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Association between sharing of toothbrushes, eating and drinking habits and the presence of *Actinobacillus actinomycetemcomitans* in Moroccan adolescents

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Background/aims: *Actinobacillus actinomycetemcomitans* is frequently detected in dental plaque collected from Moroccan adolescents, and has been shown to be associated with clinical attachment loss in this population. The aim of this study was to assess whether behaviors such as the sharing of toothbrushes, and eating and drinking habits were associated with the presence of *A. actinomycetemcomitans* in Moroccan adolescents.
Materials and methods: A total of 121 adolescents were clinically examined. Interviews regarding sharing of toothbrushes, eating and drinking habits were performed, and plaque samples were collected and analyzed for *A. actinomycetemcomitans* with different leukotoxin promoter types by polymerase chain reaction. Based on eating and drinking habits, the study population was divided in a low risk behavior group (LRB) and a high risk behavior group (HRB).
Results: No association was found between the sharing of toothbrushes and the presence of *A. actinomycetemcomitans*. The odds ratios between the HRB and LRB group for being positive for the JP2 type, for non-JP2 types, and for any type of *A. actinomycetemcomitans* were 4.74 (95% CI 0.55; 40.71), 2.49 (95% CI 1.03; 5.97), and 2.97 (95% CI 1.28; 6.91), respectively. The difference in the mean number of teeth with a clinical attachment loss of ≥ 3 mm between the HRB and the LRB group was 0.91(95% CI 0.09; 1.72).
Conclusion: Sharing of toothbrushes does not seem to be associated with the presence of *A. actinomycetemcomitans* in young Moroccans. Eating and drinking habits conducive to exchange of saliva are positively associated with presence of *A. actinomycetemcomitans*, and with a higher level of clinical attachment loss.

Key words: acquisition; *Actinobacillus actinomycetemcomitans*; attachment loss; behavior; transmission

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Recent studies have demonstrated that *Actinobacillus actinomycetemcomitans*, including the highly leukotoxic JP2 type of *A. actinomycetemcomitans*, is frequently found in Moroccan adolescents and is associated with clinical attachment loss and its progression (12–14). The association found between *A. actinomycetemcomitans* and periodontitis has raised

interest in potential modes of acquisition and transmission of this bacterium (5). Family studies have demonstrated both vertical and horizontal transmission of *A. actinomycetemcomitans* (1, 2, 6, 7, 19), possibly due to salivary transfer (4, 23–24). As it has been reported that toothbrushes are frequently contaminated with *A. actinomycetemcomitans* (17), sharing of toothbrushes may increase the risk of transmission of *A. actinomycetemcomitans* between individuals. Due to the relatively low socioeconomic and sociocultural levels in the majority of the Moroccan population, toothbrushes may frequently be shared in Moroccan families. Another type of behavior where saliva may be exchanged between individuals is the use of fingers to eat from the same plates, and the sharing of food and drinks. Such eating and drinking habits, which were frequently observed during our previous fieldwork in Morocco, may also result in transmission of *A. actinomycetemcomitans*. The aim of this study was to assess whether behaviors such as sharing of toothbrushes and eating and drinking habits are associated with the presence of *A. actinomycetemcomitans* in Moroccan adolescents.

Materials and methods

Study population

The study population comprised 121 adolescents (15–21 years old; mean: 17.6 years, SD 1.5) from eight schools located in three administrative districts of Rabat, Morocco.

Clinical attachment loss was examined on the mesial and distal surfaces of all permanent teeth present, except for third molars. Measurements were obtained from both the buccal and the oral aspects of the surfaces. The population and the method of obtaining microbiological plaque samples have been described recently (13–14). The microbiological samples were transferred to a tube containing 1 ml of 0.9% (wt/vol) NaCl and sent to Denmark for polymerase chain reaction analysis. Samples were analyzed for *A. actinomycetemcomitans* strains with the 530-bp deletion in the promoter region of the leukotoxin gene (JP2 type) and for strains without the deletion (non-JP2 type), as described by Poulsen and coworkers (20). All clinical examinations and collection of plaque samples were made by the same examiner (O.-K.E).

The examinations were performed after written informed consent from participants. Ethical clearance of the study was received from the Unité de Coordination de L'Action Pedagogique, Royaume du Maroc.

All students were interviewed regarding the availability and use of toothbrushes in their family and their habits related to the intake of food and drinks. The questionnaire was developed by native Moroccans to minimize the risk of misleading or irrelevant questions. The wording of the questions and the possible answers are shown in Tables 1 and 2. The interviews were all performed by the same interviewer (Z.I.). None of the students had diabetes and only four students (3%) reported being smokers.

Data analysis

To evaluate the risk of infection with oral microorganisms due to eating and drinking habits, an index was created. Answers to the questions could be 'daily', 'sometimes' or 'never' (Table 2), and they were coded as 2, 1, or 0, respectively. The codes were summed to obtain an index score per individual, 10 being the highest possible index score and 0 the lowest. Two groups, a low risk behavior (LRB) group and a high risk behavior (HRB) group were formed using the median of the individual index score (4.4) to define the two groups. The LRB group contained 42% of the study population (scores ≤ 4) and the HRB group 58% (scores ≥ 5).

Associations were assessed by computing the odds ratios and their 95% confidence intervals (3). The difference in the mean number of teeth with clinical attachment loss ≥ 3 mm between risk behavior groups was calculated and 95% confidence intervals were estimated (3).

Results

Two questionnaires and one plaque sample from three subjects were lost, leaving a complete set of data for 118 individuals.

Toothbrushing and presence of *A. actinomycetemcomitans*

Almost all participants (91.5%) as well as all family members (82.2%) had their own toothbrush. The majority (91.5%) brushed their teeth one or more times every day. Approximately one-fifth of the participants reported that sharing of toothbrushes took place in their family (Table 1).

Of the 118 individuals, six (5.1%) were positive for the JP2 type *A. actinomycetemcomitans*, 32 (27.1%) for the non-JP2 types of *A. actinomycetemcomitans*, and one (0.8%) for both JP2 and non-JP2 types of *A. actinomycetemcomitans*. In 79 subjects (66.9%), no *A. actinomycetemcomitans* was detectable.

A slightly negative association was found between sharing of toothbrushes

Table 1. Odds ratios for the association between behavior related to toothbrushing among 118 adolescents and the presence of *A. actinomycetemcomitans (Aa)*

Question related to toothbrushing	Possible answers	No. (%)	Occurrence of *Aa* (%)	Odds ratio [95% CI]
Do you use other family members' toothbrush?	Yes	24 (20.3)	7 (29.2)	0.80 [0.30; 2.12]
	No	94 (79.7)	32 (34.0)	
Do other family members use your toothbrush?[a]	Yes	22 (19.0)	6 (27.3)	0.76 [0.27; 2.14]
	No	94 (81.0)	31 (33.0)	

[a]Two participants did not know whether other family members used their toothbrush, leaving a total of 116 individuals to be included in the analyses.

Table 2. Distribution of subjects according to eating and drinking habits

Questions related to eating and drinking habits	Possible answers	Proportion
Do you eat from the same plate as others?	Daily	73.1%
	Sometimes	21.8%
	Never	5.0%
Do you drink of the same glass as others?	Daily	56.3%
	Sometimes	13.4%
	Never	30.3%
Do you drink from the same bottle as others?	Daily	16.8%
	Sometimes	47.9%
	Never	35.3%
Do you bite off the same food as others?	Daily	26.9%
	Sometimes	46.2%
	Never	26.9%
Do you taste the same sweets as others?	Daily	3.4%
	Sometimes	20.2%
	Never	76.5%

and the presence of *A. actinomycetemcomitans* (OR = 0.80; 95% CI [0.30; 2.12] and OR = 0.76; 95% CI [0.27; 2.14]) (Table 1).

Eating and drinking habits and presence of *A. actinomycetemcomitans*

The majority of subjects reported that they sometimes or daily ate from the same plate (94.9%), drank from the same glass (69.7%), drank from the same bottle (64.7%), or bit into the same food (banana, bread, etc.) (73.1%) as other members of the household (Table 2). In contrast, only 23.6% reported tasting the same sweets as others (ice cream, chewing gum, lollipops, etc.) sometimes or daily.

A positive association was found between eating and drinking habits and the presence of non-JP2 type and any type of *A. actinomycetemcomitans* (Table 3). The association between eating and drinking habits and presence of the JP2 type was also positive, but the number of individuals with the JP2 type was too low to allow for a precise estimate (Table 3).

Eating and drinking habits and periodontal status

The difference in mean number of teeth with clinical attachment loss ≥ 3 mm between the HRB and the LRB groups was 0.91 (95% CI [0.09; 1.72]). Figure 1 shows a higher level of clinical attachment loss among individuals in the HRB group than in the LRB group.

Table 3. Odds ratios for association between risk behavior and occurrence of *A. actinomycetemcomitans (Aa)* types

	JP2-type of *Aa*		Non-JP2 type of *Aa*		Any type of *Aa*	
	n	%	*n*	%	*n*	%
Transmission risk behavior						
HRB[a]	6	8.8	24	35.3	29	42.6
LRB[b]	1	2.0	9	18.0	10	20.0
OR[c] [95% CI]	4.74 [0.55; 40.71]		2.49 [1.03; 5.97]		2.97 [1.28; 6.91]	

[a]HRB: high transmission risk. [b]LRB: low transmission risk. [c]OR: Odds ratio.

Discussion

The present study demonstrates an association between the presence of *A. actinomycetemcomitans* and behavioral habits likely to facilitate aquisition of periodontitis-associated microorganisms, in this case *A. actinomycetemcomitans.* This association existed even for subtypes of *A. actinomycetemcomitans*. Further, the results demonstrate a positive correlation between behavior (eating and drinking habits) carrying a risk of aquisition of microorganisms, and clinical attachment loss. In contrast, we could not demonstrate any association between the presence of *A. actinomycetemcomitans* and sharing of toothbrushes with other members of the household.

The presence of *A. actinomycetemcomitans* was determined by polymerase chain reaction directly on a plaque sample in saline collected from two periodontal pockets (pooled sample). A study of the false-negative rate for detection of subgingival species by conventional culturing technique was previously performed using different sampling strategies; that study indicated that multiple plaque samples are needed to minimize false-negative rates (10). However, more sensitive methods as polymerase chain reaction have minimized the risk of obtaining false-negatives, even though we can not exclude the possibility of some misclassification (10, 20, 21, 23).

The present study is based on data obtained by interviews and may thus be subject to information bias such as under- or overreporting. On the other hand, the interviews were performed by one of the Moroccan authors (Z.I.) who through probing questions attempted to ensure that the respondents had understood the questions.

Müller and coworkers have demonstrated that *A. actinomycetemcomitans* can be detected on toothbrushes up to 1 h after toothbrushing (17). This suggests that sharing of toothbrushes within a certain time frame may be a possible route of transmission of *A.actinomycetemcomitans*. However, we were not able to demonstrate an association between the sharing of toothbrushes and the presence of *A. actinomycetemcomitans*. One explanation could be that different family members use toothbrushes at different times during the day, making transmission of viable bacteria less likely. Another explanation could be that transmission of *A.actinomycetemcomitans* actually occurs when sharing toothbrushes, but for the microorganism to colonize and establish more permanently, the individual has to be susceptible in the sense of having convenient plaque ecology and be genetically disposed for colonization. Finally, some Moroccan adolescents use a number of other oral hygiene adjuncts (e.g. mesouak, reglise, buchnikha), which have not been considered in the present study.

The present study demonstrated a positive association between the presence of *A. actinomycetemcomitans* and high values of an index that captures specific eating and drinking habits associated with

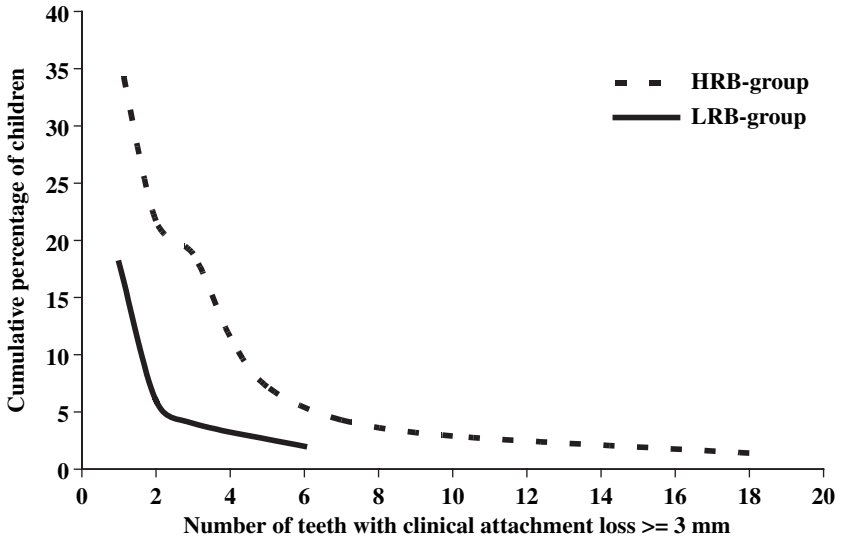

Fig. 1. Cumulative frequency distributions of subjects according to the number of teeth with clinical attachment loss ≥ 3 mm in the high risk behavior group (HRB group) and the low risk behavior group (LRB group).

interindividual salivary contact. Participants often socialize with classmates, friends, maids, and family members at lunch and dinner time. The contacts at these occasions are more intimate and of longer duration, increasing the possibility of aquisition of microorganisms. Acquisition is therefore more likely to be achieved during these behavioral events rather than by sharing of toothbrushes.

A. actinomycetemcomitans is frequently present in dental plaque of children and adolescents (8, 15, 16, 18, 22). A high prevalence of *A. actinomycetemcomitans* in the Moroccan population has also been demonstrated (14). In this study we wanted to investigate whether certain behavioral patterns supportive of transmission of *A. actinomycetemcomitans* were present in Moroccan adolescents, suggesting possibilities for acquisition other than via the familial transmission already described (5). More studies on the presence of different subtypes of *A. actinomycetemcomitans* in families may also be desirable. Several studies have suggested an age predilection for infection with the JP2 clone of *A. actinomycetemcomitans,* which should be considered when interpreting the findings in family studies, as the parents may no longer be infected (9, 11).

We also found that clinical attachment loss was more extensive in the HRB group than in the LRB group. This supports our previous finding of an association between the presence of *A. actinomycetemcomitans,* especially the JP2 clone, and clinical attachment loss in Morocco (12–14).

In conclusion, our findings indicate that certain behavioral habits likely to support aquisition of *A. actinomycetemcomitans* are associated with presence of *A. actinomycetemcomitans.*

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