A preliminary investigation of the microbiology and endotoxin content in the water reservoirs of benchtop non-vacuum autoclaves

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Summary To determine the microbial content and endotoxin concentration in the water reservoirs of benchtop autoclaves used in general dental practice.

Design The study was done in two stages. Firstly water samples were taken daily from the reservoirs of 20 autoclaves used in general dental practices for six days. The microbial content and endotoxin concentration was determined. Second the practitioners were instructed to wash and clean the reservoirs of the autoclaves each morning prior to refilling with fresh water. The reservoirs were sampled in the evenings after a day's use and the microbial content and endotoxin concentration determined.

Results The total viable count of bacteria before cleaning varied from 2300 – 8×10^4 cfu/ml and after from 0–40 cfu/ml. The endotoxin concentrations before cleaning varied from 360 – 2200 EU/ml and after 0–80 EU/ml.

Conclusions The reservoirs of non-vacuum benchtop autoclaves can become severely contaminated with micro-organisms particularly Gram-negative bacteria. The endotoxin content of the reservoirs can get to high and unacceptable concentrations. Draining the water reservoir and cleaning on a daily basis reduces the microbial content and the endotoxin concentration to acceptable levels.

Benchtop non-vacuum autoclaves are widely used for the sterilisation of dental instruments. They are usually filled with distilled or purified water which is turned into steam in the sterilisation process and condensed in a water reservoir. The recommendations in infection control guidelines are that the water in benchtop autoclaves is changed regularly.^{1,2} There have been no reported surveys on how frequently the water reservoirs in benchtop autoclaves in dental practices are changed.

Water reservoirs can become contaminated with Gram-negative bacteria.³ Gram-negative bacteria release endotoxin from their cell walls when they lyse and this can cause deleterious effects on the functioning of autoclaves.⁴ Instruments can also become contaminated with endotoxins which can cause delayed wound healing and, if introduced systemically, severe shock.

The purpose of this investigation was to determine the endotoxin and bacteriological content of water reservoirs in general dental practices.

Methods

Practices and samples taken

Twenty general dental practices on Merseyside were chosen for study. The practice policy for emptying of the autoclave reservoir was noted. All of these practices used the same model of a popular autoclave. Samples of the reservoir water (approximately 10ml) were taken aseptically from the water reservoir using pyrogen-free disposable syringes and containers (Bibby-Sterilin, Stone, Staffordshire). The water samples were used for total viable count (approximately 4ml) and endotoxin estimation (approximately 6ml). Samples were taken between 8.30am and 9.30am before the autoclave had been used. The samples were taken on the mornings of Wednesday, Thursday and Friday and on Monday, Tuesday and Wednesday of the following week.

At the second visit water samples were taken from the autoclaves approximately 1h after they had been turned off after the days surgery; this was at approximately 6.30p.m. The water samples were again assessed for endotoxin concentration and microbial content. The dental nurses were asked to evacuate the water reservoir in the autoclave before surgery began the next morning, wash out the chamber with soapy water and rinse with water to get rid of the detergent. The cleaned reservoir was then refilled with fresh distilled water.

Microbiology

The water samples were filtered through 20 µm pore size analytical test filter funnels (Techware, Poole, UK) to remove any particulate matter. The water samples (0.1ml), in triplicate, were then serially diluted ten fold with PBS and 0.1 ml samples were plated onto 5%(v/v) blood agar for total viable counts. The identity of the main colonies present was made using standard identification methods.

Endotoxin estimation

Endotoxin estimations were made using the Coatest commercial kit (Quadrantech, Epsom, Surrey). Heating all chemically clean sterile glassware used to 180°C for 4hours in a hot air oven eliminated possible contamination by pyrogens. The water samples were centrifuged at 1.75×10^5g for 15 min and filtered through a 0.2µm pore size filter (Techware, Poole, UK) to remove any residual bacteria. The supernatant was then diluted with pyrogen-free distilled water and the endotoxin concentration determined through the use of the Coatest commercial kit. The nitroaniline released by the Coatest was measured at 410nm on a microplate reader (MR700, Dynatech, Billingham, Sussex) and the endotoxin concentration calculated from a standard curve in units per ml. All samples were tested in triplicate batches which included standards with high and low endotoxin controls to check the reproducibility of the assay. All endotoxin concentrations were expressed in endotoxin units per ml (EU /ml).

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Table 1. Mean total viable counts from the autoclave reservoirs before and after cleaning. The results are expressed in cfu/ml and the range is shown in parenthesis.

Sample	Days of the week					
	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday
Before cleaning	6380 (1000–3×10 ⁴)	15761 (1000–4×10 ⁴)	351336 (2000–8×10 ⁴)	40095 (2000–5×10 ⁴)	18142 (2000–5×10 ⁴)	17333 (2000–8×10 ⁴)
After cleaning	8 (0–20)	10 (0–40)	16 (5–40)	16 (0–40)	13 (0–50)	12 (0–40)

Table 2. The mean endotoxin concentration in the autoclave water reservoirs before and after cleaning. The results are expressed in EU/ml and the range is shown in parenthesis.

Sample	Days of the week					
	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday
Before cleaning	1090 (360–2200)	1025 (360–2100)	1104 (410–2000)	1335 (560–2100)	1191 (580–2000)	1186 (610–2000)
After cleaning	24 (10–60)	26 (0–50)	30 (0–60)	27 (0–60)	27 (10–70)	25 (0–80)

Statistical analysis

The results were entered into the Statistical Package for Social Sciences (SPSS) database.⁵ From these data descriptive summaries and t-tests were undertaken.

Results

None of the 20 practices had definitive policies for the emptying of the autoclave reservoirs.

Microbiology

The mean total viable bacterial counts in the reservoirs from the first and second samples are shown for each day are shown in Table 1. The counts on the first sampling varied widely with a median of 2300 cfu/ml and a range of 1000–80,000 cfu/ml. After instituting cleaning of the reservoirs the mean counts were 12 cfu/ml with a range of 0–50 cfu/ml (Table 1). The main bacterial species isolated are shown in Table 3 together with the number of reservoirs from which it was isolated and the total number of isolations.

The endotoxin concentration before cleaning had a mean concentration of 1158 EU/ml with a range of 360–2200 EU/ml. Table 2 shows the endotoxin concentration after cleaning with a mean of 26 EU/ml and a range of 0–80 EU/ml. A significant difference was found between the first and second samples for both endotoxin concentrations (t 27.28; df 126; $P < 0.001$) and bacterial counts (t 4.466; df 126; $P > 0.001$).

Discussion

Most benchtop non-vacuum autoclaves used in dental practice contain reservoirs which supply water for the generation of steam, and for its condensation when the sterilisation process is complete. The water in most non-vacuum autoclaves is therefore re-used and it is assumed that the heat of the condensation of steam would keep the reservoir sterile. This study has shown that water reservoirs can be heavily contaminated with bacteria, particularly Gram-negatives. The condensation of water after use does not sterilise the reservoir, probably because biofilms form on the walls. It was noticeable that all the reservoirs sampled had visible evidence of biofilm formation on the walls of the reservoir. This biofilm in the reservoirs was not sampled in this study, but when removed by cleaning with detergent the bacterial counts fell to acceptable numbers, with no Gram-negative bacteria being isolated. The bacteria isolated after cleaning were mainly skin commensals, probably inoculated when the tank was filled.

Bacteria require a source of nutrients to remain viable. It is possible that the steam could carry any carbohydrates, proteins and other nutrients in the chamber into the reservoir and allow microbial growth. Another source of nutrients for the non-fastidious bacteria present in the reservoirs could be the oil used to lubricate the handpieces. Traces of oil appeared to be present on the walls of the autoclave chambers and in the reservoir water. Some of the oils used to lubricate handpieces are vegetable in origin and would provide a source of nutrient for some of the Gram-negatives.

The identity of the Gram-negative bacteria in the reservoirs is shown in Table 3. The reservoirs did contain some Gram-positive bacteria in small numbers (10 cfu/ml), but these were not considered to be of significance. The predominant bacteria isolated from the reservoirs was *Pseudomonas aeruginosa*. This Gram-negative bacterium was found in all but one of the reservoirs. *P. aeruginosa* is a non-fastidious bacterium, which generates endotoxin on lysis. Most of the reservoirs contained at least five different bacterial species (results not shown). The source of these bacteria could be derived either from the water used in the autoclave, or from the operator.

Endotoxin is a complex of carbohydrate lipid and protein that is released mainly from Gram-negative bacterial cell walls on lysis.^{4,5} Endotoxins cause pyrexia by stimulating polymorphonuclear leucocytes to produce a pyrogen and in high concentrations shock. Endotoxins can complicate the healing of wounds including those in the oral cavity and have a role in periodontal disease.⁵ More serious life threatening effects can be produced by endotoxins if systemically introduced into the body. Endotoxin occurs naturally in the mouth with the normal concentrations in children and adults

Table 3. The predominant Gram-negative bacteria found in the autoclave reservoirs in the first sampling prior to cleaning. The number of reservoirs in which each bacteria was found and the total number of isolations.

Bacterium	Number of reservoirs from which bacteria were isolated (Maximum 20)	Total number of isolations (Maximum 240)
<i>Pseudomonas aeruginosa</i>	20	211
Other <i>Pseudomonas</i> spp	8	87
<i>Klebsiella</i> spp	14	196
<i>Escherichia coli</i>	6	71
<i>Proteus</i> spp	7	98
<i>Serratia</i> spp	4	33

being about 240 EU/ml.^{6,7} The endotoxin concentrations found in the autoclave water reservoirs in the first sampling were unacceptably high. No attempt was made to measure endotoxin on the instruments being processed in the autoclaves but undoubtedly they would have become contaminated. The probable source of endotoxin was the lysis of planktonic bacteria in the reservoirs, probably by the heat of the condensing steam. The concentrations of endotoxin and bacteria after cleaning the reservoirs were reduced to acceptable levels. Distilled and potable waters all contain some endotoxin so it is probably not possible to completely permanently eradicate it from the reservoirs.

For commercial reasons the autoclaves used in this study have not been named, but they were all from a major manufacturer. Samples taken from five other makes of autoclaves (results not shown) revealed similar numbers of bacteria and endotoxin concentrations. Most manufacturers clearly state in the instructions for use of their autoclaves, that the water reservoirs must be cleaned and refilled on a regular basis.^{8,9} All the autoclaves tested in this study had removable water reservoirs which could be cleaned. Some autoclaves do not have removable water reservoirs and cannot be easily cleaned.

The cleaning of the water reservoirs in non-vacuum autoclaves is strongly recommended to prevent the build up of bacteria and endotoxin to unacceptably high numbers and concentrations.

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