving monthly benzathine penicillin and none among the 53 control subjects.

In the absence of penicillin-resistant strain, the presented population with or without chronic penicillin prophylaxis could be well covered against IE by a single 2 grams of oral amoxicillin as recommended by the American Heart Association[5] to be given one hour before a dental procedure. The mean serum amoxicillin levels at 2 and 6 hours after 2 grams of oral amoxicillin were reported to be 12.8 and 2.9 mg/L, respectively[26]. However, one still has to answer how to prophylax in instances of a repeat dental procedure or to prophylax a dental procedure while on IE therapy. With regard to the latter, the oral flora of the 2 presented IE patients changed their resistant status to intermediate after 3 days of IE treatment (18 million units/day of penicillin G) which should still be well covered by conventional prophylaxis for dental manipulation. In another patient with IE caused by *S. mitis* who was not included in this study, *S. mitis* that grew from her oral swab cultures on day 0, day 14 (the last day of treatment), day 21 and day 28 had penicillin MICs of 0.125, 0.25, 0.19 and 0.125 mg/L, respectively. However, the choice of prophylaxis in instances of repeated procedures (e.g. dental manipulation every 3 days in IE susceptible patients) is difficult. Oral antimicrobial agent itself can induce resistant strains in the oral flora within 6 hours[27] or with repeated doses[28]. Therefore, the authors' recommendation would be a) do the dental procedure 9-14 days apart[5], or b) change to a different unrelated antimicrobial agent[5]. The authors' last advice is to maintain monitoring susceptibilities of oral viridans streptococci in patients at risk for IE since this may affect the recommendation for IE prophylaxis.

ACKNOWLEDGEMENTS

This work was funded by the Faculty of Medicine, Prince of Songkla University. The authors wish to thank Woravut Jintapakorn, M.D., Noppadol Chamnarnphol, M.D. and Walla Jirathamopas, RN. from the Division of Cardiology, Department of Medicine and Somkiat Sopontammarak, M.D. from the Division of Cardiology, Department of Paediatrics for their co-operation and for helping to identify and contact the patients.

(Received for publication on March 10, 2002)

REFERENCES

1. Nissen H, Nielsen PF, Federicksen M, et al. Native valve endocarditis in the general population: A 10 years survey of the clinical picture during the 1980s. Eur Heart J 1992; 13: 872-7.
2. Bayer AS, Scheld WM. Endocarditis and intravascular infections. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 5th ed. Philadelphia: Churchill Livingstone, 2000: 857-902.
3. Rouoff KL. Streptococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yalken RH, eds. Manual of clinical microbiology. 6th ed. Washington DC: ASM Press, 1995: 299-307.
4. Wilson WR, Karchmer AW, Dajani AJ, et al. Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylococci, and HACEK microorganisms. JAMA 1995; 274: 1706-13.
5. Dajani AS, Tauber KA, Wilson W, et al. Prevention of bacterial endocarditis: Recommendations by the American Heart Association. Circulation 1997; 96: 358-66.
6. David S. Penicillin-resistant streptococci. N Engl J Med 1979; 300: 500-1.
7. Pulliam L, Hadley WK. Resistance of viridans streptococci to penicillin. N Engl J Med 1979; 300;

1442.
8. Parrillo JE, Borst GC, Mazur MH, et al. Endocarditis due to resistant viridans streptococci during oral penicillin chemoprophylaxis. N Engl J Med 1979; 300: 296-300.
9. Sprunt K, Redman W, Leidy G. Penicillin resistant alpha streptococci in the pharynx of patients given oral penicillin. Pediatrics 1968; 429: 957-68.
10. Mogi A, Nishi JI, Yoshinaga M, et al. Increased prevalence of penicillin-resistant viridans group streptococci in Japanese children with upper respiratory infection treated by beta-lactam agents and in those with onco-hematologic diseases. Pediatr Infect Dis J 1997; 16: 1140-4.
11. Guiot HFL, Corel LJA, Vossen JM. Prevalence of penicillin-resistant viridans streptococci in healthy children and in patients with malignant haematological disorders. Eur J Clin Microbiol Infect Dis 1994; 13 : 645-50.
12. Alvarez M, Alvarez ME, Maiz L, Asensio A, Baquero F, Canton R. Antimicrobial susceptibility profiles of oropharyngeal viridans group streptococci isolates from cystic fibrosis and non-cystic fibrosis patients. Microb Drug Resist 1996; 4: 123-8.
13. Nishi JI, Yoshinaga M, Nomura Y. Prevalence of penicillin-resistant viridans streptococci in the oral flora of Japanese children at risk for infective endocarditis. Circulation 1999; 99: 1274-5.
14. Appelbaum PC, Chaurushiya PS, Jacobs MR, Duffett A. Evaluation of the Rapid Strep System for species identification of the viridans streptococci. J Clin Microbiol 1984; 19: 588-91.
15. Jacobs MR, Bajaksonzian S, Appelbaum PC, Bolmstrom A. Evaluation of the E-test for susceptibility testing of pneumococcus. Diagn Microbiol Infect Dis 1992; 15: 473-8.
16. National Committee for Clinical Laboratory Standards 2001. Performance standards for antimicrobial susceptibility testing. Eleventh information. Supplement M100 - S11. National Committee for Clinical Laboratory Standards. Pennsylvania, USA.
17. Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. Am J Med 1994; 97: 256-64.
18. Classen DC, Burke JP, Ford CD, et al. Streptococcus mitis sepsis in bone marrow transplant patients receiving oral antimicrobial prophylaxis. Am J Med 1990; 89: 441-6.
19. Villablanca JG, Steiner M, Kersey J, et al. The clinical spectrum of infections with viridans streptococci in bone marrow transplant patients. Bone Marrow Transplant 1990; 5: 387-93.
20. Rapeport KB, Giron JA, Rosner F. Streptococcus mitis endocarditis: Report of 17 cases. Arch Intern Med 1986; 146: 2361-3.
21. Douglas CWI, Heath J, Hampton KK, Preston FE. Identity of viridans streptococci isolated from cases of infective endocarditis. J Med Microbiol 1995; 39: 179-82.
22. Gibbons RJ, Banghart SB. Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. Arch Oral Biol 1967; 12: 11-23.
23. Baddour LM, Christensen GD, Lowrance JH, Simpson WA. Pathogenesis of experimental endocarditis. Rev Infect Dis 1989; 11: 3452-63.
24. Douglas CWI, Brown PR, Preston FE. Platelet aggregation by oral streptococci. FEMS Microbiol Lett 1990; 72: 63-8.
25. Brown DFJ, Brown L. Evaluation of the E test: A novel method of quantifying antimicrobial activity. J Antimicrob Agents Chemother 1994; 38: 2340-5.
26. Dajani AS, Bawden RE, Berry MC. Oral amoxicillin as prophylaxis for endocarditis: What is the optimal dose? Clin Infect Dis 1994; 18 : 157-60.
27. Leviner E, Tzukert AA, Benoliel R, Baram O, Sela MN. Development of resistant oral viridans streptococci after administration of prophylactic antibiotics: Time management in the dental treatment of patients susceptible to infective endocarditis. Oral Surg Oral Med Oral Pathol 1987; 60: 414-20.
28. Woodman AJ, Vidic J, Newman HN, Marsh PD. Effect of repeated high dose prophylaxis with amoxicillin on the resident oral flora of adult volunteers. J Med Microbiol 1985; 19: 15-23.

วิริแดนส์ สเตร็พโตค็อคไคในช่องปากผู้ป่วยที่เสี่ยงต่อเยื่อบุหัวใจติดเชื้ออักเสบ ชนิดของสปีชีย์ และความไวต่อเพนิซิลลิน

พรรณทิพย์ ฉายากุล, พ.บ., ธาดา ยิบอินซอย, พ.บ.*,*
ราตรี หอทิวากุล, วท.ม., ณัฐณิชา อิงวิยะ, วท.ม.***

ผู้รายงานได้ศึกษา viridans strptococci (SV) จำนวน 57 isolate ที่ได้จากการป้ายโคนฟันของผู้ป่วยที่เสี่ยงต่อ infective endocarditis (IE) 3 กลุ่ม โดยตรวจหา penicillin MIC ด้วย E test ผู้ป่วยกลุ่มแรก (30 คน) มีลิ้นหัวใจเทียมและไม่ได้รับยาต้านจุลชีพมาก่อนในช่วง 3 เดือนก่อนหน้าการศึกษา กลุ่มที่ 2 (21 คน) มีโรคหัวใจรูมาติค และได้รับ penicillin ป้องกันโรคอยู่ กลุ่มที่ 3 (2 คน) มี IE ที่เกิดจาก SV พบ Streptococcus mitis บ่อยที่สุดจากช่องปากของผู้ป่วย (49% ของเชื้อ 51 isolate) และเป็นเชื้อที่ได้จากเลือดของผู้ป่วย IE ทั้ง 2 ราย 94% ของ SV ในช่องปากไวต่อ penicillin และที่เหลือดื้อแบบ intermediate โดยพบเท่าเทียมกันในผู้ป่วยทั้ง 2 กลุ่ม ในผู้ป่วย IE ทั้ง 2 ราย SV ในช่องปากในวันที่ 3 หลังการรักษามี MIC สูงขึ้น 3 และ 5 เท่าของค่าก่อนรักษา ควรมีการติดตามความไวของ SV ในช่องปากผู้ป่วยที่เสี่ยงต่อ IE เนื่องจากมีผลต่อการเลือกชนิดของยาต้านจุลชีพ และขนาดยาในการป้องกัน IE

คำสำคัญ : เชื้อในช่องปาก, วิริแดนส์ สเตร็พโตค็อคไค, การติดเชื้อของลิ้นหัวใจ

พรรณทิพย์ ฉายากุล, ธาดา ยิบอินซอย,
ราตรี หอทิวากุล, ณัฐณิชา อิงวิยะ
จดหมายเหตุทางแพทย์ ฯ 2545; 85: 825–830

* ภาควิชาอายุรศาสตร์,
** ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์, สงขลา 90110