Microbial contamination of removable prosthodontic appliances from laboratories and impact of clinical storage

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IN BRIEF

- Appliances returned from laboratories are often contaminated with bacteria.
- Infection control should not be left to the laboratory but the clinician treating the patient.
- A policy detailing the responsibility of the dental team in the control of infection from laboratories is advisable.

PRACTICE

Decontamination of dental instruments has recently been the subject of considerable debate. However, little information is available on the potential bacterial colonisation of dental appliances returning from dental laboratories and their need for decontamination. This study investigated the extent and nature of microbial contamination of removable prosthodontic appliances produced at different dental laboratories and stored in two clinical teaching units (CTU 1 and CTU 2) of a dental hospital and school. Forty consecutive dental prosthodontic appliances that were being stored under varying conditions in the two clinical teaching units were selected for study; the appliances having been produced 'in-house' (hospital laboratory) or 'out-of-house' (external commercial laboratory). Two appliances, that were known to have undergone decontamination before storage, were used as controls. Swabs were taken according to a standard protocol and transferred to the microbiological laboratory with bacterial growth expressed as colony forming units (cfu) per cm^2. Microbial sampling yielded growth from 23 (58%) of the 40 appliances studied (CTU 1, n = 22; CTU 2, n = 18), with 38% of these having a high level of contamination (>42,000 cfu/cm^2). The predominant bacteria isolated were *Bacillus* spp. (57%), pseudomonads (22%) and staphylococci (13%). Fungi of the genus *Candida* were detected in 38% of the samples. There was no significant difference in contamination of the appliances in relation to either their place of production or the CTU (p >0.05). However, the level of contamination was significantly higher (p = 0.035) for those appliances stored in plastic bag with fluid (n = 16) compared to those stored on models (n = 19). No growth was recovered from the two appliances that had undergone decontamination before storage. The research showed that appliances received from laboratories are often contaminated and therefore there is a need for routine disinfection of such items before use and a review of storage conditions required.

INTRODUCTION

Healthcare providers have a responsibility to minimise the potential for transmission of infectious agents between patients and staff. Therefore, a number of infection control procedure guidelines have been proposed to reduce the risk of spread of microorganisms via contaminated materials or medical devices. Sources of such information have come from the British Dental Association and the European Community Medical Devices Directive.[1,2] It should be noted, however, that such documents provide guidance and are neither statutory requirements nor directives.

Previous studies have revealed that dental laboratories are a source of contamination of,[3-6] with Wakefield (1980) finding that 90% of dentures received from dental laboratories were contaminated with potentially pathogenic microorganisms, possibly originating from other patients. Local disinfection procedures will reduce the potential for cross infection from the clinic to the laboratory and it is seen as good practice to disinfect all appliances and material being forwarded to a dental laboratory. Agents employed for decontamination of appliances include: hypochlorites, phenolics, peroxygen-based disinfectants, alcohol, aldehydes, and chlorine dioxide. The effectiveness of such disinfectants against different types of microorganism varies and this needs to be borne in mind when trying to optimise disinfection procedures. It has been shown that a ten minute exposure to a 1% sodium hypochlorite solution (10,000 ppm available chlorine) was an effective process for the disinfection of dental prostheses.[7]

A specific challenge when disinfecting prosthodontic appliances relates to potential interaction between appliance components and the disinfectant solution. For example, it has been shown that tarnish and corrosion of cobalt chromium alloy frameworks can occur following appliance exposure to hypochlorite based disinfectants.[8-10] Disinfection of dental appliances continues to provide a microbiological challenge as novel products and alternative techniques are developed.[11]

It is recognised that the responsibility for disinfection of dental appliances between the clinic and the laboratory lies with the dentist. Similarly on receipt of the appliance from the laboratory the dentist is also responsible for disinfection before insertion in the patient. A systematic

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review[12] found that overall the knowledge and attitudes of dental care professionals towards infection control procedures was poor. While in recent years there have been improvements in compliance with infection control procedures, some aspects remain problematic.[13]

Dental laboratory personnel are in a position to minimise contamination of appliances during stages of production and as such should give consideration to potential areas of improvement. Jagger *et al.*[14] indicated that there was a low level of interest by laboratory staff towards infection control among their sample of 800 dental laboratories. Only half (49%) of the laboratories that completed the questionnaire had a cross-infection policy, the majority (61%) did not use a disinfectant in their pumice, and 93% did not disinfect their polishing instruments. A number of other studies have discovered that pumice used for finishing dental appliances can be heavily contaminated with microorganisms[15-20] and therefore this substance should be considered a serious source for potential cross-infection.

While clear policies exist for disinfection of appliances before transfer from the clinic to the laboratory, an inconsistency for disinfection between laboratory and clinic exists. With dental laboratories increasingly subscribing to The Dental Laboratories Agency and their participation in schemes such as the Dental Appliance Manufactures Audit Scheme (DAMAS) in order to satisfy Medical Device Directives and Medical Devices Regulations, it was considered useful to determine the nature and extent of any microbial contamination on removable prosthodontic appliances in order to continue to assist the development of best practice protocols for dental laboratory and clinical infection control.

The aim of the present study was to assess microbial colonisation of removable prosthodontic appliances after fabrication of appliances in dental laboratories from laboratories and identify if condition of storage may impact on such contamination.

MATERIALS AND METHODS

Appliances

A total of 40 removable prosthodontic appliances, stored in one of two clinical units (CTU 1 or CTU 2) of the dental hospital and school, were studied (Table 1). The source of each appliance was recorded as either 'in-house' (prosthodontic laboratory of the dental hospital and school) or 'out-of-house' (external commercial laboratory). The type of appliance and its storage conditions were also recorded. The

Table 1 Source of production, site of storage, type of appliance and storage conditions for 42 appliances studied

Sample	Production source	Storage site	Type of appliance	Storage conditions
1	Out-of-house	Unit 1	c/- acrylic	On model
2	Out-of-house	Unit 1	p/-try in, wax	On model
3	In-house	Unit 1	-/p try-in, wax	On model
4	In-house	Unit 1	c/- try-in, acrylic + wax	On model
5	In-house	Unit 1	c/- acrylic	Plastic bag + water
6	Out-of-house	Unit 1	c/- try-in, wax	On model
7	In-house	Unit 1	p/- try-in, wax	On model
8	Out-of-house	Unit 1	-/p try-in, wax	On model
9	In-house	Unit 1	p/- try-in, wax	On model
10	Out-of-house	Unit 1	c/- try-in, wax	On model
11	Out-of-house	Unit 1	p/- acrylic + Co/Cr	On model
12	Out-of-house	Unit 1	-/p acrylic + Co/Cr	On model
13	Out-of-house	Unit 1	-/p acrylic + Co/Cr	On model
14	Out-of-house	Unit 1	p/- acrylic + Co/Cr	On model
15	Out-of-house	Unit 1	p/- acrylic	On model
16	Out-of-house	Unit 1	c/- acrylic	Plastic bag + water
17	In-house	Unit 1	c/- acrylic	Plastic bag + water
18	In-house	Unit 1	-/c acrylic	Plastic bag + water
19	In-house	Unit 1	c/- try-in, acrylic + Co/Cr	Plastic bag + water
20	In-house	Unit 1	p/- acrylic + Co/Cr	Plastic bag + water
21	In-house	Unit 1	-/p acrylic + Co/Cr	Plastic bag + water
22	In-house	Unit 1	c/- acrylic	Plastic bag + water
23	Out-of-house	Unit 2	-/p try-in, wax	On model
24	Out-of-house	Unit 2	p/- try-in, wax	On model
25	In-house	Unit 2	c/- acrylic + Co/Cr	Plastic bag
26	In-house	Unit 2	-/p acrylic + Co/Cr	Plastic bag
27	Out-of-house	Unit 2	p/- acrylic + Co/Cr	Plastic bag
28	In-house	Unit 2	p/- try-in, wax	Plastic bag
29	In-house	Unit 2	c/- acrylic	Plastic bag
30	In-house	Unit 2	-/c acrylic	Plastic bag
31	Out-of-house	Unit 2	p/- acrylic	Plastic bag + water
32	Out-of-house	Unit 2	-/p acrylic	Plastic bag + water
33	In-house	Unit 2	c/- acrylic	Plastic bag + water
34	In-house	Unit 2	p/- try-in, wax	On model
35	In-house	Unit 2	p/- try-in, wax	On model
36	Out-of-house	Unit 2	-/c wax	On model
37	Out-of-house	Unit 2	-/p acrylic + Co/Cr	Plastic bag + water
38	Out-of-house	Unit 2	p/- acrylic	Plastic bag
39	Out-of-house	Unit 2	p/- acrylic + Co/Cr	Plastic bag + water
40	In-house	Unit 2	p/- acrylic + Co/Cr	Plastic bag + water
*41	Out-of-house	Unit 2	c/- try-in, wax	Plastic bag + water
*42	In-house	Unit 2	p/- try-in, wax	Plastic bag + water

*Control appliances; c/- complete upper; -/c complete lower; p/- partial upper; -/p partial lower; Co/Cr cobalt chromium

researchers were not aware of any local decontamination procedures that may have been employed by the laboratories. Two appliances, known to have undergone disinfection as described in the protocol for laboratory work of the local NHS Trust, were also included as controls (Table 1). Disinfection of these control appliances involved immersion in a disinfectant containing 10,000 ppm available chlorine (Haz-tabs, Guest Medical, Edenbridge, Kent, TN8 6EW, UK).

Microbiological sampling

Microbiological samples were obtained by swabbing a region (a 1 × 5 cm line) of the anterior flange of the appliance (or if not present, the posterior flange) and a similar 1 × 5 cm line on the fitting surface of the appliance. Each sterile cotton swab (Medical Wire and Equipment, Corsham, UK) was moistened with phosphate buffered saline (PBS) and then applied using horizontal strokes for one minute to the swabbed areas. The swabs were transferred to 1 ml of PBS (0.85%, pH of 7.4; Oxoid Ltd, Basingstoke, UK) and maintained in this buffer for 45 minutes to standardise storage while in transit to the microbiology laboratory for processing.

Culture and identification

Culture media used included blood agar (BA; Lab M™, Diagnostic Group Plc., Bury, UK); supplemented with 5% V/V defibrinated sheep blood (TCS Biosciences Ltd., Buckingham, UK), MacConkey agar (MAC; Lab M™) and Sabouraud dextrose agar (SAB; Lab M™). Plates were inoculated with 50-ml of serial decimally diluted portions of each sample using a Don Whitley Automatic Spiral Plater (Don Whitley Scientific Limited, Shipley, UK). Replicates for each sample were performed. Plates were incubated aerobically at 37°C for 48 hours and growth recorded as total colony forming units (cfu/cm^2). Contamination of each appliance was subsequently categorised into one of four levels (I-IV) as follows; I, 0 cfu/cm^2; II, 1-2 × 10^2 cfu/cm^2; III, 2 × 10^2-4.2 × 10^4 cfu/cm^2; and IV, >4.2 × 10^4 cfu/cm^2.

Representatives of individual morphological colony types on the primary culture plates were sub-cultured on to fresh agar for pure growth and identification using a combination of Gram staining and biochemical tests. All isolates of *Staphylococcus aureus* were tested for susceptibility to meticillin using an E-test (AB-Biodisk, Solna, Sweden).

Table 2 Overall microbial count (cfu/cm^2) observed on three different culture media following inoculation with swabs from the 42 appliances studied

Sample	Blood agar	MacConkey agar	Sabouraud dextrose agar
	cfu/cm^2	cfu/cm^2	cfu/cm^2
1	32,000	26,000	24,000
2	36,000	28,000	14,800
3	24,000	5,400	3,200
9	<1	2	0
10	6,000	4,000	2,800
16	6,800	5,000	5,600
17	>42,000	>42,000	>42,000
18	5,400	7,200	2,800
19	>42,000	>42,000	3.6
20	>42,000	>42,000	3,400
21	>42,000	>42,000	6,400
22	14	5	1
27	>42,000	3,800	0
29	3	0	0
30	3	0	0
31	15,200	2,600	0
32	19,600	2,800	>42,000
33	>42,000	>42,000	0
34	17	9.6	0
37	3	<1	27
38	>42,000	>42,000	2
39	15	14	6
40	27	9	0
41	0	0	0
42	0	0	0

No counts recorded on samples 4-8, 11-15, 23-26 and 28; Samples 41 & 42 were the controls
>42,000 cfu/cm^2 represents the upper limit of quantification using the spiral plater system and the sample dilutions applied
cfu/cm^2 values represent average of replicate spiral plates
Blood agar provided data on total aerobic bacterial growth (cfu/cm^2); MacConkey agar is selective for Gram negative bacteria and able to differentiate lactose fermentation; Sabouraud's agar was used for enumerating fungal growth

Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (Chicago, IL, USA).

RESULTS

Appliances and storage conditions

Of the 40 test appliances, 22 were stored at CTU 1 and 18 at CTU 2. The type and material construction of the appliance (complete denture, partial denture, acrylic, cobalt-chrome, wax) and conditions of storage (model, plastic bag damp, plastic bag with fluid) of the test and the two control appliances are presented in Table 1.

Microbial colonisation

The microbial growth obtained from the study appliances ranged from no growth to >4.2 × 10^4 cfu/cm^2 (Table 2). No growth was recovered from 17 of the test appliances and both of the control appliances. A total of 23 (58%) of the test appliances yielded a positive growth, with 15 (38%) having a contamination at the higher levels of Category III or IV. There was no statistical difference in terms of contamination level depending on source of production (p = 0.193; Table 3).

Analysis using Chi-squared test also revealed that there was no statistically significant difference in the pattern of contamination (Categories I and II *versus* Categories III and IV) depending on site of storage in the two CTUs (p = 0.265; Table 3). However, appliances stored in a bag with fluid in both

CTUs had a significantly greater incidence ($p = 0.035$) of higher levels of contamination (Category III and IV) compared to those appliances stored on models.

Microbiological findings

A spectrum of bacterial species was recovered from the 40 appliances, the two main groups being Gram-negative rods and Gram-positive cocci. Strains of *Bacillus* spp. accounted for the majority (57%) of the isolates along with pseudomonads (22% of isolates) and staphylococci (13% of isolates). Of the staphylococci detected, one isolate was identified as being a meticillin resistant *Staphylococcus aureus* (MRSA). *Candida* species were found to be present on 38% of the appliances tested.

Table 3 Number of appliances (%) in each contamination category (I–IV) depending on source of production (in-house or out-of-house) and site of storage (Unit 1 or Unit 2)

Contamination category	Number of appliances	'In-house'		'Out-of-house'		Unit 1		Unit 2	
I	17	7	41%	10	59%	10	59%	7	41%
II	8	6	75%	2	25%	2	25%	6	75%
III	5	1	20%	4	80%	3	60%	2	40%
IV	10	6	60%	4	40%	7	70%	3	30%

Contamination category I: 0 cfu/cm^2; Category II: 1-2 × 10^2 cfu/cm^2, Category III: 2 × 10^2-4.2 × 10^4 cfu/cm^2; Category IV: >4.2 × 10^4 cfu/cm^2

DISCUSSION

Removable intra-oral prostheses usually contact intact oral mucous membranes and would not require to be sterile at point of use, however, even for healthy patients good clinical practice would dictate that such appliances are clean and disinfected before try-in. In the case of removable prosthodontic appliances, a significant number are used by older individuals and debilitated patients who may have an increased susceptibility to infection.[4] Regardless of the host status, it could be argued that the presence of any microorganisms, even those regarded as non-pathogenic, represents an infection risk that should be addressed if possible.

The present study has revealed that few of the appliances had no recorded cfu/cm^2. However, such a finding is not necessarily indicative of a total absence of microbial presence on an appliance and in this study it could be due to the limitations over swabbing efficiency and sensitivity of subsequent spiral plating. However, half of the appliances were contaminated, sometimes with relatively high levels of microorganisms under the categories defined in the study. It was noted that the differences in the levels of contamination did, in part, relate to whether the appliances were stored moist or on a model. However, another factor that could have affected the results could have been the duration of storage, although this information was not available at the time of study. Given that appliances leaving the dental laboratories can be highly contaminated (as defined in the current investigation) then this research highlights the need for appliances to be disinfected by dental care professionals and dentists either on arrival in the clinic or before being used on a patient. As shown by this research it cannot be assumed that disinfection procedures have been performed by the returning laboratory, or indeed if they have, that they are effective. It could be argued that regular spot checks or auditing of prosthodontic appliances should be performed if routine disinfection of appliances is not to be undertaken before being inserted into patients.

It is stated that 'Infection control is a dynamic and ever-changing subject and all dental staff should be kept aware of the most up-to-date procedures required to prevent the transmission of infection'.[21] It is clearly the responsibility of all the members of the dental team, including laboratory personnel, to endeavour to ensure that all appliances are correctly disinfected before they reach patients. This is of particular concern given that previous studies have shown poor levels of compliance for cross infection control protocols for appliance at the clinical/patient.[14]

On this basis of these results we would advocate a disinfection policy to be used by dental laboratories for all appliances returning to clinics or dental practices. The implementation of such a policy would be a valuable in the prevention of cross infection between the laboratory and the clinic. Consistently applied disinfection procedures at a number of clearly defined stages such as before leaving the clinic, on receipt at the laboratory, before leaving the laboratory, and before delivery to the patient would be seen as perhaps best clinical practice and reduce cross infection risks significantly.

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