Whisky, microwave or hairdryer? Exploring the most efficient way to reduce bacterial colonisation on contaminated toothbrushes

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Key points

- Toothbrush bristles are crammed with oral and environmental pathogens. Even when left untouched for 24 hours, toothbrushes remain highly contaminated.
- Hairdryer heat or immersion in whisky cannot disinfect toothbrush bristles satisfactorily within a minute.
- Microwave oven cooking seems to be an effective technique to overcome bacterial contamination of toothbrushes.

Aims It is the holiday season, but your toothbrush does not look very festive. It is damp and has been used and contaminated by someone else. To rectify this heinous crime, this study investigates the effectiveness of three household objects to disinfect toothbrushes. **Design** *In-vitro* study performed under conditions simulating everyday life. **Materials and methods** Twenty toothbrushes were contaminated using a mixture of saliva and trypticase soy broth containing *Escherichia coli* and *Enterococcus faecalis*. These contaminated toothbrushes were submerged in whisky, cooked in a microwave oven, or exposed to the hot air stream of a hairdryer, separately. Each treatment was performed on five toothbrushes for one minute. Untreated specimens (n = 5) served as controls. Toothbrushes were subsequently sonicated in sterile physiological saline, which was plated on selective agars. Bacterial counts were graded as low, medium, or high. **Results** Residual contamination was influenced by the disinfectant applied, both in *E. coli* ($p < 0.001$) and *E. faecalis* ($p = 0.019$). Microwave cooking achieved highest decontamination, while whisky had no significant effect on bacterial counts over no treatment ($p = 0.8$). Hot air showed some limited effectiveness under current conditions. **Conclusions** Microwave oven cooking appears to be a simple, cheap, and effective way to reduce bacterial contamination of your toothbrush.

Editor's note
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Introduction

There is no point denying it. Toothbrushes are dangerous.

There is abundant proof.

Severe oropharyngeal injuries[1] are caused by toothbrushes, from toothbrush swallowing to impalement.[2] Indeed, review of 31 mishaps involving toothbrushes found within the gastrointestinal tract, none of which exited spontaneously,[3] is anything but reassuring.

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Yet the most pertinent hazard related to a toothbrush is clearly its intended use. Probably too little attention has been paid to the concerns voiced by Cobb as early as 1920,[4] highlighting that the average mouth is teeming with bacteria. It is indeed hard to come to terms with the hideous, but unavoidable reality that brushing teeth causes considerable contamination of the toothbrush bristles with oral pathogens,[5] including foodborne *Escherichia coli*[6] and *Enterococcus faecalis*.[7]

Sadly, the trouble is not confined to retention and survival of oral pathogens on toothbrushes. Usually kept in bathrooms, toothbrushes are constantly exposed to environmental pathogens as well.[8] Toilet flushing is risky, as it produces potentially infectious aerosols in substantial quantities.[9] *E. coli* aerosols reportedly remain airborne and viable for at least four to six hours, and the presence of faecal organisms on all bathroom surfaces and items is incontrovertible.[10] It therefore comes as no surprise that of 26 different household objects examined, the toothbrush holder ranks second as a microbial hotspot, surpassed only by the dish sponge, but clearly outperforming the cellular phone or even the dog's bowl.[11]

With overwhelming evidence that clinically relevant bacteria can persist on dry inanimate surfaces for months,[12] the risks of self- and cross-contamination involved in tooth brushing are terrifying.[8,13] Several decontamination techniques, including chemical disinfectants, ultra violet toothbrush sanitisers or ozone toothbrushes, were indeed pompously introduced, fiercely promoted, vigorously marketed and occasionally scientifically assessed.[14] In contrast to most of the aforementioned approaches, this study focuses on three methods to reduce bacterial colonisation of toothbrushes, which are simple, accessible and time-effective.

The aim of this study was to evaluate the reduction of escherichia and enterococci colonies on contaminated toothbrushes by means of whisky, the microwave oven and the hairdryer.

Material and methods

Contamination

The heads of twenty brand-new, individually sealed toothbrushes with standardised dimensions and bristles (Candida Sensitive Premium, Mibelle Group, Buchs, Switzerland) were immersed for contamination in a particular suspension consisting of 135 mL BBL Trypticase soy broth (Becton, Dickinson and Co., Sparks MD, USA), 15 mL of whole saliva (ownership claimed by one of the authors who prefers to remain anonymous) and 0.75 mL McFarland standard for both *E. coli* (ATCC 25,922) and *E. faecalis* (ATCC 29,212). Bacterial strains were plated before and after the immersion of the toothbrushes to ascertain the contamination.

After contamination, the toothbrushes were left untouched at ambient temperature for 24 hours before disinfection.

Disinfection

Three experimental groups (five toothbrushes per group) were disinfected in one of the following manners, while the remaining five toothbrushes were saved as positive controls:

Whisky

The toothbrush heads were submerged, but neither shaken nor stirred, for 60 seconds in 20 mL of 43% vol. whisky (Dalwhinnie, Single Highland Malt, 15-year-old Scotch whisky, Dalwhinnie Distillery, Dalwhinnie, Scotland, UK) (Fig. 1).

Microwave

The toothbrushes were cooked in a commercially available microwave oven (Intertronic microwave, Interdiscount, Jegenstorf, Switzerland) on an auto-rotating glass dish for 60 seconds at 1400 W.

Hairdryer

The toothbrush heads were dried with a commercially available hairdryer (Philips Dry Care Prestige, Philips, Amsterdam, The Netherlands) from a standardised distance of 6 cm, for 60 seconds at 2300 W.

Seeding and incubation

All test and control toothbrush heads were immersed in tubes containing 20 mL of sterile physiological saline solution and sonicated for 60 seconds (BactoSonic, Bandelin, Berlin, Germany) for microbial cell detachment, using low frequency and low intensity ultrasound below the threshold of cavitation, and subsequently diluted at a 1:20 ratio. For every sample (n = 20), 10 µL aliquots of the diluted solutions were seeded onto two plates (BioMérieux, Marcy–l'Étoile, France): MacConkey agar (MCK) to differentiate E. coli; and Columbia colistin nalidixin agar (CNA) to assess *E. faecalis*. After incubation for 24 hours at 37° Celsius, the agar dishes (n = 40) were examined and the amount of colony-forming units (cfu) was counted to establish the degree of contamination (<30cfu: low contamination; 30–300 cfu: medium contamination; >300 cfu: high contamination).

Fig. 1 Images of the whisky bottle before (left) and after (right) the study, demonstrating the highly significant reduction of its content. 100 mL was used for the actual toothbrush immersion, while the rest was needed to keep the investigators' spirits up during the research

Statistical analysis

Data analysis was performed with IBM SPSS Statistical Software Package (version 23; IBM, Armonk NY, USA). The results were descriptively reviewed and are presented for each culture separately. Contingency tables were calculated and a Pearson's chi-squared test was used to disclose whether the use of a disinfection method had an effect on the degree of contamination. Individual comparisons were made using Fisher's exact test. Significance was set at $p = 0.05$.

Results

The bacterial strains seeded before and immediately after the contamination of the toothbrushes confirmed contamination of the toothbrushes of $>10^5$ cfu/ml for both *E. coli* and *E. faecalis*.

The three disinfection methods affected the two bacterial cultures differently (Fig. 2), and the observed degree of residual contamination was highly influenced by the disinfectant applied, both in *E. coli* ($p < 0.001$) and *E. faecalis* ($p = 0.019$). Generally, *E. faecalis* was more resistant than *E. coli* to all three investigated disinfection methods. The greatest effect was produced by microwave cooking, followed by hairdryer heating. Whisky immersion did not have any significant influence on bacterial growth when compared to the untreated controls ($p = 0.8$, Fisher's exact test).

Discussion

This investigation aimed to examine different methods to disinfect contaminated toothbrushes with devices or with an alcoholic beverage readily available in any ordinary household.

Entirely enteric

Some rationale may be submitted as to why toothbrushes were contaminated specifically with *E. coli* and *E. faecalis*. As noted in the introduction, these microorganisms consider the bathroom environment their home and

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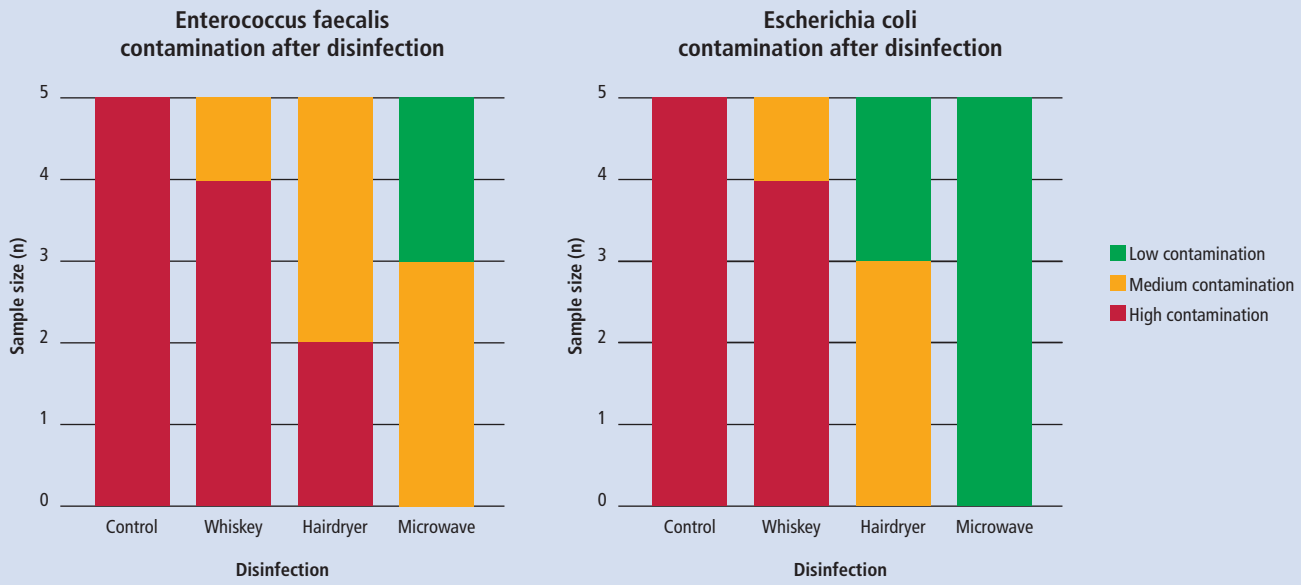
Fig. 2 The effect of different disinfection methods on the degree of contamination: *E. faecalis* (left) and *E. coli* (right)



undisputed territory. But, living from hand to mouth, these bacteria are also residents of the oral cavity,[5] relocated either through food consumption or through interactions with the lavatory milieu. *E. coli* can be found in infested raw food and water[6] and enterococci are associated with fermented European sausages, such as salami, Landjäger and chorizo, and with traditional European cheeses made from raw or pasteurised milk.[7] In fact, viable *E. faecalis* were detected in French, Italian, Swiss[15] and Irish cheese,[16] leaving little hope that national neutrality or insular withdrawal could solve any crude problems.

Enterococci, intrinsically resistant to a number of antibiotics, are opportunistic pathogens and are recognised, together with some *E. coli* pathotypes, as virulent, causing *inter alia* bacteraemia, endocarditis and urinary tract infections.[7] Virulent bacteria can persist on inert surfaces for an extended period of time,[12] and the present findings demonstrate that toothbrush bristles are no exception. All control toothbrushes, which were simply left untouched to dry for 24 hours, were still highly contaminated. Based on this disturbing result, clear-thinking people will therefore doubtlessly consider toothbrush bristles a revolting and redolent reservoir of facultative and opportunistic pathogens. Or, to put it more bluntly, the toothbrush affords the dubious opportunity to reunite daily with living organisms you surely don't wish to encounter again.

No time to waste, no stench or taste…

Some academics before us have already seriously endeavoured to find cheap and accessible alternatives to address the issues involved in bacteria-contaminated toothbrushes. Most avenues discussed are, however, rather time-consuming and of questionable applicability: Studies presenting the value of immersing toothbrushes for 20 min in Listerine or cleaning them for a whole dishwasher cycle[17] are oblivious to the time loss involved (and are incidentally rather peculiar for a non-Christmas article). A report indicating that 3% garlic concentrate is a potent antimicrobial agent for toothbrushes[18] is definitely enlightening, but probably of little societal benefit.

Brushing off the old approaches of toothbrush disinfection, our goal was to find a method to decontaminate a toothbrush within just one minute, without the use of ill-smelling agents.

Hairdryer disinfection – just a lot of hot air?

Heat kills bacterial cells by inactivation of cellular components, particularly membranes, proteins, and ribosomes. *E. coli*[19] and *E. faecalis*[20] are relatively heat sensitive organisms, so our gut-feeling was that application of some kind of heat on this enteric bacteria would prove beneficial. In our study, we differentiated between dry (hairdryer) and damp (microwave) heat. Perhaps somewhat unexpected, the hairdryer disinfection did not deliver satisfactory results (irrelevant and of no consequence, the bristle surface was sleeker and frizz free). Although reaching high temperatures at 6 cm distance, the impact of hairdryer heat on microorganisms remained limited, particularly with the Gram-positive taxon (*E. faecalis*) under investigation. The idea of using a hairdryer might be original and new, but the use of hot dry air for disinfection certainly is not. Back in 1874, correspondence on contaminated medical products and their disinfection by hot dry air was published, stating that 'to make heat penetrate, we want time.'[21] Hours are usually required to decontaminate products with dry air. Toothbrushes are no exception, rendering the hairdryer a dubious antimicrobial weapon.

Microwaving microorganisms?

Microwave radiation affects bacteria culture dramatically. On the one hand, improved heat penetration causes an immensely increased thermal effect, amplifying intracellular protein denaturation and cell lysis. On the other hand, it is also known that microwave radiation causes microbial damage at temperatures below the thermal destruction point.[22] Our results indicate that microwaving toothbrushes has a beneficial effect on decontamination, but 60 seconds seem to be insufficient to disinfect a toothbrush satisfactorily, especially in regard

to Gram-positive bacteria such as *E. faecalis*, which appear to be more heat-resistant. Studying the pertinent literature, it becomes apparent that no consensus exists on whether Gram-positive and Gram-negative colonies respond differently to microwave heating. Future research should therefore focus on this heated debate.

Don't waste your single malt!

In healthcare practice, alcohol is widely used (during working hours) as a disinfectant for hands and critical but non-sterilisable products. Alcohols are rapid broad-spectrum antimicrobial agents.[23] The underlying mechanism is not entirely clear, but is generally believed to be based on membrane damage and denaturation of proteins, with subsequent breakdown of bacterial metabolism and cell lysis.[24] While the optimal antimicrobial activity of alcohol derivates ranges between 60–90%, we thought that – when it comes to a good whisky – it would be worthwhile to give it a shot. The finding that whisky had little to offer as a disinfectant is a big relief. The haunting prospect of upper-class hospitals offering whisky for hand-disinfection is just dreadful.

Limitations

An obvious limitation is the fact that a single malt was singled out. Most obviously, many variables have not been accounted for, including whisky maturation, cask strength or specifications such as smoky versus non-peated or Speyside versus the Highlands. Conducting an elaborate study on different styles of whisky would immensely enrich our understanding in this vital area, but the authors were fully aware that consuming so much whisky for a single study would be a sacrilege that no ethics committee with any moral sense would ever approve.

In order not to obscure our research with facts, we restricted our investigation to five toothbrushes per test-group. Conducting statistical testing on such a small sample size could, however, rightfully be criticised, yet the authors found the presentation of p-values in this sanitary setting rather appropriate.

The strength of this study lies in its double-and-a-half-blinded approach. Neither the bacteria, nor the toothbrushes were fully aware of what they were exposed to and the first author's daughter also turned a blind eye when her hairdryer was temporarily utilised for the crucial advancement of scientific knowledge.

Conclusions

Imagine discovering one morning that your toothbrush is damp and must have been used by someone else before you. Instead of resorting to swearing, this innovative investigation provides a welcome alternative by demonstrating that microwave cooking offers a simple, time-effective and cheap way to significantly reduce the contamination of your toothbrush.

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