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Detecting potential respiratory pathogens in the mouths of older people in hospital

SIR—Hospital acquired pneumonia (HAP) is the third most common health care associated infection, and complicates 1.27% of UK hospital admissions [1]. The incidence is higher in groups such as the elderly, post-operative patients and those on specialist units such as liver, burns or haematology [1]. The incidence of HAP appears to increase with age [2], and the mortality in older people is thought to be between 12.2–55% depending on the group studied [3, 4].

The bacteria which cause hospital acquired pneumonia (HAP) such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* are thought to reside in the oropharynx before being aspirated into the lungs causing pneumonia [5, 6]. These bacteria are more commonly found in the mouths of sick or institutionalised persons than healthy people [7–11] but are not considered part of the normal oral flora.

Randomised controlled trials employing rigorous oral hygiene interventions have significantly reduced the incidence of HAP in both nursing home residents [12] and ventilated persons undergoing cardiothoracic surgery [13], but these studies have not identified exactly why oral hygiene may be beneficial. It has been hypothesised that heavy dental plaque load, prior antibiotics or illness may allow these 'foreign' bacteria to become part of the oral bacterial community. Currently the relationship between plaque, oral bacteria and HAP is unclear.

The next step is to investigate whether prior oral bacterial colonisation with hospital pneumonia pathogens is associated with subsequent HAP. However, the most sensitive oral sampling site for gram-negative bacilli and *S. aureus* need to be verified before this research can be undertaken. In particular clarification is needed regarding the utility of dental or denture plaque as a site for identifying these bacteria.

The aims of this study were to identify the optimal sampling method for identifying gram negative bacilli and *S. aureus* in the mouth which could cause HAP, and to explore the extent to which visible dental plaque and the number of days on antibiotics contributed to the emergence of these bacteria.

Subjects and methods

Permission to undertake this study was granted by a local research ethics committee, County Durham and Tees Valley

1. All participants gave written informed consent. Participants were a convenience sample of 29 in-patients on an orthopaedic ward at Newcastle General Hospital, aged over 65, who were able to give consent to take part in the study. Consecutive stable patients without cognitive impairment on all three orthopaedic wards were approached by the orthopaedic specialist nurse (TM) and names of interested persons were forwarded to the examiner (VE).

Examination and sampling were undertaken between 10 am and 12 pm or 2 pm and 4 pm. The mouth was examined by a single trained assessor (VE) who scored visible plaque using a dichotomous system at six surfaces of each tooth. The tooth with heaviest visible plaque was identified. Swabs were taken from four sites within the mouth (posterior palate, tongue, dirtiest tooth and denture). Cotton swabs were used to sample the posterior palate and interdental bottle brushes (TePe, Malmo, Sweden) that had been autoclaved at 121°C for 15 min were used for the other sites. Interdental brushes were placed in universal pots containing 2 ml sterile water. We also collected a 20 s oral rinse sample using 10 ml sterile water.

The assessor was calibrated for repeatability of plaque scoring just prior to the start of study and achieved kappa scores of 0.925 (very good) [14]. Demographic data and the number of days on antibiotics during hospital admission were also recorded.

Samples were transported to the laboratory within 4 h, but this was not possible for two samples, which were instead stored at 2–8°C overnight and transported to the laboratory the following day. Oral rinse samples were first centrifuged and 8 ml of the supernatant was removed. The pellet was re-suspended in the remaining 2 ml supernatant by vortexing. The samples were then plated directly onto culture media including Columbia blood agar (prepared in-house, Freeman Hospital Microbiology Laboratory, Newcastle upon Tyne, UK), ChromID CPS and ChromID *S. aureus* agar (bioMerieux, Basingstoke, UK) to select for coliforms, pseudomonads and *Staph. aureus,* which are known to cause HAP. Plates were incubated aerobically at 37°C for 48 h. Bacteria were identified to species level using standard microbiological methods. Coliforms were identified using the API 20 E® (bioMerieux, Basingstoke, UK) biochemical identification system.

Results

The mean age of the 29 participants recruited was 81.5 years (male $n = 5$, females $n = 24$) and the median number of hospitalised days prior to the study was 14 days (range 4–181). Fifteen participants had been hospitalised due to hip fracture and the others for fractured wrist, tibia/fibula, humerus or ankle. One patient was receiving intravenous antibiotics for a post-operative site infection following elective spinal decompression. All 15 hip fracture patients had received three doses of cefuroxime post-operatively as per local protocol.

Table 1 illustrates the frequency of bacteria isolated from oral sampling.

Table 1. Bacteria isolated from oral sampling

Bacteria isolated	Frequency n (%)
Either aerobic gram negative rod or *S. aureus*	13 (44.8)
Aerobic gram negative rod only	6 (20.7)
S. aureus only	7 (24.1)

Comparing identification by each site, the sensitivity of the tooth sample was 56% and the sensitivity of the denture sample was 54% in dentate and denture wearing participants, respectively. Oral rinse samples yielded respiratory pathogens more frequently than other sample types (sensitivity 92%). Tongue and throat swabs gave a sensitivity of 69% and 62%, respectively. When results from tongue and throat swabs were combined, sensitivity increased to 77%. Tooth or denture samples were only positive when other sample sites were positive, whereas tongue, throat or oral rinse samples were sometimes positive alone or in combination.

The range of the number of teeth was 0–28, with a median number of 9. Eight participants had no teeth and four of these grew pathogens including *E. coli* and *S. aureus*. All these four patients wore dentures.

The presence of pathogenic bacteria was not related to the number of teeth in dentate participants (unpaired *T*-test $P = 0.2$), nor the number of visibly clean teeth (unpaired *T*-test $P = 0.5$), nor the number of antibiotic days in all participants (Mann Whitney U $P = 0.5$).

Discussion

Other groups have reported on the presence of respiratory pathogens from one or two sites within the mouth [15–19], but to our knowledge this is the first study to compare five sample types in the detection of potential respiratory pathogens within the mouth. The oral rinse sample was most sensitive for isolating respiratory pathogens from the mouth which is comparable with other literature [20]. While this study did not include those with cognitive impairment, it would be important to do so in future studies in order to translate research into clinical practice. Participants must follow a three-stage command to produce an oral rinse sample which may be impossible for those with cognitive impairment, and there may be a risk of aspiration especially in supine patients or those with dysphagia. Combining posterior palatal and tongue swabs produced a sensitivity of 77%, and these samples may be easier and safer to obtain in those with moderate to severe cognitive impairment. The prevalence of gram-negative bacteria detected was slightly higher than the figure of 16% that was detected in another study on orthopaedic patients in 1969 [7].

It has been suggested that dental plaque acts as a reservoir for the bacteria which cause pneumonia and that subsequent periodontitis may augment the inflammatory process [6, 21, 22]. However, in our study neither teeth nor denture swabs showed high sensitivity for detecting bac-

teria compared with an oral rinse sample or combined tongue and throat sample. While the sample size is small, our findings challenge the importance of dental or denture plaque as a reservoir for these bacteria in the older, non-ventilated population and suggest that mucosal surfaces may be more important.

We tested whether dental plaque scores or the number of days on antibiotics was associated with the presence of bacteria. In our study, dentate participants harbouring pathogenic bacteria were as likely to have visibly clean teeth as visibly dirty teeth, indicating that heavy plaque did not result in a higher rate of oral colonisation with pathogenic bacteria. Other studies have found similar results in different patient groups [6]. Visual plaque scores may not detect small amounts of plaque, but in older hospitalised patients whose teeth may harbour a very heavy plaque load it is a useful way of distinguishing poor oral hygiene. Neither did we find a significant link between bacteria and the number of days of antibiotics, which echoed the findings of Johanson [7], who suggested that oral colonization with pathogenic bacteria was associated with increased illness severity, irrespective of prior antibiotics. However, most patients in this study had received at least 1 day of antibiotics and so we cannot comment on the effects of antibiotics per se. These findings need to be corroborated by a larger study in order to accept their reliability given the small sample size in our study.

Given the mortality and morbidity associated with HAP in older non-ventilated persons, further research into both the pathogenesis of HAP and the factors which influence oral colonization with pathogenic bacteria is needed in order to design appropriate comprehensive preventative strategies.

Key points

- An oral rinse sample was the most sensitive method of detecting gram-negative bacilli and *S. aureus* in the mouth.
- Neither visible plaque load nor a greater number of days on antibiotics predicted the presence of gram-negative bacilli and *S. aureus* in this study.
- Tooth or denture samples have poorer sensitivity for detecting these pathogens than other sample sites, and are unlikely to represent the sole reservoir of infection for HAP in older non-ventilated persons.

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Risk factors of new onset diabetes mellitus among elderly Chinese in rural Taiwan

SIR—Diabetes mellitus (DM) is associated with a strong negative impact on the health care system, directly causing 5.2% of all deaths in the world [1]. Certain risk factors for developing DM have been identified, including older age [2]. Among all ethnic groups studied so far, the prevalence of DM may reach 20% in older people [3–5], and older diabetic patients are more likely to develop diabetic microangiopathy, atherosclerotic vascular diseases [5] and to die from DM [6, 7]. They are also more likely to develop cardiovascular diseases than younger diabetic patients or non-diabetic elderly people [5, 8]. Moreover, older diabetic patients are more prone to have physical disabilities, cognitive impairment and depression [9]. Accurate diagnosis and appropriate intervention programmes for older diabetic patients may successfully prevent DM-related complications [10–12]. However, evidence supporting current diagnostic criteria, prevention strategies and targets for glycaemic control in the older population are not fully developed [13, 14]. Tight glycaemic control may successfully reduce the risk of microvascular and macrovascular complications in adults [15], but we lack evidence that these effects are relevant in the older population. For example, older men with late onset DM had similar mortality to non-diabetic subjects in long-term follow-up [16]. Thus, strategies regarding diagnosis, screening and treatment in later onset DM remain controversial.

The main purpose of this study was to evaluate risk factors for the development of DM in older rural Taiwanese residents.

Methods

In 2000, people aged over 40 living in three major townships of I-Lan County, who participated in the annual physical examinations, were invited for study. The study protocol has been reported before [4, 17]. Subjects aged 65 years were selected from the primary cohort and followed up in 2005. In 2000, research staff performed a thorough physical examination, and fasting samples were taken to estimate fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), creatinine and FI.

Obesity was defined as the body mass index (BMI) over 25 kg/m^2, and overweight was defined when $23 < \text{BMI} < 25$ kg/m^2 [18]. Insulin resistance was determined by homeostasis model assessment (HOMA-IR) [19], and the insulin resistant state was defined as the highest quartile of HOMA-IR among the lean subjects (BMI <25 kg/m^2) [20]. The estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study, and chronic kidney disease (CKD) was defined when eGFR <60 ml/min/1.73 m^2 [21]. Overt proteinuria was determined by dipstick urine analysis. Metabolic syndrome (MS) was defined according to the Adult Treatment Panel III standard, and the cutoffs of waist circumferences were modified, according to Asian-Pacific recommendations, to 90 cm in males and 80 cm in females.

With assistance from local health stations and county government, research staff followed up the subjects in 2005. All subjects in the primary cohort were surveyed by telephone contact and a detailed health record review or personal visits. DM status was determined by the medical records (both medication use and laboratory testing), self report or plasma glucose testing. New onset diabetes (NOD)