ISOLATION AND GENOTYPIC COMPARISON OF ORAL STREPTOCOCCI FROM EXPERIMENTAL BITEMARKS

L.M. Borgula,[1] F.G. Robinson,[2] M. Rahimi,[3] K.E.K. Chew,[4] K.R. Birchmeier,[5] S.G. Owens,[6] J.A. Kieser,[3] G.R. Tompkins[3]

1. Moonee Ponds Dental Group, Moonee Ponds, Victoria, Australia.
2. Department of Oral Health Practice, University of Kentucky, Lexington, KY., USA.
3. Department of Oral Sciences and Orthodontics, University of Otago, Dunedin, New Zealand.
4. Team Dental, Christchurch, New Zealand.
5. Department of Oral Biology, Medical College of Georgia, Augusta, GA., USA.
6. Department of Health and Human Performance, Western Carolina University, Cullowhee, NC., USA.

ABSTRACT

The feasibility of recovering and genotypically comparing oral bacteria from bitemarks for forensic purposes was assessed experimentally. Volunteers firmly bit their own upper arms and bitemarks were sampled at intervals to recover viable *Streptococcus* isolates. The recoverability of bacteria decreased over time but an average of more than one thousand viable organisms was recovered 24 hrs after biting, provided the site remained relatively undisturbed. Physical exertion, manual rubbing and application of moisturizing lotion all decreased bacterial recoverability compared to controls. Streptococci could also be recovered from bites inflicted on various fabrics. Genomic profiles (DNA "fingerprints") of bacteria recovered from bitemarks could be identified exclusively with those from the teeth of the individual responsible. These findings suggest that a bacterial genotyping approach to bitemark analysis could have forensic application in situations where the perpetrator's DNA cannot be recovered from an oral contact site. **(J Forensic Odontostomatol 2003;21:23-30)**

Key words: DNA typing, bitemark, oral bacteria, genotype, *Streptococcus*.

INTRODUCTION

Sexually abusive crimes against women and children have been reported with increasing frequency over the last two decades[1] and estimates indicate that between 9% and 24% of women will be assaulted at least once in their lifetime.[2] Bitemarks are associated with both attempted rape and child abuse, and forensic examination of human bitemarks is often a central issue in the identification of the perpetrators of such crimes. Bitemarks, however, are complex injuries involving a number of factors and can be very difficult to analyze,[3] interpretation frequently requiring a degree of subjective judgment which is often challenged.[4] Nevertheless, as biting may be the principal forensic evidence of such attacks, all opportunities to acquire information from such lacerations should be explored. Advances in molecular biological techniques now offer a further dimension to forensic analyses but because human saliva and skin secretions contain nucleases, recovery of human DNA fragments from recent bitemarks may not always be successful.[5]

The human oral cavity maintains a large and varied community of bacteria, many of which are unique to this habitat.[6] The predominant genus is *Streptococcus* which includes several benign species[7] universally present in the human oral cavity and which express adhesins mediating attachment to the salivary macromolecules selectively adsorbed onto the tooth surface.[6] These adhesins facilitate re-colonization of the tooth surfaces within minutes of professional cleaning.[8] Because these bacteria are found on all tooth surfaces, even on those not prone to plaque accumulation such as the incisors,[6] streptococci are likely to be initially present in essentially all bitewounds inflicted by humans. The oral streptococci are genotypically extremely diverse which has limited attempts to speciate and classify this group of bacteria, even by modern molecular

methods. For example, Alam *et al.*,[9] compared 72 isolates by randomly-primed polymerase chain reaction (PCR) and found no two strains with more than 90% similarity. Other PCR-based studies have further emphasized the genotypic variety of the oral streptococci.[10,11] Whereas this diversity has hampered identification and taxonomy of these bacteria it may prove advantageous for forensic purposes.

The aims of this study were: (i) to assess the practicability of recovering oral streptococci from bitemarks inflicted on human skin and clothing; and (ii) to evaluate the feasibility of matching *Streptococcus* isolates recovered from bitemarks with those recovered from the incisor teeth of the perpetrators, using a genomic comparison (DNA "fingerprinting") approach.

MATERIALS AND METHODS

Healthy volunteers bit themselves in the biceps region of the upper arm, maintaining as much pressure as they could tolerate for 10 seconds. This resulted in an imprint lasting for at least ten minutes and often produced mild bruising. Each bitemark was sampled only once and, if not sampled immediately, was covered by the volunteers' clothing until the appropriate time. No sites were bitten twice in the same day and sampling times were arranged such that at least one hour elapsed between each bite inflicted by the same individual. This was to ensure an adequate bacterial load for each bite. Participants were directed not to undertake any strenuous activity nor to interfere with the bitemark (unless specifically directed) between the time of biting and the time of sampling. Bacteria were recovered from bitemarks by swabbing the area with a sterile cotton-tipped applicator moistened in sterile tryptic soy broth (TSB).* The swab was placed in 5 mL of sterile TSB and vortexed vigorously for 1 minute to dislodge bacteria. The suspension was serially diluted in TSB and 100 µL volumes plated onto Mitis-Salivarius (MS) agar* plates which were then incubated at 37°C under anaerobic conditions (10% [v/v] hydrogen, 5% [v/v] carbon dioxide in nitrogen) (MS agar is selective for streptococci[12]). Four days later, the agar plates were removed from the anaerobic atmosphere, the bacterial colonies enumerated and the number of colony-forming units recovered by the swab, calculated.

The initial trial determined the length of time following biting that viable bacteria could be recovered from bitemarks. Subsequently, various treatments of the bitemark area, which we surmised might adversely affect bacterial recoverability, were assessed by variations of the above method. To determine the influence of preservative-containing moisturizing lotion, volunteers applied Nivea® Body cream† to the upper arm one hour prior to biting; the bitemark was sampled for bacteria three hours following biting. As a natural reaction to biting, fresh bitemarks were briefly rubbed manually immediately following biting, to the extent that the participant gained some degree of perceived relief from the infliction, and the bitemarks sampled three hours later. The effect of brief physical exertion was assessed by requiring the participants to run on a treadmill for ten minutes at 75% of their age-predicted maximal heart rate[13] immediately following biting; bitemarks were sampled three hours later. To assess bacterial recoverability from various fabrics, a freshly laundered square (approximately 15 cm^2) of fabric was pinned to the volunteer's shirt-sleeve and the bite inflicted through the fabric square. Five fabric types were tested for each of six participants. The volunteers wore the squares until the appropriate sampling time when the squares were removed, immersed in 100 mL of 0.3% TSB and agitated (230 rpm) for five minutes on an orbital shaker.‡ Dislodged bacteria were collected by passing the TSB through a 0.45 µm cellulose nitrate filter§ and the filter then vortexed in 3 mL of TSB. A sample of the TSB was subsequently diluted and plated as above.

Bacterial strains from eight volunteers were compared by whole genomic "fingerprinting" according to the following method. Bacterial cultures from bitemarks (six hours old) and from the lower incisors were examined under a dissecting microscope.¶ At least ten colonies (all less than 2 mm diameter) were isolated from the two sites from

* *Difco, Becton Dickinson Co., Sparks, MD.*
† *BDF Beiersdorf, AG Hamburg, Germany.*
‡ *Queue Systems, Parkersburg, WV.*
§ *Sartorius, Goettingen, Germany.*
¶ *SZ-CT, Olympus, Japan*

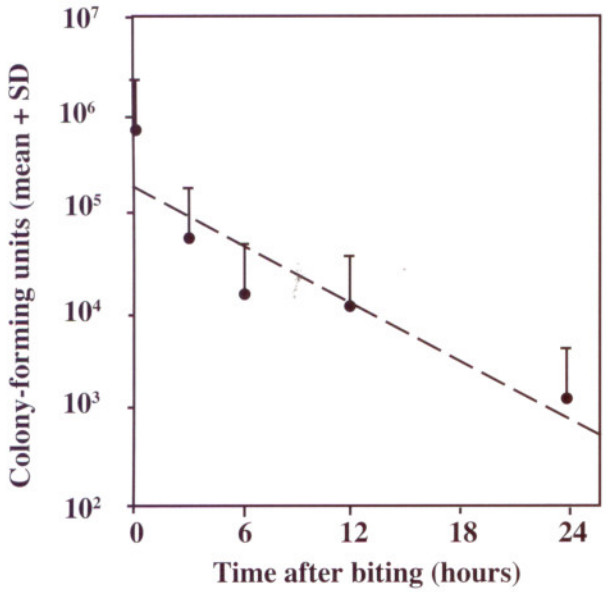

Fig.1: *Recovery of viable oral streptococci from bitemarks inflicted on the upper arms of 13 volunteers (mean + standard error). Bacteria were recovered and enumerated at intervals by swabbing and plating onto Mitis-Salivarius agar. The exponential line (dashed) was fitted by CA-Cricket Graph III (Computer Associates, Vancouver, Canada).*

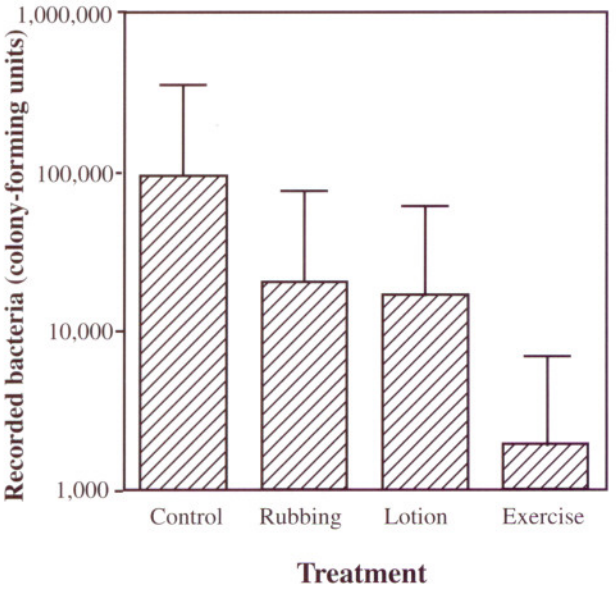

Fig.2. The effects of (1) brief manual rubbing, (2) commercially available moisturizing lotion, and (3) physical exercise, on recovery of oral streptococci from self-inflicted bitemarks inflicted on the upper arms of ten volunteers. The control bites were inflicted on untreated sites and remained undisturbed until sampling. Bacteria were recovered and enumerated three hours after biting by swabbing and plating onto Mitis-Salivarius agar.

each participant by re-streaking onto MS agar. Overnight cultures of purified bacterial strains, grown in brain-heart infusion (Difco) supplemented with 0.5% yeast extract (BHI-YE), were used to inoculate pre-warmed BHI-YE (8 mL) which was incubated at 37°C until reaching an absorption (A540) ≈ 0.4. Solid glycine (0.5 g) was added and incubation continued for a further 45 minutes. Cultures were cooled in iced water and the bacterial cells washed and lysed by the method of Macrina *et al.* [14] The released DNA was then purified and concentrated according to the procedure of Marmur.[15]

Several restriction endonucleases (*Eco* R1, *Hind* III, *Hae* III, *Sac* II, *Not* I, *Eag* I, *Nde* I, *Sal* I, *Pvu* II) were considered for DNA digestion but, using a test strain of *Streptococcus gordonii*, *Pvu* II* was chosen as it produced a greater number of clearly resolved larger sized fragments. Purified chromosomal DNA (≈ 500 μg/mL) was digested with *Pvu* II (200 unit/mL) in NE2 buffer* by incubating at 37°C for 2 hours. The DNA fragments were separated by electrophoresis at 40V through an agarose gel (0.5%) for approximately 5 hrs using 40 mM Tris acetate buffer (pH 8.0) containing 2 mM Na_2EDTA.[16] Gels were calibrated with a *Hind* III digest of lambda phage DNA.† DNA digests of strains isolated from the teeth and bitemarks of the same individual were always compared on the same gel.

Gels were stained for 10 minutes with aqueous ethidium bromide (5 μg/mL) and destained in distilled water for 15 minutes before photography with ultraviolet trans-illumination. The DNA fragment patterns of bacterial isolates were compared visually. Participants in this study were not undergoing antibiotic therapy and were not using antiseptic mouth rinses. The involvement of human participants was approved by the Medical College of Georgia Human Assurance Committee and by the University of Otago Ethics Committee.

* *New England Biolabs, Inc. Beverley, MA.*
† *Life Technologies, Grand Island, NY.*

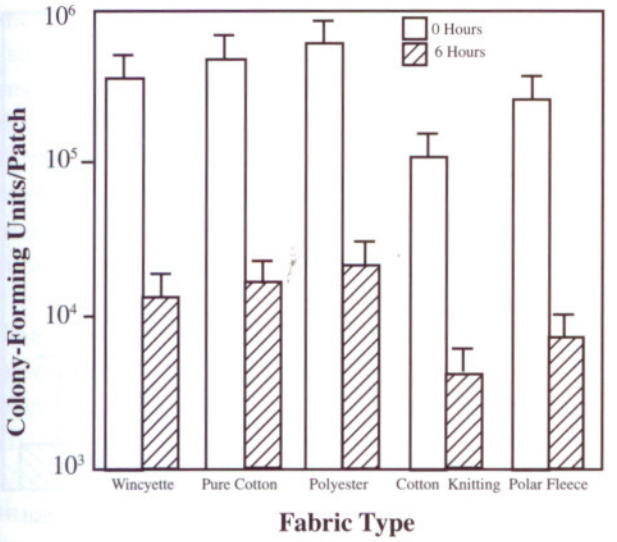

Fig.3: Recovery of oral streptococci from various types of fabric following biting. Bacteria were recovered by agitating the fabric in TSB, concentrating by filtration and plating onto Mitis-Salivarius agar.

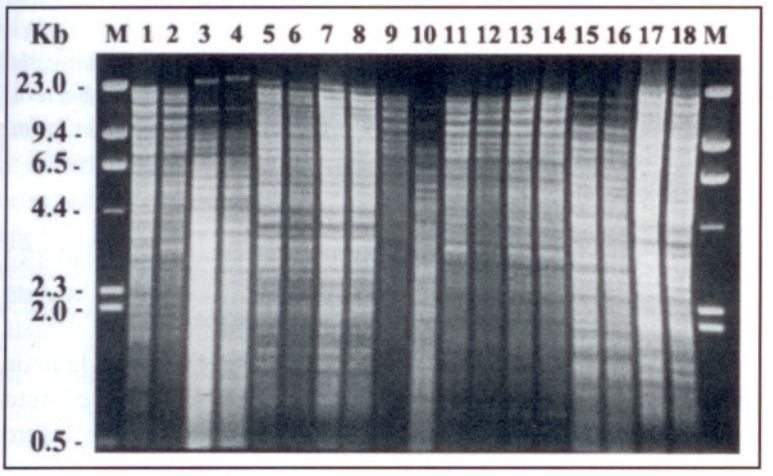

Fig.4: DNA profiles of bacteria isolated from the incisors and bitemark from one subject. The first and last lanes (designated M) contain the Lambda phage DNA calibration markers. Odd numbered lanes contain digested DNA from incisor isolates and even numbered lanes digested DNA from bitemark isolates.

RESULTS

Oral streptococci recoverable from the skin following biting decreased exponentially over time (Fig 1). The average number of colonies recoverable after 24 hrs was slightly more than one thousand, provided that the skin remained relatively undisturbed (Fig. 1). The decrease over the first three hours was 30.8%/hr but this was reduced to 5-6%/hr between hrs 6 and 24. Samples taken from control (unbitten) sites of each of thirteen participants failed to produce more than two colonies/site cultivable on MS agar and ten of the thirteen control samples produced no colonies, indicating negligible background contamination.

Manual rubbing of the freshly bitten sites resulted in a decrease of approximately 80% in recoverable bacteria (Fig. 2). Similarly, the pre-application of moisturizing lotion containing various solvents, oils and preservatives, caused a decrease in recoverable streptococci of just over 80% (Fig. 2). Moderate physical exertion for ten minutes was even more detrimental, resulting in a decrease in recoverable bacteria of more than 95% (Fig. 2). However, despite large proportional decreases in bacterial numbers following exercise, greater than a thousand colonies could still be recovered from most subjects (Fig. 2) which would be more than required for genotypic analysis. All tested fabric types retained viable oral streptococci over the six-hour period examined though the recoverable number of organisms decreased by more than 90% when compared to the initial sample (Fig. 3). Streptococci were not recovered from control fabric squares that had not been bitten.

From eight volunteers, the DNA "fingerprints" (genotypes) of 58 streptococcal isolates from the teeth and 54 from the bitemarks were compared. A total of 60 distinct patterns were identified. Between 2 and 8 genotypically distinguishable strains were isolated from each dental source and also from each bitemark (Table 1). A minimum of 1/6 and a maximum of 4/4 bitemark genotypes could be matched with a genotype from the corresponding incisors (Table 1). No bacterial genotypes could be matched between individuals (Table 1). An example of a genomic DNA gel comparing bacterial isolates from the bitemark and incisors is shown in Fig. 4.

A frequent problem encountered in the development of this procedure was the difficulty of successful subculturing from the primary isolation plate. Although we aimed to recover at least ten strains from

Table 1: *Genotypic comparison of oral streptococci recovered from bitemarks and from the teeth responsible for inflicting the bite.*

	Participant															
	1		2		3		4		5		6		7		8	
	Number and source of isolates[1]															
	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T
genotype a	2	2	2	2	3	3	3	3	1	2	4	1	1	2	2	3
genotype b	1	1	2	2	1	1	2	3	1	0	1	2	1	0	1	1
genotype c	1	1	1	1			1	1	1	0	1	0	1	0	1	1
genotype d	1	1	1	1			1	0	1	0	1	0	1	0	1	1
genotype e	1	1	1	1			1	0	1	0	1	0	0	1	1	0
genotype f	1	0	1	1			0	1	1	0	0	2	0	1	0	2
genotype g	1	0	1	0					0	1	0	1	0	1	0	1
genotype h	1	0	0	1					0	1	0	1				
genotype i	0	1							0	1	0	1				
genotype j	0	1							0	1						
genotype k	0	1														
Number of genotypes from bitemarks	8		7		2		5		6		5		4		5	
Number of genotypes from incisors	8		7		2		4		5		6		4		6	
Matching bitemark genotypes/total genotypes[2]	5/11		6/8		2/2		3/6		1/10		2/9		1/7		4/7	
Matching bitemark isolates/examined bitemark isolates[3]	6/9		8/9		4/4		6/8		1/6		5/8		1/4		5/6	

(1) For each of eight study participants, the number of isolates of each genotype (designated a, b, c, etc.) recovered from the bitemark (B) and from the teeth (T) is shown. Note that genotypes designated a, b, c, etc. from one subject were distinct from those with the same letter designation isolated from all other subjects.
(2) The number of genotypes recovered from the bitemark that were indistinguishable from a genotype recovered from the teeth/total number of genotypes (from both teeth and bitemarks).
(3) Number of isolates recovered from the bitemark that were genotypically indistinguishable from a tooth isolate/number of examined isolates from the bitemark.

both the bitemark and the tooth primary isolation plates, failure either to subculture several strains or to recover sufficient DNA for endonuclease digestion resulted in fewer DNA profile comparisons (Table 1).

DISCUSSION

The various species of non-pathogenic streptococci inevitably comprising the human oral microbiota are rarely found in other environments such as the skin.[6] Recovery of oral streptococci from the skin (or other surfaces) would therefore seem to imply contact with either oral surfaces or saliva (which may in itself provide compelling evidence of oral involvement in a nondescript laceration). As these organisms are not adapted to live on the skin, their survival in this environment is limited. Nevertheless, under favourable conditions, streptococci were recovered from bitemarks in large numbers for up to 24 hrs after biting. On average, women over the age of 20 yrs seek medical assistance seven hours after a sexual assault and younger women eleven hours after assault[17] and it should therefore be feasible to recover oral bacteria originating from the perpetrators of most crimes involving biting. Oral bacteria were also recovered from various fabrics from which it may

be difficult to obtain other forensic information. Again the recoverability of bacteria decreased fairly quickly but the nature of the fabric seemed to have little effect on survival and large numbers of streptococci could be recovered from all fabric types.

Conditions associated with the commitment of violent crimes are unlikely to be as conducive to bacterial survival as those of a laboratory investigation. Whereas it is impossible realistically to simulate the stress experienced by the victims of violent crime, a degree of physical activity seems almost inevitable and, as this study demonstrated, brief exertion markedly diminished the survival of oral bacteria in bitemarks. Furthermore, application of moisturizing lotions and the natural response of simply rubbing the afflicted site also reduced bacterial recoverability. The loss of viable bacteria during exercise is probably due to the anti-microbial effects of sebaceous secretions. Other factors that induce sweating, such as a warm humid climate, will almost certainly diminish recovery of oral bacteria from bitemarks also. Nevertheless, even after the most detrimental treatments such as physical exertion, bacteria were recoverable in large numbers.

The current investigation attempted to replicate natural conditions of bacterial deposition as closely as possible by actual biting. Although the number of organisms in the bitemarks could not be standardized, we felt that the fact that bacteria had been deposited authentically was very important. The arms are the second most frequently bitten site after the breasts in assaults,[18] but obvious ethical and anatomical considerations restricted the experimental bite sites. All bitemarks were covered by loose clothing until sampling and this may have enhanced bacterial survival by reducing desiccation. Brown *et al.*[19] performed similar experiments by placing measured volumes of saliva on the chest of a volunteer and were able to recover streptococci for at least six hours with a similar exponential decrease over time.

Elliot *et al.*[20] demonstrated that strains of *Streptococcus salivarius*, the most prominent species in saliva, could be distinguished by pyrolysis mass spectroscopy and advocated recovery of these bacteria from bitemarks for comparison with oral isolates for forensic purposes. However, the technique involves specialized equipment and the interpretation of results is complex,[20] possibly resulting in the method not being widely adopted. The genotypic approach described in the current study involves relatively straightforward and frequently used molecular biological techniques requiring inexpensive apparatus.

Our findings indicate that, under controlled laboratory conditions, it is feasible to match one or more bacterial strains recovered from a bitemark with those from the teeth of the perpetrator. We had anticipated that sampled tooth surfaces would be dominated by two or three strains of streptococci and therefore selected the relatively labour-intensive whole genomic method in preference to a PCR-based approach. PCR may have facilitated analysis of greater numbers of bacteria but at the possible expense of resolution. In fact, there were sometimes as many as eight abundant bacterial colony types in a sample and the dominant genotypes from the bitemark were generally not the dominant strains on the corresponding tooth site. These findings, together with the survival data shown in Fig. 1, suggest that the skin imposes significant challenges to these organisms. Furthermore, microscopically indistinguishable colonies from the two sources from the same individual were often genotypically distinct. Thus strains dominating the tooth surface will not necessarily be prominent in the bitemark and a more extensive comparison of strains will be necessary to evaluate the probability of recovering identical strains from two individuals. Nevertheless our results indicate that development of a microbial genome-based approach has the potential to provide strongly supportive forensic evidence. As streptococci survive for several hours on inanimate surfaces,[21] a genomic identification method could also be applied to support analysis of bitemarks imprinted on materials other than skin.

The application of bacterial genotypic analysis for forensic purposes cannot indisputably link a suspect to a crime because the genetic material is not the suspect's. The likelihood of recovering the perpetrator's own genetic material from a bitemark is diminished by the presence of nucleases in saliva which rapidly degrade naked DNA.[22] Sweet *et al.*[23] have attempted to overcome this problem by recovering intact epithelial cells deposited in the bitemark (by the aggressor) and amplifying the DNA

protected within the cell. Identification using the perpetrator's own DNA is, potentially, an almost infallible forensic aid but even under controlled experimental conditions, more than 20% of attempts to amplify salivary DNA deposited on the skin of cadavers (presumably maintained at 4°C) were unsuccessful.[23] Under field conditions the success rate is likely to be less. Therefore the bacterial approach described has the potential to provide supportive evidence and a valuable back-up measure.

To be of value in a courtroom, it will be necessary to determine the frequency with which bacterial strains of indistinguishable genotype occur among the human population (which we cannot do from the data currently available). This may be significant among siblings[24] and perhaps close relatives but among unrelated individuals there could be a statistically sound opportunity to obtain supportive evidence from bacterial genotypes. Furthermore, the occurrence of multiple matches among strains from bitemark and tooth site from the same individual (which occurred in six of the eight participants) would further increase the statistical resolution.

In conclusion, this study demonstrates that bacteria, unambiguously derived from the oral cavity, can be recovered from bitemarks impressed on human skin for up to 24 hrs. Because of the extreme genotypic diversity of the oral streptococci, bacteria recovered from bitemarks could be matched exclusively to the teeth of the "perpetrator" in each of eight samples, indicating that a bacterial genotyping approach has the potential to support the identification of the perpetrators of crimes involving biting. We are currently assessing approaches to optimize and expedite molecular identification of streptococcal isolates from bitemarks and teeth as well as examining the long-term genotypic stability of naturally occurring oral streptococcal populations.

ACKNOWLEDGEMENTS

This study was supported by the New Zealand Dental Research Foundation Board, the New Zealand Lottery Grants Board and the Department of Oral Rehabilitation, Medical College of Georgia, (U.S.A.)

REFERENCES

1. Corwin DL, Olafson E. Overview: clinical identification of sexually abused children. Child Abuse and Neglect 1993;17:3-5.

2. Koss MP. Detecting the scope of rape: a review of prevalence research methods. J Interpersonal Violence 1993;8:198-222.

3. Rothwell BR. Bite marks in forensic dentistry: a review of legal, scientific issues. J Am Dent Assoc 1995;126:223-32.

4. Whittaker DK, MacDonald DG. A colour atlas of forensic dentistry. London: Wolfe Medical, 1989.

5. Sweet DJ. Human bitemarks: examination, recovery, and analysis. In: Bowers CM, Bell GL. Eds. Manual of forensic odontology. Ontario: Manticore, 1997:148-69.

6. Marsh P, Martin MV. Oral Microbiology. 4th edn. Oxford: Reed, 1999.

7. Kilian M, Mikkelsen L, Henrichsen J. Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis* (White and Niven 1964), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrewes and Horder 1906). Int J Syst Bacteriol 1989;39:471-84.

8. Bowden GHW, Ellwood DC, Hamilton IR. Microbial ecology of the oral cavity. Adv Microbiol Ecology 1979;3:135-217.

9. Alam S, Brailsford SR, Whiley RA, Beighton D. PCR-based methods for genotyping viridans group streptococci. J Clin Microbiol 1999;37:2772-6.

10. Rudney JD, Larson CJ. Identification of oral mitis group streptococci by arbitrarily primed polymerase chain reaction. Oral Microbiol Immunol 1999;14:33-42.

11. Wisplinghoff H, Reinert RR, Cornely O, Seifert H. Molecular relationships and antimicrobial susceptibilities of viridans group streptococci isolated from blood of neutropenic cancer patients. J Clin Microbiol 1999;37:1876-80.

12. Chapman GH. The isolation of oral streptococci from mixed cultures. J Bacteriol 1944;48:113-4.

13. Kenney WL. Guidelines for exercise testing and prescription. 5th edn. American College of Sports Medicine. Baltimore: Williams and Wilkins, 1995.

14. Macrina FL, Reider JL, Virgili SS, Kopecko DJ. Survey of the extrachromosomal gene pool of *Streptococcus mutans*. Infect Immun 1977;17:215-26.

15. Marmur J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 1961;3:208-18.

16. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd edn. Cold Springs Harbor: Cold Springs Harbor Laboratory Press, 1989.

17. Peipert JF, Domagalski LR. Epidemiology of adolescent sexual assault. Obstetrics and Gynecology 1994;84:867-71.

18. Pretty IA, Sweet D. Anatomical location of bitemarks and associated findings in 101 cases from the United States. J. Forensic Sci. 2000;45:812-4

19. Brown KA, Elliot TR, Rogers AH, Thonard JC. The survival of oral streptococci on human skin and its implication in bite-mark investigation. Forensic Sci Int 1984;26:193-7

20. Elliot TR, Rogers AH, Haverkamp JR, Groothuis D. Analytical pyrolysis of *Streptococcus salivarius* as an aid to identification in bite-mark investigation. Forensic Sci Int 1984;26:131-7.

21. Tagg JR, Ragland NL. Applications of BLIS typing to studies of the survival on surfaces of salivary streptococci and staphylococci. J Appl Bacteriol 1991;71:339-42.

22. Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA, Flint HJ. Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. Appl Environ Microbiol 1999;65:6-10.

23. Sweet D, Lorente JA, Valenzuela A, Lorente M, Villanueva E. PCR-based DNA typing of saliva stains recovered from human skin. J Forensic Sci 1997;42:447-51.

24. Kulkarni GV, Chan KH, Sandham HJ. An investigation into the use of restriction endonuclease analysis for the study of transmission of mutans streptococci. J Dent Res 1989;68:1155-61.

Address for correspondence:
Geoffrey Tompkins,
Department of Oral Sciences and Orthodontics,
School of Dentistry, University of Otago,
PO Box 647,
Dunedin,
New Zealand.