Concerning the survival of saliva borne oral bacteria on patient notes

Madam,

Hospital patient notes have already been confirmed as potential fomites for pathogenic bacteria.[1, 2] In order to ascertain if this was an infection control issue with respect to dentistry, we spent time in one of the restorative clinics at the Liverpool University Dental Hospital taking swabs whenever a student touched their notes with a (potentially) saliva contaminated gloved hand. These samples were plated onto *Mitis salivarius* agar (MSA) supplemented with 1% tellurite solution; a medium that is selective for *Streptococcus* spp. [3] Streptococci are a ubiquitous component of the oral microbiota and as such represent a reasonable indicator of contamination by salivary bacteria, whereas sampling with non-selective media might yield confounding or inconclusive environmental cultures. No viable streptococci were detected using this method.

A laboratory model was developed to address our continued concerns with regard to the potential contamination of patient notes with salivary bacteria. The hypothesis was that an extended period of desiccation would render oral bacteria unrecoverable – which may constitute latent protection against cross-contamination via patient notes. Squares of normal office paper (≈25 mm x 25 mm; Impega Premium 80g) were soaked in 100% ethanol and then oven dried. A 70% ethanol mixture proved unsuitable since the water content distorted the structure of the paper. Representative paper samples were assayed to confirm that this process rendered the paper sterile. The remaining papers were individually placed into Petri dishes before being inoculated with 20 µl of saliva and then incubated at room temperature. A parallel study showed that such a 20 µl saliva droplet

took 45 minutes to dry out in these conditions; this was assessed using a microbalance to monitor the decreasing mass of the sample due to evaporation over time. Papers were collected at various time points and vigorously vortex mixed in sterile phosphate buffered saline (PSB) to rehydrate the saliva sample and release bacteria from the paper. The number of viable oral streptococci in the PBS was then determined by serial dilution of the samples and spread plating onto MSA. After 6 hours desiccation, only 4.58% of the bacteria found in the initial saliva sample were recoverable (Figure 1). Viable *Streptococcus* spp. were recovered at 24 hours, but were uncountable by the PBS vortex mixing method beyond this time point. Culture techniques, namely incubating papers in brain heart infusion (BHI) broth, were also unable to detect viable cells beyond 24 hours.

In order to mimic a 'worst-case scenario', in terms of this perceived infection control issue, similar saliva-drying experiments were conducted using pure cultures of *Enterococcus faecalis* (*Ef*) (NCTC 775) which were grown in BHI liquid broth before being washed and re-suspended in filter-sterilised saliva. *Ef* is a facultatively anaerobic, Gram-positive coccus found in the oral cavity that is implicated in persistent root canal infections[4], endocarditis[5] and the proliferation of antibiotic resistance genes through the oral metabiome. [6] *Ef* is a non-spore forming bacterium that has been shown to be extremely resistant to dessication. [7] Physiologically and genetically, *Enterococcus spp.* are very similar to *Streptococcus spp*.; indeed *Ef* was previously classified as belonging to the Group D streptococci. [8] *Ef* proved to be far more resiliant to desiccation on paper than the *Streptococcus spp.* indicator organisms that were selected for in the experiments using whole saliva (Figure 1). *Ef* was enumerable by the vortex mixing technique up to 51 hours after deposition and remained recoverable for a total of 24 days using culture techniques.

The results obtained using a pure culture of *E. faecalis* suspended in saliva reinforce the precautions that should already be undertaken to prevent the deposition of saliva onto patient case notes, or indeed other fomites, as it poses a potential cross-infection risk for pathogenic oral microorganisms for an extended period of time. Desiccation offers very little protection.

Conflict of interest statement

None declared

Funding sources

None

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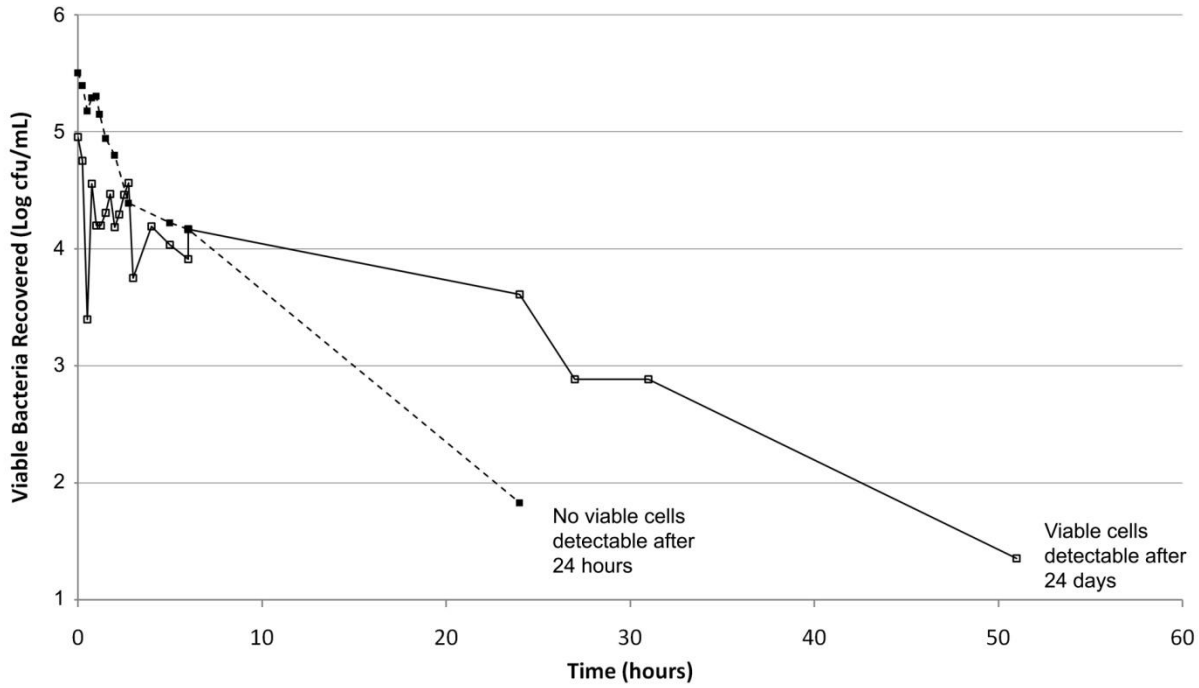

Figure 1. The number of viable bacteria recovered after deposition of whole saliva (closed squares – dotted line) and *Enterococcus faecalis* suspended in sterile saliva (open squares) onto paper over time.

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