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BACTERIOLOGY

Isolation of filter passing bacteria from a range of dental clinic surfaces

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Abstract. Filter passing bacteria have been isolated from a variety of natural environments, appearing as a mixture of Gram-positive and Gram-negative, as well as nano-forms and wall-free species. In this study, filter passing bacteria were isolated from surfaces located in various dental departments at the College of Dentistry, King Saud University Hospital. Surface samples were obtained by using Q-tip swabs, with ten different surfaces being sampled in each clinic during pre-patient and post-patient visits. Filterable bacteria (using 0.4 and 0.2 micron filters, but not 0.1 micron filter) were isolated, being mainly Gram-positive cocci. Isolation results of filterable bacteria were compared before and after patient treatment in the clinic. More frequently, filter passing bacteria were isolated on clinic surfaces after patient treatment. The results show that dental settings are contaminated with filterable bacteria which may act as a reservoir for the wider contamination of hospital environments.

Key words: filter passing bacteria, filterable bacteria, nanobacteria, dental clinics.

1. INTRODUCTION

Filter passing bacteria, which are also known as ultra-micro cells, dwarf cells, nanobacteria, nanoarchaea, and 'nanobes', have been widely isolated. The term nano-archaea relates specifically to the phylum Nanoarchaeota which includes organisms in the nano-range (i.e. 50–400 nm). Ultra-small-sized organisms are part of pico-plankton (0.2–2.0 μm) or femtoplankton (0.02–0.2 μm) communities [1–4], but they are not part of nanoplankton (2.0–20 μm in size). Ultra-microbacteria generally appear as a mixture of Gram-positive, Gram-negative and cell-wall-lacking species, and are either free-living or occur in symbiotic relationships. Even the smallest of cells can contain sufficient material to sustain life, having densely packed spirals of DNA, a very small number of ribo-somes, hair-like appendages, and a stripped-down meta-bolism that in some cases makes them rely on other bacteria for their existence.

Filter passing bacteria have been isolated from freshwater [5–7], hypersaline lakes [8], open oceans [9–12], acid mine drainage settings (AMD), glacial ice, permafrost [13], subterranean bedrock [14] and the human body. Earlier studies which used such environ-ment for genomic data suggest that there is a wide range of bacteria possessing small genomes, e.g. Pelagibacter ubique (1.3 Mb) [10] as well as candidate phyla radiation WWE3, OD1, OP11, and TM7 (0.7–1.1 Mb) [15–18]. These reports show that filter passing bacteria are ubiquitous (occurring also in humans) and likely play an

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important role in microbial communities, environments and possibly in human disease. Surfaces and other fomites may act as a reservoir for ultra-small bacteria, allowing them to infect patients, notably when immuno-compromised.

The aim of this study was to isolate filter passing bacteria from a variety of dental hospital clinics prior to and after patient treatment.

2. MATERIALS AND METHODS

2.1. Collection of samples

Three clinics were selected from the dental departments at King Saud University (Dental Implant Clinic, Paediatric Clinic and Restorative Clinic). Sixty samples were collected during the pre-patient and post-patient visits. Q-tips were used to swab the following surfaces: an armrest of a dental chair, the floor beneath a dental chair, a sink/faucet, a towel dispenser, a handle of a dental instrument table, a headrest, a handle of a light source, an X-ray viewer, paper dental records, and a bench. The Q-tip swab was then placed in phosphate-buffered saline and sent to the microbiology lab for culturing.

2.2. Isolation of filter passing bacteria from swab samples

Filters, 0.2μm, contained in sterile plastic bags (Nalgene, USA) were utilized. While the filter apparatus (pore size of 0.2 micron) was inside its sterile closed bag, the screw lid was removed. A sterile hypodermic syringe (20 mL) was then used to inject the LB medium, after which a piece of autoclave tape was applied to cover the syringe-made hole. The orientation of the filter was vertical. The collected swap sample in phosphate-buffered saline was injected into the filter apparatus by using a 2.5 mL sterile syringe. The hole made in the polythene bag was again sealed by autoclave tape. The sample was allowed to pass through the filter under continuous suction. After that the filter apparatus was removed and the bottle was closed, using a screw lid. The bottle was incubated at the temperature of 37 °C for different time periods (one to five weeks) after removal of the plastic bag. The turbid growth which appeared after a period of one week of bacterial incubation was cultured on LB plates. Bacteria were then sub-cultured. Bacterial isolates were not identified at this stage.

Aliquots of the bacterial growth culture solution were further cultured in nutrient broth (NB) for a period of 24 h and subsequently filtered through micropore filters with the pore size of 0.4, 0.2, and 0.1 micron into fresh sterile NB and incubated until cloudy.

3. RESULTS AND DISCUSSION

The results indicated in Table 1 show that 0.4 and 0.2 micron filterable bacteria were isolated from the surfaces of the dental operatories, including an armrest of a dental chair, the floor beneath a dental chair, a sink/faucet, a towel dispenser, a handle of a dental instrument table, a handle of a light source, paper dental records, a headrest, a bench and an X-ray viewer. The isolated bacteria were mainly Gram-negative cocci, but an occasional Gram-negative rod was also detected. In contrast, no bacteria grew from any of the samples which passed through 0.1 micron filters (Table 1).

The question arises whether the isolates obtained are true ultrasmall bacteria or large bacteria that can pass through small holes under suction. True ultrasmall bacteria are stable small forms which pass through a filter with little or no suction and remain the same size when viewed before and after filter passage. The filterable bacteria isolated in this study could be true filterable bacteria or those which can squeeze through filters under pressure, appearing as normal-sized bacteria before and after being sucked past the filter. Alternatively, there may be small forms of large bacteria which pass through the filter and regrow as normal-sized bacteria. The fact that filtrates were obtained when 0.1 micron filters were used failed to yield bacterial growth points to the latter explanation.

In general, the size of laboratory-cultivated bacteria is around 1 micron. Therefore, it is not surprising that the highest numbers of bacteria passed through 0.4 and 0.2 micron pores, but not 0.1 micron pores. The authors were unable to determine and confirm if such passage of bacteria was by 0.4 and 0. 2 intact bacteria, smaller life-cycle forms or viable bacterial cell fragments. However, a bacterial form which was able to grow in the culture medium below the membrane was able to pass through these filter membrane pores. The results reported here show that filter passing bacteria contaminate the surfaces of the dental operatories and act as a reservoir for filter passing bacteria.

Since filterability of large bacteria is considered a statistical event when passing through membranes, some of the filter membranes may have expanded pores that can allow a large bacterium to pass through the filter. As a result, the presence of ultra and nano-bacteria (small filterable forms) is ideally confirmed, for example, by using a scanning electron microscope.

4. CONCLUSIONS

In conclusion, our findings show that bacteria passing through filters were isolated from surfaces located in various dental departments at the College of Dentistry, King Saud University Hospital. Such passage of bacteria

Table 1. Filterable bacteria isolated from patients of Dental Implant Clinic, Paediatric Clinic and Restorative Clinic

Clinic Surfaces	Dental Implant Clinic						Paediatric Clinic						Restorative Clinic					
	Pore size of filter						Pore size of filter						Pore size of filter					
	0.4 µm		0.2 µm		0.1 µm		0.4 µm		0.2 µm		0.1 µm		0.4 µm		0.2 µm		0.1 µm	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
Bench	–	+	–	+	–	–	–	+	–	+	–	–	–	+	–	+	–	–
Sink/faucet	–	+	–	+	–	–	–	+	–	+	–	–	+	+	–	+	–	–
Armrest of dental chair	+	+	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	–
Floor beneath dental chair	–	+	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	–
Headrest	–	+	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	–
Handle of light source	+	+	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	–
Paper records	+	+	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	–
Instrument table	–	+	–	+	–	–	–	+	–	+	–	–	–	+	–	+	–	–
Towel dispenser	+	+	–	+	–	–	–	+	–	+	–	–	–	+	–	+	–	–
X-ray film viewer	+	+	_	+	–	–	–	+	–	+	–	–	–	+	–	+	–	–

(–) No filter passing bacteria colonies. (+) Detection of filter passing bacteria colonies.
BT = Before treatment.
AT = After treatment.

through small holes may be of great importance in the development of infections in body organs, especially in areas that can be subjected to high blood pressure and blood flow, such as kidneys. The existence of the bacteria on surfaces in dental clinics may, therefore, be of considerable medical significance.

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Filtrit läbivate bakterite isoleerimine hambaravikliinikute pindadelt

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Uuriti filtrit läbivate bakterite isoleerimist Kuningas Saudi ülikooli haigla hambaravikliinikutes, kogudes pinnaproove tööruumide erinevatelt pindadelt. Proovid koguti vatitikkude abil enne ja pärast patsiendi raviprotseduure. Filtreeritavate bakterite isoleerimiseks keskkonnast kasutati filtreid poorisuurusega 0,4 ja 0,2 mikronit, nende hulgas olid enim levinud bakterid grampositiivsed kokid. Uurimistulemused näitasid, et hambaravi protseduuride tegemise vahetu keskkond on saastunud filtrit läbivate bakteritega, mis võivad toimida haiglakeskkonna ulatuslikuma saastumise allikana.