Bacterial colonization status of cystic fibrosis children's toothbrushes: A pilot study

Contamination des brosses à dents par *Pseudomonas aeruginosa* ou *Staphylococcus aureus* dans la mucoviscidose

A. Genevois[a,1], C. Roques[b,2], C. Segonds[c,2], L. Cavalié[c,2], F. Brémont[a,3], L. Maubisson[d,f,4], E. Mas[e,3], M. Mittaine[a,*,1]

[a] *CRCM pédiatrique, service de pneumologie, hôpital des Enfants, 330, avenue de Grande Bretagne, TSA 70034, 31059 Toulouse cedex 9, France*
[b] *Laboratoire de génie chimique, UMR5503, faculté des sciences pharmaceutiques, université Toulouse III, 35, chemin des Maraîchers, 31062 Toulouse cedex 4, France*
[c] *Laboratoire de bactériologie hygiène, pôle biologie, institut fédératif de biologie (IFB), 330, avenue de Grande Bretagne, TSA 40031, 31059 Toulouse cedex 9, France*
[d] *Centre de recherche en management, UMR CNRS 5303, IAE de l'université Toulouse I, 2, rue du Doyen Gabriel Marty, 31042 Toulouse cedex 9, France*
[e] *CRCM pédiatrique, service de gastroentérologie hépatologie, hôpital des enfants, 330, avenue de Grande Bretagne, TSA 70034, 31059 Toulouse cedex 9, France*
[f] *Université François Rabelais de Tours, université d'Orléans, Vallorem EA6296, 50, avenue Jean Portalis, 37200 Tours, France*

Summary

Background. *Pseudomonas aeruginosa* and *Staphylococcus aureus* toothbrush contamination in cystic fibrosis (CF) is unknown. This pilot study aimed to determine their prevalence and the potential involvement of toothbrushes in pulmonary infection.

Methods. Toothbrush bacteriological analysis for children aged 8 18 years was conducted on 27 CF patients, 15 healthy siblings, and 15 healthy children from the general population.

Results. *S. aureus* was detected on 22% of the patients' toothbrus hes, and 13% of healthy children's toothbrushes and *P. aeruginosa* on 15% of patients' toothbrushes and 0 13% of healthy children's toothbrushes. There was no statistical correlation between pulmonary colonization and toothbrush contamination. *P. aeruginosa* genotyp ing showed two identical clones on the patients' toothbrushes and in their sputum, and between one patient's sputum and his sibling's toothbrush.

Résumé

État actuel du problème et objectifs du travail. La contamina tion des brosses à dents par *Pseudomonas aeruginosa* ou *Staphylo coccus aureus* dans la mucoviscidose est inconnue. Notre étude pilote a pour objectif de déterminer la prévalence de ces germes et l'implication potentielle de ce matériel dans la colonisation de la flore respiratoire.

Matériel et méthodes. Analyse microbiologique de brosses à dents d'enfants entre 8 à 18 ans : 27 patients mucoviscidosiques, 15 mem bres sains de leur fratrie, et 15 enfants sains hors fratrie.

Résultats. Le *S. aureus* a été identifié sur 22 % des brosses à dents des malades, et 13 % des enfants sains, sans détection de souche résistante à la méticilline. Le *P. aeruginosa* a été trouvé sur 15 % des brosses à dents chez les malades, et de 0 % à 13 % chez les enfants sains. Nous n'avons pas trouvé de relation statistique entre la colonisation bronchique et la contamination des brosses à dents.

* Corresponding author.
e-mail: mittaine.m@chu-toulouse.fr (M. Mittaine).

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[2] Microbiological analysis.
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Conclusion. *S. aureus* and *P. aeruginosa* can colonize CF patients' toothbrushes. The impact on pulmonary colonization remains unk nown. Toothbrush decontamination methods need to consider these bacteria in CF patients.

Le génotypage des souches de *P. aeruginosa* a mis en évidence la présence chez 2 malades d'un même clone bactérien sur la brosse à dent et dans les expectorations, et une correspondance génotypique entre l'expectoration d'un malade et la souche isolée sur la brosse à dent de son frère.

Conclusion. *S. aureus* ou *P. aeruginosa* peuvent coloniser la brosse à dents de patients mucoviscidosiques. L'impact de ce phénomène sur la colonisation bronchique est inconnu. Les méthodes de dés infection des brosses à dents doivent être testées spécifiquement sur ces germes dans la mucoviscidose.

1. Introduction

Bronchial colonization by pathogens is a recognized cause of morbidity and mortality in patients with cystic fibrosis (CF). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the main bronchial pathogens in CF. Chronic *P. aeruginosa* lung colonization is associated with a decline in lung function, increased morbidity, and a shorter life expectancy [1,2]. In 2013, 39.7% of the French CF patients had *P. aeruginosa* isolated from their sputum [3]. It is a ubiquitous environmental bacteria, surviving in still water for a long time, and is recognized as a potential pipe contaminator [4]. *S. aureus* is another major pathogen in CF. Its impact on lung function and its survival is more controversial than that of *P. aeruginosa*. Colonization by multi-drug resistant *S. aureus* seems to have an impact on lung function, exacerbation frequency, and survival [5,6]. Recently, a study has shown that the coinfection by *P. aeruginosa* and *S. aureus* was associated with worsened lung function [7]. *S. aureus* has been isolated in 62.1% of the sputum of French CF patients [3].

In healthy children's nasopharyngeal passages, *P. aeruginosa* colonization is rare, not exceeding 5% [8,9], but *S. aureus*, which is one of the nasopharyngeal commensal bacteria, is detected in 27–49% of cultures [9,10]. In CF children, *P. aeruginosa* nasopharyngeal carriage is up to 14% [8], and *S. aureus* carriage is also significant (Odds ratio, 2.4) [10]. Since the oropharynx can serve as a reservoir for pathological microorganisms, it could lead to lower airway bacterial infection in CF patients [11]. The patient's environment is considered as a potential source of contamination and many recommendations have been made to reduce the risk of this potential pathogen, especially by increasing the process of cleaning and disinfection. Hence, oral and dental hygiene must be considered. The Haute Autorité de Santé (HAS) in France recommends the following: at least two tooth brushings per day with a fluoride toothpaste. However, toothbrush maintenance is not specified, resulting in a variety of protocols being used. According to the literature, toothbrushes can be contaminated by microorganisms, which can originate from the oral cavity, the environment, the hands, aerosol contamination, contact with other toothbrushes, or from storage containers [12–14]. There is limited research on microorganisms, most particularly *S. aureus* and *P. aeruginosa* toothbrush contamination after use [13,15]. Since toothbrushes can be a potential vector for CF bronchial contamination by *S. aureus* and *P. aeruginosa* and since no data exist on CF toothbrush bacterial contamination, we conducted a descriptive pilot study to assess the prevalence of two pathogens – *S. aureus* and *P. aeruginosa* – on the toothbrushes of CF children vs their siblings and healthy controls. Finally, we compared the toothbrush contamination profiles with the pulmonary colonization of CF children.

2. Methods

2.1. Study design and patient population

Participation in the study was proposed to each CF patient aged from 8 to 18 years, and their parents, during the follow-up visits at Toulouse Children's Hospital CF Center from 17 December 2013 to 21 February 2014. CF patients were asked to complete a questionnaire about individual toothbrush care and other oral care issues, and to bring their currently used toothbrush (< 24 h after the last use). CF diagnosis was confirmed by two sweat chloride tests > 60 mEq/L and/or genetic testing positive for two known CF-causing mutations. To compare with the general population, we also proposed this study to their healthy siblings, who had a known normal sweat chloride test, and to healthy volunteers 8–18 years old, selected from the relatives of the staff working at the hospital or the bacteriology institute.

For each CF patient, bacterial contamination of the toothbrush was compared to the bacterial analysis of the sputum sample performed on the same day, and to the sputum bacterial analysis of the preceding year. According to the EuroCareCF recommendations, chronic *P. aeruginosa* infection was defined as 50% or more if these samples had been positive in the preceding 12 months [16]. If *P. aeruginosa* was isolated both on the CF patient's toothbrush and in sputum, bacterial genotyping was conducted to compare the strains. For the sibling group, the toothbrush strain was compared with their CF sibling's sputum strain.

Written informed consent was obtained from each patient and his or her parents before the currently used toothbrush was provided, regardless of type, design, duration of use, or toothbrush care habits. The Toulouse University Hospital Research Ethics Committee approved this study.

2.2. Questionnaire

A questionnaire was used to obtain information from each child about home toothbrush care and other oral care issues that may have influenced the bacterial contamination of the toothbrush (data not shown).

2.3. Toothbrush bacterial isolation and identification

Used toothbrushes were recovered within 24 h after the last use and were transported in separate single-use sealed plastic bags. Quantitative toothbrush cultures were performed in the Fonderephar Laboratory in Toulouse and in the microbiology laboratory at Toulouse Purpan University Hospital Federative Biology Institute.

The toothbrush head was retrieved and soaked in 20 mL of Tryptone-sel and vortexed for 30 s to dislodge bacteria. Serial tenfold dilutions were made and 100-μL aliquots were spread-plated onto blood agar and incubated under aerobic and anaerobic conditions for 5–7 days at 36 ± 1 °C. One milliliter of the initial solution was filtered on 0.45-μm membrane (Millipore), the membrane was deposited, and 100-μL aliquots were also spread-plated onto Cetrimide agar and Chapman 2 agar, for 3–5 days under aerobic incubation at 36 ± 1 °C.

After incubation, the total viable count of each distinct colony type was determined and all isolates present with a colony count greater than 1 colony forming unit (CFU)/Petri dish were identified at the species level using morphological (Colony appearance, Gram staining), respiratory type, and biochemical (oxydase, catalase, API systems) criteria and agglutination for staphylococci (Staphyslide, Biomérieux). *S. aureus* strains were also checked for methicillin resistance.

The total bacterial group (streptococci, etc.) contamination level was expressed as CFU/toothbrush. For specific pathogens such as *S. aureus* and *P. aeruginosa*, the CFU log/toothbrush was completed by detection/no detection criteria.

2.4. Bacterial analysis of expectorated sputum

On each visit, an induced sputum sample was collected for bacteriological analysis, performed according to the national guidelines for CF sputum microbiology (in REMIC, Société française de microbiologie Ed; 2010, 99–103), with a detection threshold of 10^2 CFU/mL for *S. aureus* and *P. aeruginosa*.

S. aureus was identified by means of positive DNAse and agglutination (Prolex® i2a, Perols, France) tests. *P. aeruginosa* was identified using Vitek2GN cards (BioMérieux, Marcy l'Étoile, France).

2.5. Strain genotyping

The PFGE procedure was carried out using the Bio-RAD protocol. In brief, whole-cell DNA from *P. aeruginosa* isolates was digested with SpeI (for 20 h at 37 °C) after lysis by lysozyme and proteinase K. Electrophoresis of enzyme-generated fragments was performed with a contour-clamped electric field GenePath System apparatus (Bio-Rad) through a 1% agarose gel. Migration was performed for 20 h at 14 °C, with an electric field of 6 V/cm. DNA banding patterns were visualized with GelRed and digitally photographed. DNA band size was determined from the Lambda Ladder PFG Marker (Biolabs®) (size range: 50–1000 kb).

2.6. Statistical analysis

Concerning statistical analysis, we constituted three groups: group A (CF patients), group B (healthy siblings), and group C (healthy volunteers). Appropriate statistical analysis was performed using SPSS software. Categorical variables were compared using a Chi^2 test. The degree of linear dependence between two quantitative variables was studied using the Pearson's correlation coefficient.

The relation between a quantitative variable and a dichotomous categorical variable was calculated using Student *t*-tests for independent samples, whereas the relation with more than two-modality categorical variables was investigated with analysis of variance (Anova). A 95% confidence interval was selected for statistical significance.

3. Results

3.1. Patient population

Fifty-seven children were included in this study and were divided into three groups:

- group A: 27 CF patients out of the 29 CF families to whom the study was proposed. Their median age was 14 years (± 2.7) and sex ratio 1.1. Of these patients, 26 were able to perform routine spirometry, median FEV_1 was 92% (range: 39–148%) of the predicted level, the nutritional status (estimated by the ratio of actual weight to ideal weight at the same age) was 98% (range, 74–135%) of the predicted level. Over the past year, 17 of group A CF patients had at least one *P. aeruginosa*-positive culture (eight of them having chronic *P. aeruginosa* infection), 23 had at least one *S. aureus*-positive culture, and 13 children had at least one positive culture of each. The presence of methicillin-resistant *S. aureus* (MRSA) in respiratory cultures from three CF patients should be noted;
- group B: 15 healthy siblings, whose median age and sex ratio were, respectively, 12.6 years (± 3) and 1.5;
- group C: 17 healthy volunteers: their median age was 13.2 years (± 2.3) and their sex ratio was 1.1.

There was no significant difference in sex or median age between children with CF and the two other groups ($P > 0.05$). According to the answers of the corresponding questionnaires, the toothbrush duration of use differed greatly for the 57 toothbrushes collected: minimum 2 days and maximum 1 year. There was a significant difference in the mean value ($P = 0.042$) between groups, which was lower in group A with 42 days of use, compared to 91 days in group C. There was no significant difference in tooth brushing frequency, time, autonomy, or toothbrush storage. The mean value of tooth brushing frequency was 1.8/day (range: 0.5–4). Every child used toothpaste and rinsed his brush thoroughly with tap water afterwards.

3.2. Toothbrush bacterial contamination

3.2.1. Oral flora bacteria

Microbiological analysis of toothbrushes on blood agar identified a large number of bacterial species, which corresponded to the main oral flora genera (*Streptococcus*, *Actinomyces*) sometimes with the detection of anaerobic strains belonging to *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*, or *Prevotella* sp. There was no significant difference in these bacteria's distribution among the three groups ($P > 0.05$).

The mean CFU value was 5.8×10^5/mL, with a minimum survival count under the threshold of detection and a maximum of 6.2×10^6 CFU/mL *(fig. 1)*. There was no significant difference among the three groups ($P > 0.05$); however, group A tended to harbor a lower count (2.5×10^5 CFU/mL) than group C (1.3×10^6CFU/mL). Toothbrush contamination with oral bacteria significantly increased with toothbrush duration of use ($P < 0.01$).

3.2.2. S. aureus

S. aureus contamination was observed in ten out of the 57 toothbrushes (17%) with no significant difference between the three groups ($P > 0.05$); however, *S. aureus* detection frequency tended to be higher in group A (6/27, 22%), than group B (2/15, 13%) or group C (2/15, 13%) *(fig. 2)*. The *S. aureus* bacterial count was low, ranging from 1 to 60 CFU/mL. *S. aureus* detection frequency was lower as toothbrush duration of use increased ($P = 0.029$) and as the oral flora bacterial count increased (CFU/mL) ($P = 0.005$).

3.2.3. P. aeruginosa

P. aeruginosa was detected in six of the 57 toothbrushes (10%), with no significant difference between the three groups ($p > 0.05$); however, *P. aeruginosa* detection frequency tended to be higher in group A (4/27, 15%) than in group B (2/15, 13%) or group C (0/15) *(fig. 3)*. The *P. aeruginosa* bacterial count was low, ranging from 1 to 80 CFU/mL. *P. aeruginosa* detection frequency was lower as the oral flora bacterial count increased (CFU/mL) ($P = 0.007$).

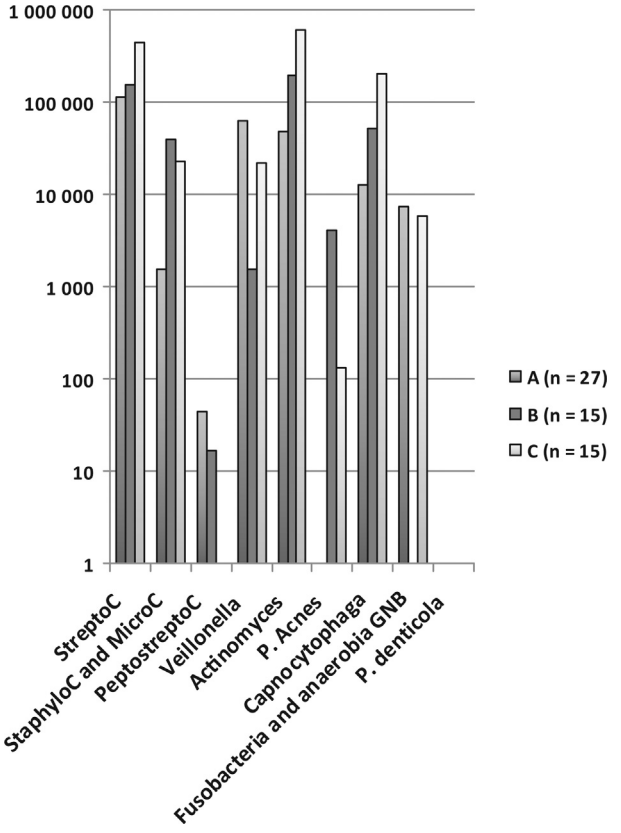

Figure 1. Oral flora bacterial species identified on blood agar (CFU/mL). Group A: CF patients; group B: healthy siblings of CF patients; group C: healthy volunteers.

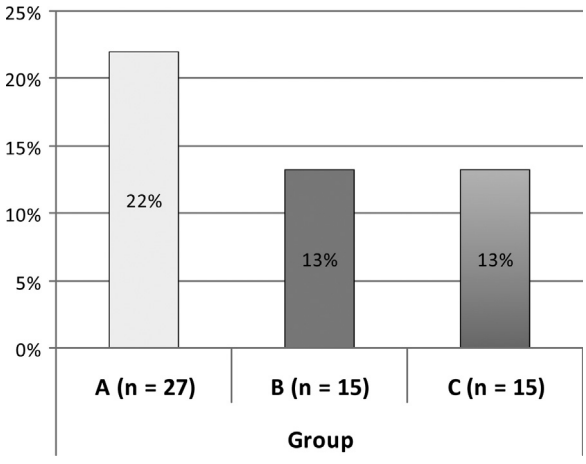

Figure 2. Percentage of toothbrushes with positive *S. aureus* culture. Group A: CF patients; group B: healthy siblings of CF patients; group C: healthy volunteers.

3.2.4. Contamination with S. aureus and P. aeruginosa

Among the 57 toothbrushes, three were simultaneously contaminated by *S. aureus* and *P. aeruginosa*: two in group A and one in group B.

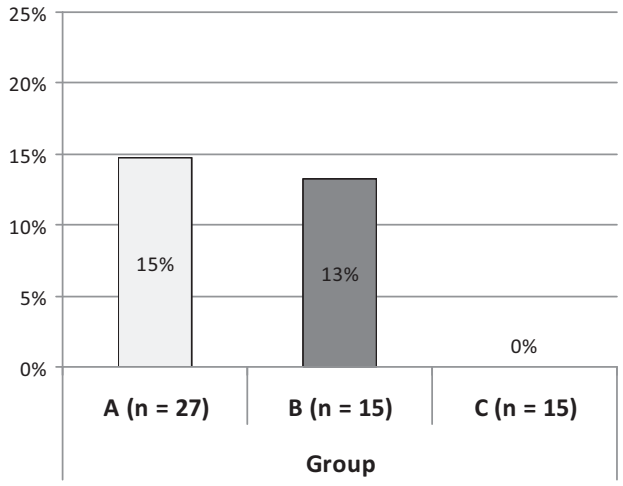

Figure 3. Percentage of toothbrushes with positive *P. aeruginosa* culture. Group A: CF patients; group B: healthy siblings of CF patients; group C: healthy volunteers.

3.3. Bacteriological sputum analysis and correlation with toothbrush colonization

We did found no concordance between sputum culture results and data from the patients' toothbrushes ($P > 0.05$), with the result of the sample collected the same day as their toothbrush, the retrospective samples collected in the preceding 12 months, or the pulmonary colonization status (chronic or intermittent) ($P > 0.05$). No MRSA was isolated on toothbrushes, even in the case of the three CF patients known to have MRSA in their sputum cultures. *P. aeruginosa* was found on the toothbrush of a single CF patient who had never had *P. aeruginosa* isolated in his sputum. For two patients, the *P. aeruginosa* toothbrush clone was compared with *P. aeruginosa* isolated in the sputum collected on the same day. For the third patient the toothbrush clone was compared to the last positive sputum sample for *P. aeruginosa*.

In group A, toothbrush and sputum *P. aeruginosa* genotyping confirmed the presence of the same clone in both samples for two of the three CF patients considered (*table I*). Of the two identical clones, one *P. aeruginosa* was from the same day's sputum and the other was from a previous sample. In group B, one of the two strains of *P. aeruginosa* isolated from the siblings' toothbrushes was considered genetically identical to the strain isolated in his CF sibling's sputum (*table II*).

Table I
***P. aeruginosa* genotyping: bacterial strain comparison in a single subject: expectorated sputum vs toothbrush culture.**

Subject	Group	Sample	Ribotype of *P. aeruginosa*
Subject 2	A	Expectorated sputum	a
		Toothbrush culture	b
Subject 14	A	Expectorated sputum	c
		Toothbrush culture	c
Subject 5	A	Expectorated sputum	d
		Toothbrush culture	d

Group A: CF patients.

Observed genotypes varied from one patient to another, confirming no prevalence of any specific genotype, regardless of the group considered.

4. Discussion

This study is the first documentation of CF patients' toothbrush contamination by bronchial pathogens such as *S. aureus* and *P. aeruginosa*. This pilot study found a prevalence of 15% for *P. aeruginosa* on CF patients' toothbrushes and 22% for *S. aureus*. In the healthy adults, bacterial contamination of toothbrushes is known to occur soon after initial use [12], increases with repeated use, and can persist from 1 to 5 days [12,17–19]. As described in the literature, we observed oral flora colonization of toothbrushes increasing with the duration of use ($p < 0.01$): no significant difference in the contamination level was noted between the three groups even though we surprisingly noted a lower mean value in the CF patient group. Concerning *S. aureus* and *P. aeruginosa* contamination, the three groups were equally concerned, even though we noted a higher rate of contamination on CF patients' toothbrushes. This difference was not significant, possibly due to the small size of the population included. Variations in pathogen detection may stem from bacterial growth competition on toothbrushes between oral flora, *S. aureus* and *P. aeruginosa*. Total bacterial counts increased with the duration of use of the toothbrushes with a concomitant decrease in *S. aureus* and *P. aeruginosa* detection. Repetitive antibiotic therapy (or occasional antibiotic use for non-CF groups), or chronic CF bronchial colonization could also have directly influenced survival of bronchial pathogen bacteria on toothbrushes.

Table II
***P. aeruginosa* genotyping: bacterial strain comparison among sibling pairs: patient expectorated sputum vs sibling toothbrush culture.**

Siblings	Subject	Group	Sample	Ribotype of *P. aeruginosa*
Couple 1	Subject 11	A	Expectorated sputum	e
	Subject 31	B	Toothbrush culture	f
Couple 2	Subject 15	A	Expectorated sputum	g
	Subject 37	B	Toothbrush culture	g

Group A: CF patients; group B: healthy siblings of CF patients.

The actual impact of toothbrush contamination on bronchial primary colonization by *S. aureus* or *P. aeruginosa* seems to be very difficult to assess, because bronchial colonization can occur from different sources and is highly dependent on the child's pulmonary status. Indeed, nebulizers, toilets, and washbasins have been described as potent sources of *P. aeruginosa* aerosolization [20]. However, it is very interesting to note that the same *P. aeruginosa* clone was identified on toothbrush and sputum in three cases: two CF patients and surprisingly in the sputum of one CF patient and his brother's toothbrush. No clonal prevalence was noted. At this time, considering the environmental variability of *P. aeruginosa*, no conclusion as to the origin of toothbrush contamination (the patient's sputum or the environment) or the repercussion on the patient's lung colonization (direct or indirect colonization) can be drawn. In a recent report using multilocus sequence typing, Kidd et al. [21] found that even if several CF strains were frequently encountered in multiple ecological settings, the most frequently encountered CF strains were confined to CF patients with a non-clonal epidemic structure. It was earlier demonstrated [22] that the microbial communities that chronically infect the airways of patients with CF vary little over a year despite antibiotic perturbation. The present species tended to vary from one subject to another, with less variation within each patient, suggesting that each CF airway infection is unique, with relatively stable and resilient bacterial communities. Thus, the potential role of external reservoirs in maintaining pathogens in CF patients must be considered. With the description of various genotypes between CF patients but similarity between strains isolated from sputum and toothbrushes, our observations emphasize that toothbrushes may constitute another pathogen reservoir among all those described in the literature.

Unrelated CF patients may acquire *P. aeruginosa* from the environment or by cross-infection, for example during nosocomial outbreaks. The efficacy of measures to prevent nosocomial acquisition of *P. aeruginosa* was earlier demonstrated [23–25]. Currently, CF patients should follow the general recommendations of the health authorities including the Centers for Disease Control and Prevention, suggesting that patients not to share toothbrushes, rinse their toothbrush thoroughly with tap water following brushing, not routinely cover the toothbrush or store it in closed containers, allowing it to air-dry, and renew the toothbrush every 3 or 4 months, sooner if the bristles appear worn or splayed.

CF patients are supposed to use their toothbrushes at least twice a day, which are, as demonstrated in the present study, potentially contaminated with bronchial pathogens. Many different toothbrush maintenance methods are available [14,15,18,26–29], including cleaning and/or disinfection/sterilization, which still need to be tested on bronchial pathogens such as *P. aeruginosa*. Other studies are needed to determine the actual impact of oral-dental hygiene habits on bronchial colonization even if the ubiquity of *P. aeruginosa* certainly limits conclusions. Simple oral-dental hygiene recommendations should be discussed, such as more frequent toothbrush renewal (once per month) and/or after *P. aeruginosa* primary colonization treatment.

5. Conclusion

Toothbrush hygiene might be considered as one of the reasonable actions to be taken by parents to avoid contact with environmental *P. aeruginosa*.

Disclosure of interest

The authors declare that they have no competing interest.

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References

[1] Sagel SD, Gibson RL, Emerson J, et al. Impact of Pseudomonas and Staphylococcus infection on inflammation and clinical status in young children with cystic fibrosis. J Pediatr 2009;154:183–8.

[2] Emerson J, Rosenfeld M, McNamara S, et al. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 2002;34:91–100.

[3] Registre français de la mucoviscidose, bilan des données 2013 [database on the internet]; 2015, Available from : http://www.vaincrelamuco.org/sites/default/files/registre 2013.pdf. [Visited on 05/11/2015].

[4] Barbeau J. Waterborne biofilms and dentistry: the changing face of infection control. J Can Dent Assoc 2000;66:539–41.

[5] Ren CL, Morgan WJ, Konstan MW, et al. Presence of methicillin resistant Staphylococcus aureus in respiratory cultures from cystic fibrosis patients is associated with lower lung function. Pediatr Pulmonol 2007;42:513–8.

[6] Dasenbrook EC, Merlo CA, Diener West M, et al. Persistent methicillin resistant Staphylococcus aureus and rate of FEV1 decline in cystic fibrosis. Am J Respir Crit Care Med 2008;178:814–21.

[7] Hubert D, Reglier Poupet H, Sermet Gaudelus I, et al. Association between Staphylococcus aureus alone or combined with Pseudomonas aeruginosa and the clinical condition of patients with cystic fibrosis. J Cyst Fibros 2013;12:497–503.

[8] Carlson D, McKeen E, Mitchell M, et al. Oropharyngeal flora in healthy infants: observations and implications for cystic fibrosis care. Pediatr Pulmonol 2009;44:497–502.

[9] Rosenfeld M, Bernardo Ocampo C, Emerson J, et al. Prevalence of cystic fibrosis pathogens in the oropharynx of healthy children and implications for cystic fibrosis care. J Cyst Fibros 2012;11:456–7.

[10] Ridder Schaphorn S, Ratjen F, Dubbers A, et al. Nasal Staphylococcus aureus carriage is not a risk factor for lower airway infection in young cystic fibrosis patients. J Clin Microbiol 2007;45:2979–84.
[11] Komiyama K, Tynan JJ, Habbick BF, et al. Pseudomonas aeruginosa in the oral cavity and sputum of patients with cystic fibrosis. Oral Surg Oral Med Oral Pathol 1985;59:590–4.
[12] Wetzel WE, Schaumburg C, Ansari F, et al. Microbial contamination of toothbrushes with different principles of filament anchoring. J Am Dent Assoc 2005;136:758–65 [quiz 806].
[13] Frazelle MR, Munro CL. Toothbrush contamination: a review of the literature. Nurs Res Pract 2012;2012:420630.
[14] Nelson Filho P, Faria G, da Silva RA, et al. Evaluation of the contamination and disinfection methods of toothbrushes used by 24 to 48 month old children. J Dent Child (Chic) 2006;73:152–8.
[15] Mehta A, Sequeira PS, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. N Y State Dent J 2007;73:20–2.
[16] Pressler T, Bohmova C, Conway S, et al. Chronic Pseudomonas aeruginosa infection definition: EuroCareCF working group report. J Cyst Fibros 2011;10(Suppl. 2):75–8.
[17] Quirynen M, de Soete M, Pauwels M, et al. Bacterial survival rate on tooth and interdental brushes in relation to the use of toothpaste. J Clin Periodontol 2001;28:1106–14.
[18] Efstratiou M, Papaioannou W, Nakou M, et al. Contamination of a toothbrush with antibacterial properties by oral microorganisms. J Dent 2007;35:331–7.
[19] Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. J Dent Res 1977;56:706.
[20] Doring G, Hoiby N. Early intervention and prevention of lung disease in cystic fibrosis: a European consensus. J Cyst Fibros 2004;3:67–91.
[21] Kidd TJ, Ritchie SR, Ramsay KA, et al. Pseudomonas aeruginosa exhibits frequent recombination, but only a limited association between genotype and ecological setting. PLoS One 2012;7:e44199.
[22] Stressmann FA, Rogers GB, van der Gast CJ, et al. Long term cultivation independent microbial diversity analysis demonstrates that bacterial communities infecting the adult cystic fibrosis lung show stability and resilience. Thorax 2012;67:867–73.
[23] Ullrich G, Wiedau S, Schulz W, et al. Parental knowledge and behaviour to prevent environmental P. aeruginosa acquisition in their children with CF. J Cyst Fibros 2008;7:231–7.
[24] Wiehlmann L, Cramer N, Ulrich J, et al. Effective prevention of Pseudomonas aeruginosa cross infection at a cystic fibrosis centre – results of a 10 year prospective study. Int J Med Microbiol 2012;302:69–77.
[25] Doring G. Prevention of Pseudomonas aeruginosa infection in cystic fibrosis patients. Int J Med Microbiol 2010;300:573–7.
[26] Aysegul O, Elgin IE, Gulcin A, et al. The efficacy of chlorhexidine spray vs mouthwash in the microbial contamination of child toothbrushes. J Dent Child (Chic) 2007;74:177–81.
[27] Nelson Filho P, da Silva LA, Ds Silva RA, et al. Efficacy of microwaves and chlorhexidine on the disinfection of pacifiers and toothbrushes: an in vitro study. Pediatr Dent 2011;33:10–3.
[28] Boylan R, Li Y, Simeonova L, et al. Reduction in bacterial contamination of toothbrushes using the violight ultraviolet light activated toothbrush sanitizer. Am J Dent 2008;21:313–7.
[29] Turner LA, McCombs GB, Hynes WL, et al. A novel approach to controlling bacterial contamination on toothbrushes: chlorhexidine coating. Int J Dent Hyg 2009;7:241–5.