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Microbial contamination of laboratory constructed removable orthodontic appliances

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Abstract

Objectives: To determine whether laboratory constructed removable orthodontic appliances are free from microbial contamination prior to clinical use and to evaluate the dental hospital cross-infection procedures to ensure patient-derived contamination does not enter the construction process, thereby propagating a cycle of cross-contamination.

Materials and Methods: The construction process of removable orthodontic appliances from three individuals was evaluated at every stage, from impression to final delivery of the appliance using molecular microbiological techniques. The bacterial profiles at each stage of appliance construction were obtained using Denaturing Gradient Gel Electrophoresis, along with the bacterial profiles of the three participants' saliva. This enabled the bacterial profiles found at each stage of construction to be compared directly with the saliva of the person for whom the appliance was being constructed. Bacteria were identified at each stage by using 16S rDNA PCR amplification and sequence phylogeny.

Results: There was no evidence of bacterial cross-contamination from patients to the laboratory. The current process of disinfection of impression appears to be adequate. Contamination was found on the final removable appliances ($0.97x10^{2}$-$1.52x10^{3}$ cfu $ml^{-}1$) and this contamination occurred from within the laboratory itself.

Conclusions: Every effort is made to reduce potential cross-infection to patients and dental professionals. Newly constructed removable appliances to be shown not to be free from contaminated with bacteria prior to clinical use, but this contamination is environmental. Further studies would be required to determine the level of risk this poses to patients.

Clinical significance: Dental professionals have a duty of care to minimise or eradicate potential risks of cross-infection to patients and other members of the team. To date much less attention has been paid to contamination from the orthodontic laboratory so contamination and infection risks are unknown.

Key words: Cross-contamination, orthodontic laboratory, orthodontic removable appliances, disinfection

Introduction & Literature review

For cross-infection controls to be effective it is important to implement best-practice in disinfection and sterilisation procedures, both in the clinic and the dental laboratory[1-2]. Within medicine and dentistry the aim has been to reduce the risks of cross-infection for both patients and healthcare workers, particularly with the emergence of more virulent strains of bacteria and viruses. A recent example in dentistry is the emergence of the possibility of prion contamination of dental instruments[3]. Whether it is bacteria, viruses or fungi, it is important to minimise the transmission of such organisms within the clinical setting, particularly during interventional procedures (*e.g*. surgery).

Intraoral impressions of patients in orthodontics are required for the construction of orthodontic appliances and the production of study models. These appliances are then manufactured according to EC Medical Devices directives (Directive 93/42/EEC) number 10 and, in the United Kingdom (UK), the regulations covering these provisions are the Medical Devices Regulations 2002 (SI 2002 No 618). Although the directives concerning these custom-made devices include a note about cleanliness and cross-infection controls (Annex 1.11 8), there is no specific guidance issued. Most orthodontic impressions are taken using alginate (irreversible hydrocolloid), with a much smaller, but increasing percentage, taken using silicone impression materials. Whatever material is used, dental impressions are usually cold sterilised/disinfected prior to being sent to the dental laboratory, using one of a range of disinfectants such as sodium hypochlorite, aldehydes, phenols, and quaternary ammonium compounds. A number of studies have investigated the effectiveness of these sterilisation/disinfection procedures on dental impressions[4-6] and have found generally high levels of disinfection, although some bacterial content may remain. When looking more specifically at the practice of dentists when handling impressions, much lower levels of disinfection have been reported[7], with bacterial growth recorded in 61.3% of the disinfected impressions. This may be due to poor compliance with disinfection procedures rather than a failure of the disinfectant solution. The different disinfection protocols used in each of the dental practices were not ascertained in this investigation, or the exact nature of the organisms found. Dental impressions are disinfected prior to dispatch to the laboratory, in order to minimise the cross-infection risk to the laboratory staff, who may not wear protective clothing. Some authors have questioned the need for disinfection of impressions as it was felt the risk to the technician was very low[7]. Instead they advised a greater emphasis on the use of protective clothing and barriers within the laboratory. This study and others[8] have not only indicated a poor compliance by dentists when it comes to the disinfection of impressions, but also a lack of awareness within dental laboratories about the implications of poor disinfection. The risks are real, with pathogenic

bacteria such as *Mycobacterium tuberculosis* having been isolated on dental impressions taken from patients known to be infected with the organism[9]. Some laboratories disinfect the impressions received[8], which itself may lead to problems, as repeated disinfection may lead to distortion of the impression, especially hydrophilic impression materials[10].

It is clear that there is a potential for transmission of patient derived micro-organisms to the orthodontic laboratory via poorly disinfected impressions. An investigation into the transmission of artificially applied bacteria from different types of dental impressions without disinfection (alginate, polyvinylsiloxane and polyether), found that there was very little transmission of bacteria to the poured stone models. Viable bacteria present on models were significantly reduced in numbers from those initially present on the impression using standard bacterial culture[11]. As a result they concluded that there is little need to disinfect impressions, as the bacterial numbers carried onto dental stone are very low and present only a small, possibly negligible, risk to the technician. This may be partially explained by the fact that the creation of a gypsum-based stone model involves an exothermic setting reaction and this may reduce the viable bacterial content on the impression as well as the model. This will further reduce, but not necessarily eliminate any potential contamination risks[12]. Although the evidence is contradictory, there appears to be a possible continuation of the cycle of cross-infection onto the dental cast, which is then used to construct an orthodontic appliance.

There are many opportunities for cross-contamination along the construction process. Unlike in the dental surgery, where most equipment is sterile and cleaned thoroughly between patients, orthodontic laboratories do not follow the same cross-infection protocols, mainly due to the lack of patient contact and the assumption that everything used in the laboratory has not been contaminated by patient saliva or blood products[13]. If there is any contamination, this has the potential to be transmitted around the laboratory, either by direct contact or via the production of aerosols when grinding or polishing[14]. Previous work using settle plates around the polishing lathe has shown that the aerosols produced can contain pathogenic bacteria such as staphylococci and pseudomonads[14]. Furthermore, as in the dental surgery environment, aerosolised particles and splatter can spread over large distances[15].

Orthodontic appliance construction is under the guidance of the medical devices directive and should be returned to the patient free from contamination. They should therefore be cleaned and disinfected prior to dispatch to the clinic for fitting[14]. The process of cleaning and polishing in the dental laboratory has been found to introduce more contamination than was already present. Previous investigations into the microbial contamination of pumice used for polishing, has revealed high levels of contamination[16]. As a result,

disinfectants that are both bactericidal and virocidal should be added to the pumice slurry prior to use. Accumulation of microbes can occur in the slurry over time even in the presence of a disinfectant[17] and an increase has been noted after 3 days of use. This has been attributed to microbes from the skin and air[14]. The use of cold-cured acrylic in the construction of most orthodontic appliances requires the use of a water bath heated to a temperature of 45°C to accelerate the polymerisation reaction of the polymethylmethacrylate. Investigation of the bacterial content of such heated water baths has also revealed the presence of bacteria such as *Rhodotorula rubra*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus capitis*, *Pseudomonas putida* and *Hafnia alvei*[14].

Local guidance at University Hospitals Bristol requires that all custom made orthodontic appliances are visually checked for cleanliness to ensure that they are free from contamination and are rinsed and decontaminated using an approved disinfecting agent (*e.g.* 0.5% aqueous chlorhexidine) prior to final packaging and dispatch. This latter immersion process has however been omitted as chlorhexidine has been found to adversely affect the surface of the acrylic causing degradation of the polished surface.

Objectives

Although attention is paid to disinfecting orthodontic impressions prior to sending to the dental laboratory for appliance construction, little is known of the likely bacterial load of the appliances once constructed and prior to fitting back in the patients mouth. This investigation therefore aimed to determine the bacterial load, and identity of predominant contaminating bacteria, at each stage of the fabrication process and finally to determine whether laboratory constructed removable orthodontic appliances are free from microbial contamination prior to clinical use.

Materials and Methods

Ethical approval was granted from the North Somerset & South Bristol Research Ethics Committee (10/H0106/5) and University Hospitals Bristol Research & Development Department. Initially, 10 volunteers were to be recruited from the Specialist Registrars in Orthodontics at Bristol Dental Hospital to take part in the study. Participant invitations were given to potential candidates and they were then provided with participant information sheets so that they could be given sufficient information about the study and the extent of their participation. Once potential participants indicated that they would like to take part in the study, informed consent was gained. A unique and anonymous identification number was assigned to each participant and

recorded in the site file along with the signed informed consent form. Only three subjects were used in the final study. This will be discussed later in this paper.

Participants were required to provide a sample of un-stimulated saliva by spitting into a 50 ml sterile collection tube. A 200μl sample of saliva was centrifuged (Biofuge® fresco, Heraeus®) at 13000 rpm for 5 minutes to collect any bacterial cells for DNA extraction. Two dental impressions were taken of each of the three participants. One was used to determine the efficacy of the disinfection protocol and one for the laboratory to construct an orthodontic appliance. Impressions were disinfected in a solution of sodium dichloraisocyonurate (Haz-Tabs, 500ppm available chlorine) for ten minutes and then rinsed under cold running water, as per the normal hospital protocol, before transportation to the hospital dental laboratory within a polyethylene bag. The disinfected impression was cast as per standard practice, using dental gypsum to create a stone model on which the removable appliance could be constructed. The construction process and sampling intervals are outlined in **Figure I**. The resultant cast model base was trimmed to size on a Trim Pro (Wehmer Corporation, Illinois, USA) and was then used to construct an upper removable appliance to a standard design. The cold cure acrylic base was laid down and cured using a pressure polymerisation chamber (Palamat Elite® Polymerisation unit, Heraeus Kulzer, USA). Once cured, the appliance was removed, trimmed and polished using acrylic trimmers. It was then polished using a slurry of pumice on a Poliereinheit PE 5 polishing machine followed by polishing with Canning Polishing Compound on the same machine (Degussa, Frankfort, Germany). Following this it was finally cleaned in an ultrasonic bath (QC Ultrasonic Cleaner, Walker, Newark, UK) containing an alkaline chelating detergent (Micro-90® concentrated cleaning solution, International Products Corporation, USA) and then with a toothbrush under running water prior to packaging for delivery on the clinic.

Sampling intervals

To sample the impression, 50ml of sterile10mM:1mM Tris-EDTA was pipetted into a sterile 500ml glass beaker. The disinfected impression was transferred from the polythene bag to the beaker containing the buffer solution. A 20 ml sterile syringe was used to irrigate the solution all around the impression to dislodge any adherent bacteria. The resulting solution was centrifuged (5,000 rpm for 3 minutes using Rotina 380R, Andreas Hettich GmbH, Germany) to concentrate any bacteria in the base of the tube. The supernatant was removed leaving 1ml of solution. This solution was then vortexed using a Fisons Whirlimixer™ for 60 seconds.

Sterile swabs (Fisher) were taken of the stone model once the setting reaction was complete, as well as the final removable orthodontic appliance. These were placed, separately, in 1ml 10mM:1mM Tris-EDTA in a sterile

1.5ml Eppendorf tube. Samples from the impression, final stone models and the standardised constructed removable appliances were then cultured for potential bacterial contamination. 100µl of the resulting sample was plated onto blood-rich media blood agar base No. 2 (LAB M Limited, Bury) and fastidious anaerobe agar (LAB M Limited, Bury). Plated samples from each specimen were then cultured: i.) aerobically, ii.) in an oxygen-depleted candle jar, and iii.) in a Don Whitley Mark II Anaerobic Cabinet (N_2: H_2: CO_2 at 8:1:1). All samples were incubated at 37°C for 5 days together with uncontaminated controls. These were then visually assessed to detect and enumerate any bacterial colonies indicative of sample contaminants. The numbers of colony forming units per millilitre (cfu/ml) present were determined. Cultured bacteria were Gram stained to aid differentiation.

Following appliance construction samples of the stone trimming slurry, the solution within the pressure polymerisation chamber, the pumice polishing slurry and the ultrasonic cleaning fluid were taken after the appliance had been processed through each of these stages. Each solution was centrifuged at 5,000rpm for 3 minutes to allow collection of 1ml of the solution containing potential bacterial cells. These 1ml solutions were further centrifuged (Biofuge® fresco, Heraeus®) at 13,000 rpm for five minutes to collect any bacterial cells for DNA extraction.

All samples underwent DNA extraction using GenElute™ (Sigma-Aldrich) or GeneReleaser™(BioVentures Inc) as per the manufacturers' instructions. PCR amplification of 16S rRNA whole gene sequences was undertaken for the microbiology samples using universal 16S rRNA gene oligonucleotide primers 27 Forward (27F: AGA GTT TGA TCC TGG CTC AG) and 1492 Reverse (1492R: TAC GGG TAC CTT GTT ACG ACT T) within a 50µl reaction containing 1.25 units Go *Taq* DNA polymerase (Promega), 10µl supplied buffer (final $MgCl_2$ concentration 1.5mM), 0.2mM each dNTP, 1.0mM each primer and approximately 20µl GenElute™ extracted template DNA or 5µl GeneReleaser™extracted template DNA. Negative controls using ultrapure PCR H_2O and positive controls using previously validated bacterial DNA samples were included in all experiments. The following protocol within a PTC-100TM thermocycler (MJ Research Inc.) was used, employing a pre-denaturation step of 94°C for 2 minutes, followed by 34 cycles of 94°C for 30 seconds, 56°C for 60 seconds, 72°C for 2 minutes each, and a final extension step of 72°C for 10 minutes, after which the temperature was held at 10°C. This was followed by agarose gel electrophoresis, purification of the rRNA (QIAquick PCR purification kit, Qiagen) and DNA sequencing (Source BioScience, University of Oxford, UK). The resultant sequence was then compared with existing databases of bacterial DNA sequences (http://blast.ncbi.nlm.nih.gov). All samples underwent amplification of the 16S rRNA gene V2-V3 region using

universal oligonucleotide Denaturing Gradient Gel Electrophoresis (DGGE) primers 2 and 3[18] within a 50µl reaction containing 1.25 units Go *Taq* DNA polymerase (Promega), 10µl supplied buffer (final $MgCl_2$ concentration 1.5mM), 0.2mM each dNTP, 1.0mM each primer and approximately 20µl template DNA. A touchdown protocol within a PTC-100TM thermocycler (MJ Research Inc.) was used, employing a pre-denaturation step of 94°C for 2 minutes, then 15 cycles of 94°C for 30 seconds, primer annealing steps of 30 seconds starting at 65°C and descending 1°C each cycle, and extension at 72°C, followed by 10 cycles of 94°C, 50°C, 72°C for 30 seconds each, and a final extension step of 72°C for 10 minutes, **Figure II**.

Denaturing Gradient Gel Electrophoresis was undertaken to provide a molecular DGGE profile or signature representing the bacterial species present along the construction process, which allowed tracking of potential contaminants. Saliva was taken as a baseline of bacteria derived from the participant. PCR amplicons were separated on 10% polyacrylamide gels containing a linear gradient of urea (40 to 70%) using a CBS Scientific DGGE electrophoresis system, run initially at 30V for 1 hour, then at 60V overnight at a constant temperature of 60°C in 22 litres if 0.5x Tris-acetate-EDTA (TAE) buffer, stained for 30 minutes in 1x TAE containing SYBR green nucleic acid gel stain (10:4 dilution) and visualised using an ultraviolet transilluminator and Kodak EDAS image capture. A profile marker was constructed of known bacterial species with distinctly separate bands on the DGGE to monitor appropriate electrophoresis of DNA within the gel. These 5 bacteria were: *Actinomyces viscosus*, *Tannerella forsythia*, *Eikenella corrodens*, *Fusobacterium nucleatum* and *Streptococcus constellatus.*

Results

Samples from the impression, cast and appliance were cultured under aerobic, oxygen-depleted and anaerobic conditions for each participant. The most significant contamination was found on the constructed orthodontic appliance. This was consistent across all three appliances investigated. Only one impression for participant number three showed any contamination, but this was at a very low level. Bacteria were cultured in the oxygen-depleted environment from only one of the casts. The results are displayed in **Table I**.

Twelve phenotypically different colonies were identified from bacteria cultured from the stone cast and the removable appliance for participant 1. Six phenotypically different colonies were identified from the cultures from the appliance for participant 2 and eight phenotypically different colonies were identified from the cultures from the impression and the appliance from participant 3. DNA extraction and 16S rRNA gene amplification allowed identification, and separately, separation by DGGE of the V2 to V3 region of the gene, enabled comparison of those species identified by microbiological methods and those identified by molecular

techniques. These bacteria were identified to be species either within the *Kocuria*, *Rothia*, *Micrococcus*, *Brachybacterium*, *Staphylococcus, Actinomyces* or *Klebsiella* genera.

Molecular techniques

DNA extraction from the eight samples taken along the construction process of an orthodontic appliance followed by PCR amplification of the 16S rDNA using DGGE primers, and subsequently, electrophoresis on agarose gel allowed visualisation by ultraviolet transillumination. The agarose gels for each participant are shown in **Figure III**. The molecular techniques are more sensitive in detecting bacteria, although it is not possible to differentiate between viable and non-viable bacteria, thus there is a potential to over-estimate the bacterial content.

PCR products were obtained for more samples from participant 1 and 3 than participant 2 (**Figure III**). Bacterial contamination, as indicated by a PCR fragment from a sample, was always detected by 16S rDNA PCR amplification and DGGE for saliva and the pumice solution for each participant. The Palamat and ultrasonic solutions appeared to have significant bacterial loads for participants 1 and 3. Unfortunately, PCR products were not always detected for the final appliance, even though this was shown by microbiological techniques. Due to the poor isolation of DNA from the final appliance, a swab of the bacteria grown from the appliance was also used (8S) and DNA extracted to allow comparison of those cultured bacteria on the DGGE.

The PCR amplified 16S rDNA, obtained using DGGE primers from each stage of the construction process, was further analysed by DGGE for each participant. 16S rDNA from cultured bacteria were also run alongside each stage, to potentially identify the genotype of similar bands. Similar results were achieved for each participant with bacteria identified at the same points along the construction process. An example is shown below for participant 3. DGGE profiles (**Figure IV**) reflect bacterial diversity from samples taken during the construction of an orthodontic appliance. There are a large number of bands in the saliva sample reflecting diversity of the bacteria present. Distinct DGGE profiles were obtained for the Palamat solution, pumice and ultrasonic solution. These appear to relate to the bacterial profile of the final appliance (swab) indicating the potential source of contamination of the final appliance. There are very faint bands present in the samples from the impression and cast, which shows that DNA products are present in these solutions but suggesting very low numbers of bacteria present. The microbiology showed that there were bacteria present on the impression but not on the cast.

Statistical comparison of the lanes that each sample created on the DGGE gives some indication of the overall

similarity between the samples, thus indicating whether the bands (showing a specific bacterial DNA signature) are similar and therefore if the bacteria common between samples (**Figure V & Table II**).

The dendogram (**Figure V**) shows the similarity between the DGGE profiles and thus between the samples. The saliva sample, impression and cast all appear on the same branch of the tree (Lanes 1-3) indicating that these are most similar to each other, showing that bacteria is passed from saliva to impression to the cast. The similarity between saliva and the impression is 0.3 and 0.21 with the cast (**Table II**). This similarity is weak but the diversity of bacteria present in saliva is not found in the other two samples as they only show three weak bands. The microbiology technique has confirmed that only a few bacterial colonies were found on the impression in this case and contamination was minimal. The bands that appear on the DGGE could represent dead or denatured bacteria, which could not be cultured. Importantly these bacterial signatures were not found further into the appliance construction process, indicating that the bacteria from the patient are not passed into the laboratory and therefore cannot potentially cross-contaminate appliances being constructed for other patients. Greater similarities were found between samples from solutions in the orthodontic laboratory with the Palamat and ultrasonic solutions having the greatest similarity (0.8) in terms of bacterial signatures. The bacterial signatures on the final appliance also had the greatest similarity with the Palamat solution (0.67) and the ultrasonic solution (0.57).

Further investigation of the potential sources of contamination of the final appliances was undertaken using microbial culture of the solutions from the orthodontic laboratory. The previous molecular techniques proved difficult and unreliable for extraction of DNA from the bacteria present within the solutions. This was due to difficulties in obtaining the bacteria without contaminating the GenElute© membranes with other particles, for example the pumice. Microbiological culture (as previously described) of the solutions from the Palamat pressure polymerisation chamber, pumice and the ultrasonic solution, enabled subculturing of individual species, DNA extraction and 16S rRNA gene amplification (with universal 27F and 1492R primers) for bacterial identification. The species identified were unique to each solution, with those isolated from the Palamat polymerisation chamber being mainly *Cupriavidus, Pseudomonas, Corynebacterium,* and *Sphingomonas* genera. The ultrasonic solution had a different microflora of *Micrococcus, Candida, Kocuria* and *Gordonia* genera and the pumice solution contained *Brevundimonas, Corynebacterium* and *Klebsiella* genera. Bacteria identified within the solutions used in the orthodontic laboratory correlated with most of the bacteria found on the constructed appliances, demonstrating that the source of contamination was most probably from these solutions.

Discussion

Contamination within the dental laboratory has previously been shown to occur with inadequately disinfected impressions[4], by bacterial transfer from impressions to stone casts[12] and from the pumice slurry[17,19]. Our investigations into the potential contamination from patient to final orthodontic removable appliance construction found there to be very little contamination associated with disinfected impressions, with only one of the three impressions investigated showing any contamination (2.66×10^{1} cfu ml^{-1}). This contamination was not transferred to the stone cast. Only one cast was found to have any bacterial growth, and again this was extremely low with no growth identified from the initial impression. Therefore this contamination can only be assumed to have been from within the laboratory[14]. Molecular analysis of the process of construction of an orthodontic appliance clearly showed that there was no contamination from the patients' saliva to the dental laboratory and that the disinfection procedure was effective. Some weak bacterial signatures were found in the solutions from the impression and the stone cast when none were identified by standard microbiological techniques. This can be explained by the nature of the experiments. DNA extraction from a solution containing bacteria does not discriminate between viable and non-viable organisms. Therefore dead or denatured bacteria, not capable of further growth, will still be present and DNA will still be extracted, leading to a potential overestimation of bacteria present within the solution. These tests are highly sensitive and capable of amplifying very small samples of DNA present. The use of molecular techniques has shown far superior results in identification of the bacterial species present in a sample in comparison to bacterial cultures on enriched media [18]. The use of PCR and DGGE combined with GC-rich primers at the 5′ end has been shown to identify almost all of the sequence variations in a sample [20] .This method allows direct identification and relative abundance of the bacterial species present in a sample, without the risks of inability to directly culture by conventional methods only [18]. Some deficiencies of DGGE are that multiple species may be present in one band when examining complex microbial communities [21], but PCR amplification and identification were undertaken of the samples taken at each stage in the construction process to enable identification of the species present. These were found not to be salivary in origin, which corroborates the DGGE profiles. Overestimation can also be a problem when using PCR-amplified 16S rDNA due to the possibility of heterogeneity of the 16S rDNA amplicons. This is reduced using specific touchdown protocols as employed in this study, which reduces spurious amplification during PCR [21].

Solutions investigated within the laboratory showed significant contamination (using both microbiological and molecular approaches), which is consistent with the findings of previous studies[14,17,19,22]. Within the dental hospital orthodontic laboratory, solutions are changed on a weekly basis, but the receptacles are not thoroughly cleaned with antibacterial agents at the same time. One study investigating potential contamination from the pressure polymerisation unit[22] revealed *Candida albicans, Pseudomonas sp.*, and an unidentified gram-negative rod to be present. Our results showed slightly different flora including *Cupriavidus, Pseudomonas, Corynebacterium,* and *Sphingomonas* genera, but confirmed the pressure polymerisation unit as a potential contamination source. Bacteria identified from the pumice solution were of the *Brevundimonas, Corynebacterium* and *Klebsiella* genera. The ultrasonic solution revealed *Micrococcus, Candida, Kocuria* and *Gordonia* genera. Although these bacteria may be considered as environmental, being found in soil and water systems, they may be potentially pathogenic in susceptible individuals. Case reports have shown infectivity by several of these bacteria, but they are generally catheter-related bacteraemias[23,24] or infections in immunocompromised individuals[25], with one report of fatal infection[26]. The actual risk these organisms pose to technicians or patients is unknown, but reducing the risk of potentially infective solutions would be advisable. The bacteria/fungi identified in this experiment are unlikely to be exhaustive, as DNA extraction was not successful in all cases. Further work would be required to fully establish the identities of other potential bacteria.

The number of investigations was reduced to three volunteers due to the results found during these investigations. It was clear that our disinfection protocols were satisfactory and that the contamination of the final appliances was from the laboratory. The laboratory is a busy department, constructing orthodontic appliances for use with patients. During this study, it was felt that we needed to change the practice within the laboratory on the preliminary findings of this investigation to protect patients from potential harm due to cross-contamination from the orthodontic laboratory to the final appliance.

This study into cross-contamination across the orthodontic laboratory has shown some interesting areas that need to be resolved. A similar study investigating the contamination across the prosthetic dental laboratory found that cross-contamination of non-disinfected patient's dentures onto sterile dentures was possible from the polishing wheels[27]. This study also showed aerosolisation of these bacterial contaminants to the technician. Unfortunately this study did not identify the cultured bacteria. Investigations into contamination by commercial dental laboratories found that 90% of sterile denture repairs were returned with bacterial contamination[28]. The final appliance, in our study, ready for placement in patients was found to be the most contaminated of the steps

investigated using microbiological techniques ($1.3x10^2$-$1.61x10^3$ cfu ml^{-1}). The bacteria identified were of the *Kocuria*, *Rothia*, *Micrococcus*, *Brachybacterium*, *Staphylococcus*, *Klebsiella* and *Actinomyces* genera. The potential pathogenicity of these organisms is unknown from contamination of removable orthodontic appliances, but has been shown to be infective in susceptible, immunocompromised patients. However, this is generally related to invasive medical treatment, indwelling urinary catheters[23] or central venous catheters. *Klebsiella* has been implicated in nosocomial infections, particularly neonatal bacteraemias[29] and there has been one report showing *Staphylococcus pasteuri* bacteraemia possibly due to sausage consumption in an immunocompromised individual[30]. The presence on a removable appliance could therefore potentially cause infection in susceptible individuals.

Conclusion

To date, no other studies have been identified which quantify and identify whether orthodontic appliances are contaminated prior to placement in patients. Examination of the construction process in this study has demonstrated removable orthodontic appliances to be potential sources of contamination, although the contamination itself is from within the laboratory, rather than cross contamination from patient's impressions. It is also unknown whether the levels of bacteria identified in this study have the potential to cause any ill effects in patients. Nevertheless appliances leaving the laboratory should be free from contamination when delivered to the patient. The final stage in the orthodontic laboratory production protocol for the disinfection of appliances involves immersion in 0.5% aqueous chlorhexidine solution, but this stage was found to degrade the acrylic and has been omitted from current practice at University Hospitals, Bristol, UK. The acrylic used in the construction of orthodontic appliances is cold cure acrylic (polymethyl methacrylate) rather than the heat-cured alternative used for the construction of dentures. Although the chemistry is the same for both acrylics, cold-cure acrylic has a lower molecular weight, which adversely affects the strength and also has increased levels of uncured resin. This may account for the degradation noted by the laboratory staff. It is clear from this study that an effective, alternative final decontamination solution should be identified for use with orthodontic removable appliances that will not affect the acrylic. Commercially available orthodontic cleaning products containing hypochlorite may be more appropriate to ensure disinfection.

Conflict of interest

The authors declare there are no conflicts of interest. This research forms part of the thesis of the first author for the degree of Doctor of Dental Surgery (D.D.S.) of the University of Bristol.

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