Effect of daily manual toothbrushing with 0.2% chlorhexidine gel on pneumonia-associated pathogens in adults living with profound neuro-disability

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Abstract

Purpose. To investigate the effect of daily toothbrushing with 0.2% chlorhexidine digluconate (CHX) on the colonization of dental plaque by pathogens associated with pneumonia amongst non-ventilated adults with a neuro-disability.

Methodology. Forty-nine patients living in long-term care were recruited. Daily toothbrushing with 0.2% CHX gel was conducted for 48 weeks. Plaque accumulation was assessed and microbiological sampling was undertaken every 6 weeks.

Results. At any one time point at least 65% (n=32) of subjects were found to harbour respiratory pathogens. Although there were significant changes in the proportion of individuals colonized over time with Gram-negative bacilli and Pseudomonas aeruginosa, the changes were not sustained. By week 48 there was no significant difference from the levels that had been recorded at baseline.

Conclusions. Bacteria known to be causal in pneumonia are present and colonize the dental plaque of non-ventilated adults with a neuro-disability. Daily toothbrushing with 0.2% CHX gel did not produce a sustained reduction in intra-oral respiratory pathogen counts after 48 weeks.

INTRODUCTION

Aspiration pneumonia (AP) is an acute inflammatory condition that can arise from the aspiration of colonized oropharyngeal material into the distal airways and lung alveoli. It is of clinical importance due to its high morbidity and mortality rates [1]. The surfaces of the oral cavity are contiguous to those in the lower respiratory tract, making aspiration of oral bacteria possible [2], but infection can be prevented by efficient immunological and mechanical defence mechanisms [3]. The onset of pneumonia and its severity is a balance between the strength of the host's defences and the virulence and inoculum size of the pathogen [3, 4]. Individuals with neurological conditions, such as dementia, stroke and multiple sclerosis, are predisposed to developing aspiration pneumonia due to the presence of dysphagia and the challenges they encounter in maintaining oral hygiene. In a meta-analysis conducted amongst frail elderly people, dysphagia was identified as a significant risk factor for AP [odds ratio (OR)=9.84; 95% confidence interval (CI): 4.15–23.33] [5]. Damage to either the central or peripheral nervous system can delay the initiation of the swallowing reflex and weaken the swallowing action, resulting in material spilling into the airway [6]. As a result, many adults who are living with a neuro-disability are fed by non-oral routes, for example via percutaneous endoscopic gastrostomy (PEG).

Accumulation of dental plaque as a result of poor oral hygiene is believed to promote the growth of intra-oral respiratory pathogens by means of (1) periodontal disease-associated enzymes in saliva modifying the mucosal surfaces and promoting the adhesion of and colonization by respiratory pathogens, (2) cytokines originating from periodontal tissues altering the respiratory epithelium to promote infection by respiratory pathogens and (3) periodontal disease-associated enzymes destroying protective host components (e.g. mucins), which normally help to clear respiratory pathogens [7]. Dental plaque is primarily removed from the surfaces...
of the oral cavity by toothbrushing and interdental cleaning [8]. For persons with a neuro-disability impaired motivation, cognitive decline and reduced manual dexterity/voluntary motor function can hamper individual efforts in oral care [9]. Dependence on a third party for oral care has been associated with less than optimal oral hygiene because of barriers such as lack of training, fear of causing pain and uncooperative patients [10]. Langmore et al. [6] found that dependence for oral care is one of the strongest predictors for aspiration pneumonia (P < 0.05), while other studies have demonstrated the colonization of dental plaque by respiratory pathogens amongst dependent adults residing in the community [11] or amongst those who are admitted to intensive care units [12].

A review of the evidence [13] highlighted a reduction in the incidence of pneumonia in community and hospital patients after the implementation of oral care measures. A large proportion of the studies reviewed were set within intensive care units. Amongst individuals who are mechanically ventilated the use of oral hygiene practices that include chlorhexidine has been suggested to prevent 1 person developing pneumonia for every 17 individuals who are mechanically ventilated for more than 48 h [14]. In contrast, there is limited evidence with respect to individuals who receive enteral feeding and are not ventilated.

**Study aim**

The aim of this study was to evaluate the impact of manual toothbrushing with 0.2% chlorhexidine digluconate (CHX) gel on the colonization of dental plaque by bacterial pathogens associated with pneumonia amongst PEG-fed adults with a neuro-disability.

**Ethical approval**

The study was approved by the National Research Ethics Service Committee South Central – Oxford C (16/SC/0235). Information about the study was provided to participants verbally and via information sheets, and written consent was obtained. For individuals who lacked the capacity to consent to participation, as recommended by the Mental Capacity Act [15], advice was sought from the next of kin or the ward manager responsible for the patient’s care (not a member of the study team). No participant was recruited against consultee advice.

**METHODS**

**Population sampling**

A convenience sample of n=49 adults with a neuro-disability was recruited from the long-term wards at the Royal Hospital for Neuro-Disability (RHN), Putney, London, UK. The RHN provides care for those with progressive neurological conditions such as Huntington’s disease, and for individuals with an acquired brain injury who require 24 hour care. Recruitment was conducted between September 2016 and October 2016.

The inclusion criteria in this study were that participants were dentate, aged 18 years or above and receiving nutrition via PEG or a combination of PEG and oral feeding. Patients with a known allergy to CHX or those who had used CHX orally in the year prior to the study were excluded.

**Treatment**

The test intervention was a combination of mechanical and chemical plaque control and comprised of manual toothbrushing of the teeth and the gums with 0.2% CHX gel (Periokin, Pamex, Ireland) in the morning and fluoride toothpaste in the evening. All study participants required third-party assistance for oral care and all staff responsible for providing oral care on the wards received training from a dentist and a dental nurse on appropriate techniques. For staff unable to attend due to scheduling issues, training was cascaded by the ward managers. The new oral care regime was implemented for 1 year (February 2017–January 2018).

**Microbiological sampling**

The primary outcome of the study was the assessment of the presence and colonization of supra-gingival dental plaque by bacterial pathogens associated with pneumonia. The typical pathogens that cause bacterial pneumonia have been identified as: Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Haemophilus influenzae, Gram-negative bacilli (such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter species, Proteus species and Acinetobacter species), Legionella pneumophila, Chlamydia pneumoniae and Mycoplasma pneumoniae [16]. Patients were only considered to be colonized by respiratory pathogens when one of the above was identified from the samples provided.

Pooled samples of supragingival plaque were collected from the buccal surfaces of the upper and lower teeth using a Transwab gel medium without charcoal (Medical Wire, UK). The swabs were transported to the laboratory (TDL, London, UK) for analysis on the same day that they were taken. Samples were collected prior to the implementation of the intervention (baseline) and then every 6 weeks thereafter, resulting in nine data points. Swabs were taken at different times in the morning to accommodate the patient’s care routine and were all undertaken by the lead researcher. One swab was used for each patient at each time point. As part of a larger study, the presence of plaque and periodontal pocketing was documented at each time point, and medical records were examined for the occurrence of episodes of pneumonia.

**Microbiological isolation**

After collection, swabs were plated directly onto the following selective media and incubated at 36–37°C for 48 h.

- Blood agar/CHOC agar (incubated in 5% CO2/air) – for H. influenzae, S. pneumoniae, M. catarrhalis and S. aureus.
- CLED agar/MacConkey agar (aerobic incubation) – for Acinetobacter species, P. aeruginosa and Gram-negative bacilli.
Following incubation, the various colony types were identified using tests as per laboratory procedure – optochin disc and bile solubility test, Staph Latex kit – and confirmed by DNase, oxidase and pigmentation on centrimide agar plates and an API strip system (API 20 NE). *L. pneumophila, C. pneumoniae* and *M. pneumoniae* were not assessed, as culturing these organisms was either not possible or not reliable.

### Data analysis
Cochran’s Q tests were initially performed to evaluate if there were any changes in the proportion of individuals positively colonized with respiratory pathogens over time. When these returned a statistically significant result, further post hoc analysis was conducted via McNemar’s tests, with Bonferroni correction being applied. The weeks in which no patients were colonized with the respective pathogen were omitted from the comparison. The level of significance for all tests was set at 0.05, except those in which multiple pairs of data were analysed simultaneously, whereby Bonferroni correction was applied, resulting in a significance level set at $P=0.001$. Data were analysed using IBM SPSS Statistics version 25 software.

### RESULTS
Fifty patients were recruited, but one participant had all their remaining teeth extracted between baseline and week 6 and was excluded from further participation and data analysis. Therefore, a total of $n=49$ patients partook in the study, all of whom had been patients within the long-term care wards at the RHN for at least 2 years. Eighty per cent (39 patients) were assessed at all nine time points. Samples were not gained from one participant at baseline, two at week 24, one at week 30, three at week 36 and three at week 48. This was due to lack of patient co-operation or patient absence, (hospitalization, social reasons or death).

The participants ranged from 25 to 82 years old, with a mean age of 53 years ($sd=11.8$). There were 28 females and 21 males. Forty-two (84 %) were PEG-fed only compared to seven (14 %) who were PEG- and oral-fed. In addition to their neurological condition, 17 patients (35 %) were diagnosed with medical conditions that could increase their risk of developing pneumonia [17] (Table 1). In terms of oral health, all participants had five or more teeth and relied on a third party to deliver oral care. Six (12 %) participants were recorded as being dependent on a third party for oral care. Six (12 %) participants were recorded as being dependent on a third party for oral care.

### Microbiological findings
At any one time point at least 32 patients (65 %) were found to harbour respiratory pathogens. Only one patient (2 %) remained negative for respiratory pathogen colonization for the entirety of the study period. The most common micro-organism isolated was Gram-negative bacilli (GNB); at its most prevalent it was identified in 90 % of participants. Named micro-organisms included *P. aeruginosa, Proteus mirabilis, K. pneumoniae, Citrobacter koseri, E. coli* and *Acinetobacter* species, of which *P. aeruginosa* was the most frequent, with 41 % of the patients sampled colonized with the pathogen at one point. *S. pneumoniae* was isolated in three (6 %) individuals and *S. aureus* was identified in eight (16 %). No samples were positively colonized with *M. catarrhalis* or *H. influenzae* (Table 2).

Statistical analysis was used to test whether the proportion of individuals positively colonized with respiratory pathogens changed significantly during the assessed 48-week period. Only data from individuals who were assessed across all nine time intervals were included in the analysis. Cochran’s Q test determined that there were no significant changes in *S. pneumoniae* [$X^2 (2)=0.779, P=0.500$], *S. aureus* [$X^2$
Table 2. Number of participants positively colonized with GNB, P. aeruginosa, Acinetobacter species, S. pneumoniae and S. aureus at 9 time points

|                | Baseline, n (%) | Week 6, n (%) | Week 12, n (%) | Week 18, n (%) | Week 24, n (%) | Week 30, n (%) | Week 36, n (%) | Week 42, n (%) | Week 48, n (%) |
|----------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| GNB            | 30 (61)        | 27 (55)       | 35 (71)       | 32 (65)       | 36 (73)       | 44 (90)       | 42 (86)       | 41 (84)       | 35 (71)       |
| P. aeruginosa  | 18 (37)        | 12 (24)       | 18 (37)       | 1 (2)         | 14 (29)       | 15 (31)       | 16 (33)       | 19 (39)       | 19 (39)       |
| Acinetobacter species | 4 (8)       | 2 (4)         | 2 (4)         | 0 (0)         | 3 (6)         | 2 (4)         | 3 (6)         | 3 (6)         | 2 (4)         |
| S. pneumoniae  | 1 (2)          | 0 (0)         | 0 (0)         | 0 (0)         | 1 (2)         | 2 (4)         | 0 (0)         | 0 (0)         |               |
| S. aureus      | 0 (0)          | 2 (4)         | 3 (6)         | 0 (0)         | 4 (8)         | 0 (0)         | 4 (8)         | 2 (4)         | 1 (2)         |

Clinical findings

Dental plaque accumulation was found to reduce significantly after 12 weeks of CHX use and toothbrushing $[X^2 (8)=113.318, P=0.001]$. This improvement was sustained for the remainder of the study period. Thirty-three patients (67%) developed pneumonia during the course of the clinical trial.

DISCUSSION

This study provides evidence that non-ventilated adults with a neuro-disability, who are hospitalized and PEG-fed, are vulnerable to colonization of the oropharynx by respiratory pathogens, and that plaque may be considered to act as a reservoir for such bacteria. The prevalence of oral GNB was found to be higher when compared to studies conducted amongst a similar population group. Lam et al. [18] and Ab Malik et al. [19], whose patients consisted of those undergoing hospital-based rehabilitation for stroke, documented figures of 58 and 73.1%, respectively, at baseline. The high rates of colonization noted within the present study are indicative of the predisposition of adults with neuro-disability to develop pneumonia and are evidenced by the number of patients that experienced an episode of respiratory infection during the clinical study.

Due to widespread, often prolonged, use of antibiotics, GNB and P. aeruginosa are pathogens that the Centers for Disease Control and Prevention (CDC) [20] have identified as acquiring multi-drug resistance and, as a consequence, they are more difficult to treat. Management is therefore not only limited to accurate diagnosis and treatment, but the application of preventive strategies that target modifiable risk factors [21]. This includes modulating colonization by means of oral antiseptics [22]. CHX has been a natural choice due to its widespread antibacterial action against Gram-positive and Gram-negative bacteria, viruses, yeast, fungi and dermatophytes [23].

There have been few published studies that have assessed the action of 0.2% CHX on pneumonia-associated pathogens amongst non-ventilated persons with a neuro-disability. In the 3-week randomized control trial conducted by Lam et al. [18], twice daily rinsing with 0.2% CHX did not have a significant effect on the prevalence of oral opportunistic pathogens. The short duration of the clinical trial, insufficient frequency of 0.2% CHX use and inadequate CHX concentration were cited as potential factors. The present study increased the duration of 0.2% CHX use to 48 weeks, and yet this also failed to improve outcomes, with no significant impact on pathogen prevalence found when comparing baseline to study end data. Any potential positive impact associated with an increase in clinical trial duration may have been mitigated by our choice to restrict the use of 0.2% CHX to once a day. Due to its ability to adhere to hard and soft tissues, CHX has been found to suppress salivary bacterial counts for several hours [24]. It may be inferred that increasing the frequency of exposure to CHX would maintain CHX concentrations within the oral cavity at a level at which it continues to be active. However, it was important that the research intervention could be readily accepted by the nursing staff. By limiting its use to once daily there was minimal disruption to the nursing schedule, thereby helping both acceptability and compliance with the research programme.

Beyond this specific cohort of individuals, 0.2% CHX has also been incorporated into the oral care routine of other medically compromised patient groups, namely those in intensive care units. In these instances, variable results do not allow us to draw firm conclusions on the effectiveness of 0.2% CHX. Cabov et al. [25] reported a significant reduction in oropharyngeal colonization, Fourrier et al. [26] recorded an initially significant decrease between days 5–7, but this did not continue for the remainder of the study (30 days in total), whilst Grap et al. [27] suggested that although CHX may delay pathogen colonization, it did not significantly reduce it. At higher concentrations, Ab Malik et al. [19] documented significant reductions in plaque scores, aerobic and facultative GNB and S. aureus in stroke patients when using 1%
CHX gel in conjunction with a powered toothbrush over 6 months. However, this reduction in GNB was not significantly different from that observed in the control group, where oral hygiene instruction (OHI) and manual toothbrushing with a commercial toothpaste was used. In ventilated patients, CHX concentrations of as high as 2% have been evaluated [28, 29], and although they have reduced the incidence of Gram-positive bacteria, the effect is less pronounced with GNB. Furthermore, a greater number of adverse effects (tooth discoloration and oral mucosa irritation) were associated with 2% CHX compared to 0.2% [29]. In the present study no adverse events associated with prolonged 0.2% CHX use were reported by patients or staff.

One explanation for the lack of impact on oropharyngeal colonization is that there are factors beyond that of poor oral hygiene that can promote the growth of respiratory pathogens. Although the patient’s own endogenous flora is considered to be the primary source of opportunistic pathogens, Lam et al. [18] acknowledged that the hospital environment itself can act as a reservoir of bacteria, potentially compromising the effectiveness of any oral care intervention. A finding shared by the present study and that of Lam [18] is that despite a sustained, significant drop in plaque scores, at study end there was no significant effect on pathogen prevalence when compared to baseline. Patients within a hospital are exposed to a number of different sources of infection, including healthcare devices and the environment (air, water, equipment and formites), while there is also the potential for cross-infection between patients via the hands of healthcare workers [21, 22]. Studies [12, 30] have found that hospitalized patients have greater levels of pathogen colonization than outpatients. In addition, the high need for antibiotic therapy amongst hospitalized patients, whether given prophylactically to prevent infection or to treat an existing infection, can promote the growth of respiratory pathogens. In a comparative study [12] conducted within a medical intensive care unit, the dental plaque in patients who had been recently treated with antibiotics had a greater chance of being colonized with respiratory pathogens than those who had not. It is suggested that antibiotics inhibit the commensal flora, which typically compete with and exclude pathogens [31]. Besides being vulnerable to pneumonia infections, the patients recruited to the present study were also vulnerable to urinary tract infections (treated with trimethoprim, nitrofurantoin and ciprofloxacin) and infections around their PEG site (treated with fluocoxacillin and mupirocin). The potential impact of this confounding factor necessitates the need for it to be recorded in future work.

A further limitation of the study is that overall microbial counts may have been influenced by inherent weaknesses within the study design. Due to variability in the patient’s morning routine, oral swabs were undertaken at different times at each time point. Therefore, it is not known if they occurred before or after toothbrushing with CHX. A further weakness of the study was the lack of named individuals to perform the daily toothbrushing with CHX. The study relied on various members of the nursing team providing the oral care. Although face-to-face training on the new oral care regime was delivered by dental professionals, no formal records were kept as to when toothbrushing was performed.

**CONCLUSIONS**

This study showed that causative bacteria associated with pneumonia colonize the dental plaque of non-ventilated adults with a neuro-disability. In the case of GNB and *P. aeruginosa*, colonization occurred in large numbers. Twice daily toothbrushing with a 0.2% CHX gel used at the morning toothbrushing did not produce a sustained reduction in intraoral respiratory pathogen counts after 48 weeks.

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**Conflicts of interest**

A percentage of Periokin tubes were donated to the RHN by PAMEX for use within the hospital, while the remainder were purchased.

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