Oral Streptococcal DNA Genotype Profiling as a Promising Tool for Forensic Personal Identification

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Abstract: Background: Bacterial DNA is protected from degradation by salivary enzymes. Additionally, oral streptococcus species are unique to each person. So, oral streptococcal genotype profile analysis could be proposed as a method for personal identification by isolation of bacteria from the bite marks in case of crimes. **Aim of the study:** This study aimed to investigate the medico-legal application of oral streptococcal genotype profile for personal identification, besides evaluating the effect of oral antibiotic intake on feasibility of its use for matching with existing bite mark evidence. **Subjects and methods:** Twelve volunteers were included in the current study. Each volunteer was asked to inflict a bite mark on his upper limb. Six of them were going to take prophylactic oral antibiotic after dental manipulations. Swabs were taken from the following sites; the incisors biting margins, the skin before bite infliction, the skin after bite infliction and the incisors biting margins one month later. Arbitrarily-PCR was used for streptococcal genotype profile analysis recovered from these swabs. **Results:** The genotype profile isolates from swabs of each participant were not identified among the isolates of any other participant. After one month, the percentage of genotype profile matching was 100% for those who didn't receive antibiotic and 74%-88% for those who received it. **Conclusion:** Oral streptococcal genotype profiling could be considered a promising forensic tool for personal identification even with the use of antibiotics.
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1. Introduction

Biting is a common finding in different crimes such as; murder, robbery, sexual assaults and child abuse (1). The pattern left by the pressure of teeth on different objects or tissues is known as a bite mark. Human bite marks take an elliptical or round pattern and consist of abrasions and contusions that associate the indentations (2). Proper analysis of bite marks can provide beneficial information about the type and time of the crime and can help in identification of the assailant (1).

Dentition is unique for each person and so the bite marks. Additionally some individuals may show some dental traits like crowdedness, fractured teeth, spacing, proclined or retroclined teeth that makes photographing, scanning and dental casting beneficial methods for bite marks comparison and identification (3).

Biological evidences can be obtained from swabbing of bite marks as about 0.3 mL of saliva is deposited on the skin and spread over about 20 cm^2 during the act of biting. Initially saliva can be collected for ABO antigen grouping and to obtain DNA for forensic purposes (4,5). With advance of polymerase chain reaction (PCR) technology, DNA analysis plays an important role in forensic science (6).

However, the utility of DNA recovery from saliva can be restricted by its degradation by nucleic acid degrading enzymes (nucleases) which are present in the saliva. This degradation can be accelerated by ambient temperature when saliva present on the skin of the living victim (7).

The human mouth harbors a diverse species of bacteria that varies from one individual to another depending on the oral hygiene, dental status and whether prosthesis are present or not (8,9).

Unlike human DNA, bacterial DNA is protected from degradation as it is enclosed within the cell envelop. It was found that, Streptococcus species present in the human oral cavity are unique to each person. This can propose oral streptococcal analysis as a method for personal identification by isolation of bacteria from the bite marks in case of crimes (10,11).

Previous studies assessed the natural distribution of oral streptococcal genotype and examined the retention of identifiable genotype after 12-month period, but information about antimicrobial treatment in this period was missing (11).

Therefore, the present study aimed to investigate the medico-legal application of oral streptococcal

genotype profiling for personal identification, besides evaluating the effect of oral antibiotic intake on feasibility of its use for matching with existing bite mark evidence.

2. Subjects and Methods

Study population and swabs:

The current study was conducted on twelve volunteers during the period from January, 2018 to June, 2018. The volunteers were visitors of dental clinic of Faculty of Dentistry - Tanta University. They didn't receive antibiotics during the previous three months. They were divided into two groups; participants of the first group (six volunteers) were going to be subjected to dental manipulations followed by prophylactic use of antibiotics, the second group (six volunteers) were not in need to receive antibiotics. The antibiotic prescribed was Augmentin 1 g (Amoxicillin 875 mg + Clavulanic acid 125 mg manufactured by Medical Union Pharmaceutical, Egypt) given twice daily for 5 days.

Before any dental maneuver, a swab was taken from each participant from the biting margin of the incisor teeth. Then, each volunteer was asked to inflict a bite on his upper arm in a force that induced a clear bite marks. Two swabs were obtained from the skin at the biting site; one before bites infliction and the second after bites infliction. In a step to simulate a real forensic situation, a swab was taken from the biting margin of the incisor teeth of each participant after one month (for those who received antibiotic course, this month was after cessation of antibiotic use). During this interval, participants didn't receive any antibiotics. Bacterial DNA profiles were analyzed by arbitrarily-PCR technique.

Steps of arbitrarily-PCR:

Swabs were vigorously inoculated in 5 ml of brain heart infusion broth (BHI broth; Qiagen Co., USA). Serial dilution of the swabs (10 folds) was done then plated onto Mitis-Salivarius agar. The agar plates were anaerobically incubated for 3 days at 37°C (9). Twenty five colonies 2 mm or less in diameter were randomly obtained from each sample to be re-incubated. Using a sterile toothpick, small amounts of bacterial growth were recovered to be suspended in molecular biology-grade nuclease-free deionized water (50µl). DNA extraction from purified colonies was done using pure gene kits for extraction of bacterial DNA (produced by Qiagen Co., USA).

The following protocol used the primer 5′-TGCCGAGCTG-3′ (OPA-02; Invitrogen Life Technologies, Auckland, New Zealand) as this primer was found to give the most consistent and informative results and hence adopted for the current study (12). The PCR reagents were from the same commercial source (MasterTaq Kit, Eppendorf).

Reaction preparations (25 µl) was formed from bacterial suspension (2·5 µl) with $MgCl_2$ (4 mmol l^{-1}), primer-deoxynucleotide mixture (200 µmol l^{-1}), Taq polymerase (1·25 units), and primer (4 µmol l^{-1}). Each run of AP-PCRs included a tube containing S. mitis strain I18 as an internal control (13). One tube containing all reagents except the bacterial suspension served as the negative control.

Denaturation of preparations was done through several steps started by heating at 94°C for 2 min. That was followed by 35 amplification cycles of denaturation at 94°C for 30 s, annealing at 36°C for 1 min and extension at 72°C for 2 min, Then, a final polymerization step at 72°C for 10 min was done. Agarose (1·0%) electrophoresis was used to resolve the obtained amplicons.

The amplicons were stained with ethidium bromide and photographed by UV transillumination (14). DNA bands relative migration distances were normalized against DNA molecular weight markers (1 kb Plus DNA Ladder, Invitrogen Corp., Carlsbad, CA, USA) included in each gel. A 'binning' method was used to compare amplicon profiles (15). The most prominent bands of each profile were expressed as an 'X' in the corresponding bins, that were ascribed molecular size ranges of 0·2 kb. This helped finding of similar amplicon profiles of different individuals that appeared on separate gels. Matched AP-PCR profiles were electrophoresed again on a second gel for direct comparisons.

Ethical consideration:

The study was approved by the research ethics committee of Tanta Faculty of Medicine (Approval Number: 31695/08/17). A written informed consent was obtained from each participant after receiving detailed information about the aim of the study. Code numbers were given to participants to maintain confidentiality.

3. Results

Swabbed samples taken from the skin, the biting edges of teeth and from bite marks were taken from twelve participants and analyzed by arbitrarily-PCR technique.

Swabs from the skin served to exclude micro-organisms inhabitants of the skin, and test the match between swabs from the teeth and swabs from the bite-marks.

Swabs from the teeth and bite-marks of the twelve participants were analyzed to yield 102 genotypically distinguishable streptococcal strains. The letters A, B, C,……etc. were given to different genotype strains.

The streptococcal genotype profile of all participants in the initial setting comprised of multiple strains ranged from 8 to 20 (8-12 for participants of

group 1 and 11-20 for participants of group 2). The genotype of each participant had a dominant strain with percentage ranged from 38% to 82%, and less dominant strains with varying percentages. Table (1) and Fig. (1).

The genotype profile isolates from swabs of each participant were not identified among the isolates of any other participant over the entire study period; the genotypes harbored by participant (X) were not the same harbored by participant (Y).

Swabs of second setting taken a month later, revealed a perfect match100% for participants of group 2 who didn't receive antibiotic treatment, and numbers of isolated genotype strains were the same 11-20.

For participants of group 1, swabs taken one month later after five days of antibiotic use, revealed decreased percentage of perfect match to results of initial setting to be 74%-88% and accompanied by decreased number of genotypically isolated strains ranging (4-6) that were indistinguishable from genotype strains of the initial setting for each participant. It is noted that this match depended more on the dominant strains in the initial setting. Table (2) and Fig. (2).

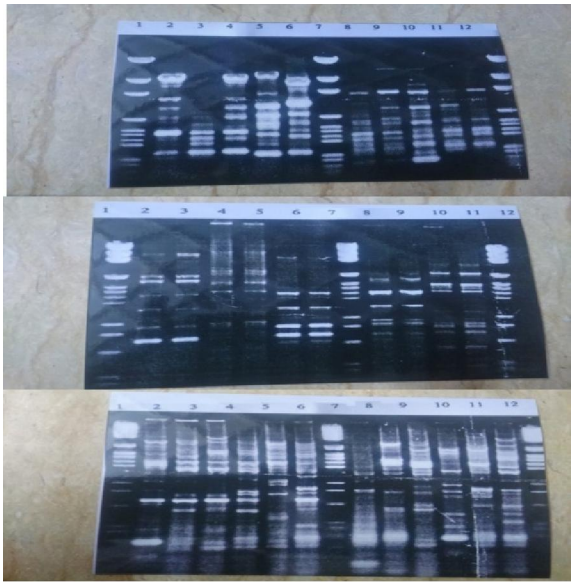

Fig. (1): AP-PCR amplicon profiles are shown in fig. (1) Arbitrary primed PCR amplicon profiles of streptococcal isolates from skin and bite-marks with those from teeth in initial setting.

Table (1): Percentage of different streptococcal strains isolates in the genotype profile of each participant in initial setting:

Genotype*	Participant Number											
	Group 1						Group 2					
	1	2	3	4	5	6	7	8	9	10	11	12
A	79	82	38	60	40	42	42	38	40	35	36	41
B	6	4	23	10	32	32	30	10	12	24	20	26
C	5	3	22	6	6	6	6	8	6	16	18	10
D	2	3	4	4	6	4	4	8	6	10	8	7
E	2	2	4	4	4	2	2	6	4	4	6	4
F	2	2	3	4	2	2	2	6	4	4	2	2
G	2	2	2	4	2	2	2	6	2	2	2	2
H	2	1	2	2	2	2	2	4	2	2	2	2
I		1	2	2	2	2	2	4	2	1	2	2
J				2	2	2	2	2	2	1	2	2
K				2	2	2	2	2	2	1	2	2
L						2	2	2	2			
M							2	2	2			
N								2	2			
O									2			
P									2			
Q									2			
R									2			
S									2			
T									2			
Total#	8	9	9	11	11	12	13	14	20	11	11	11

*A, B, C….etc. indicates different genotype strains for each individual in a descending order of percentage.
Total number of different strains in each individual genotype profile.

Table (2): Percentage of different streptococcal strains isolates in the genotype profile of each participant in second setting a month later:

Genotype*	Participant Number											
	Group 1 (with antibiotic)						Group 2 (without antibiotic)					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A_1	79	82	38	60	40	42	42	38	40	35	36	41
A_2	73	75	34	55	36	36	42	38	40	35	36	41
B_1	6	4	23	10	32	32	30	10	12	24	20	26
B_2	6	2	20	8	27	28	30	10	12	24	20	26
C_1	6	3	22	6	6	6	6	8	6	16	18	10
C_2	5	3	20	6	6	6	6	8	6	16	18	10
D_1	2	3	4	4	6	4	4	8	6	10	8	7
D_2	2	0	4	4	6	4	4	8	6	10	8	7
E_1	2	2	4	4	4	2	2	6	4	4	6	4
E_2	2	2	4	4	4	0	2	6	4	4	6	4
F_1	2	2	3	4	2	2	2	6	4	4	2	2
F_2	0	0	3	4	0	0	2	6	4	4	2	2
G_1	2	2	2	4	2	2	2	6		2	2	2
G_2	0	0	0	0	0	0	2	6		2	2	2
H_1	2	1	2	2	2	2	2	4		2	2	2
H_2	0	0	0	0	0	0	2	4		2	2	2
I_1		1	2	2	2	2	2	4		1	2	2
I_2		0	0	0	0	0	2	4		1	2	2
J_1				2	2	2	2	2		1	2	2
J_2				0	0	0	2	2	2%	1	2	2
K_1				2	2	2	2	2	for each strain till srtain T	1	2	2
K_2				0	0	0	2	2		1	2	2
L_1						2	2	2				
L_2						0	2	2				
M_1							2	2				
M_2							2	2				
N_1								2				
N_2								2				
Total#	5	4	6	6	5	4	13	14	20	11	11	11
Total % of match##	88	82	85	81	79	74	100	100	100	100	100	100

*A, B, C….etc. indicates different genotype strains for each individual in a descending order.
1 results of initial setting
2 results of second setting a month later after 5 days of antibiotic treatment.
Total number of different strainsin each individual genotype profile in correct match with results of initial setting.
percentage of correct matching with initial setting.

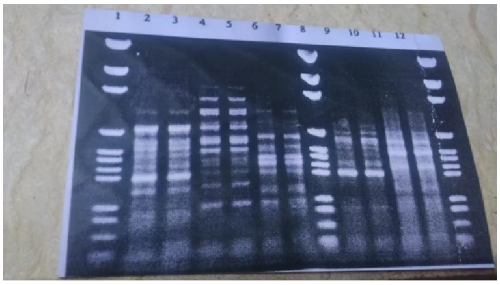

Fig. (2) Confirmation of identity of apparently matching AP-PCR of streptococcal isolates a month later after 5 days of antibiotic use for participants of group 1in second setting.

4. Discussion

Personal identification constitutes important scope of forensic investigations. Bite marks are valuable for identification in instances of crimes, but unfortunately human DNA recovered from bite marks are rapidly degradable because of nuclease enzymes in saliva that breakdown DNA rapidly (16, 17).

Recent research proposed oral streptococcal DNA genotype profile as a promising tool for personal identification in forensic investigations in cases when human DNA cannot be recovered from bite marks for any reason (11, 18), as streptococcal DNA is retrievable for up to 24 hours (9).

In a previous study, Borgula et al. 2003 demonstrated the feasibility of recovering oral streptococci from bite marks and matching their genomic finger prints to isolates exclusively from the teeth of the biter (9,19).

To the best knowledge of the authors, no previous study examined the impact of antibiotic treatment on the usefulness of oral streptococcal genotype profile for identification in forensic investigations.

The present study aimed to confirm usefulness of oral streptococcal genotype profile for personal identification, besides evaluating the effect of oral antibiotic intake on feasibility of its use for matching with existing bite mark evidence.

Twelve subjects participated in the present study, first swabs were taken from all subjects initially, then second swabs were taken a month later. Half subjects had taken oral antibiotic for five days after the first swab (Augmentin as it is of common use in the population of study).

The finger printing method involving extraction, purification and restriction endonuclease digestion of genomic DNA is labor and hence restrictive with the respect to the number of the examined isolates (19, 20).

On the contrary, the AP-PCR method adopted in the current study, facilitates faster analysis of larger numbers with no evidence of sharing of genotype profile between individuals, as suggested by previous studies that this method could afford forensic application due to its discriminatory power (18).

In the present study, PCR amplicon genotype profile for the first swabs revealed a unique genotype for each individual. Moreover, the genotype for each streptococcal strain was distinguished and unique for each individual. No genotype for any streptococcal strain was shared between any of the participants. This result confirms results of previous studies (11, 18).

Previous studies proposed that the likelihood of retaining some of the predominant streptococcal strains on the teeth of the perpetrator of a bite-mark for a prolonged period (12 months) would be 20%-78%, but accurate information about consumption of antibiotics during this period was missing.

In the present study, genotyping of second swabs taken a month later revealed identifiable matching with the genotype profile of the same person 100% for participants who didn't take antibiotic treatment. For those who went under antibiotic treatment, changes in the percentage of indistinguishable isolates didn't hinder proper matching with previous genotype profile of the same participant as the matching was 79-88%.

Conclusion

The study herein presents oral streptococcal PCR amplicon genotype profile as a promising tool for personal identification for forensic purposes that could add valuable information in instances when human DNA cannot be retrieved, and still the results can be matched with confidence.

Recommendations

Further studies to investigate personal oral streptococcal profile after intake of other types of antibiotics and after longer intervals are recommended.

Conflicts of interest

The authors declare that there are no conflicts of interest

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