The Relationship Between Holding Time and the Bacterial Load on Surgical Instruments

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Abstract The aim of this investigation was to determine the bacterial load on used instruments and to evaluate the relationship between the bacterial load and the holding time prior to cleaning. Thirty six sets were evaluated to establish the average number of bacteria per square centimeter. For the experimental study, three different bacteria were prepared in sheep blood and used to contaminate sterile stainless steel pieces with the surface of 10 cm². After incubation at room temperature for 2, 4, 6, 8, 12, 24, 36, and 48 h, colonies were counted and compared to time zero. Bacterial counts were between 10 and 250 CFU/cm², depending on the operation site. Bacterial load was found to have increased after 6 h. An increase of 3log₁₀ CFU/cm² was measured after 12 h. It is imperative to clean surgical instruments in the first 6 h to ensure effective disinfection and sterility.

Keywords Surgical instruments · Bacterial load · Bioburden · Holding time · Contamination

Introduction

After each intervention all surgical instruments are contaminated to a certain degree. This bacterial load depends on the site of the operation. Earlier studies have shown that cleaning is critical in removing gross debris, preventing cross contamination, protecting health

care workers and maintaining the medical instruments. The number of bacteria contaminating medical devices will determine how long it will take for a cleaning agent or disinfectant to destroy them. This is due to the genetically heterogeneous bacterial populations [1]. Very few data providing accurate estimates of the levels and the types of contamination on used surgical instruments are available. Previous studies have reported that the bacterial load was lower than 10⁴ on used surgical instruments [2–4].

The contamination may have an adverse effect on the instruments if they are not cleaned as soon as possible after use [5]. Proper cleaning prolongs the life of the instruments, keeps them in optimal condition and allows the sterility assurance level (SAL) to be reached more easily by reducing the bacterial load by 3log₁₀ and by shortening the time required to reach SAL [6].

However, the direct relationship between holding time prior to cleaning and disinfecting and bacterial load on contaminated instruments has not been established up to now. In our study, we aim to determine the bacterial load on surgical instruments and to evaluate the relationship between bacterial load on stainless steel instruments and holding time prior to their cleaning.

Materials and Methods

Evaluation of Median Numbers of Bacteria on Surgical Instruments

Thirty six different instrument sets were evaluated in order to find the average number of bacteria per square centimeter on a used instrument. The bloodiest instruments from each

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set were selected. A cotton swab which was saturated with physiological saline was scrubbed on 1 cm² of the instrument's surface. The cotton swabs were immediately put into 1 mL physiological saline and were vortexed. Immediately after vortexing, 100 µL solution from each tube was spread onto sheep blood agar. Agar plates were incubated at 37 °C for 24 h and colonies were counted as colony forming units per square centimeter (CFU/cm²).

Experimental Study to Evaluate the Relationship Between Holding Time and Bacterial Load on Stainless Steel Instruments

Three different inocula including 5×10^3 CFU/mL of *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 strains were prepared in sheep blood. They were used to contaminate steam sterilized stainless steel pieces with the surface of 10 cm². The contaminated pieces were incubated in sterile petri dishes under room temperature. After the incubation for 2, 4, 6, 8, 12, 24, 36, and 48 h, the pieces were sonicated in physiological saline and 100 µL of each solution inoculated onto sheep blood agar.

The colonies were counted and compared to time zero after overnight incubation on the basis of the time-kill kinetic assays [7]. For every incubation time the tests were repeated three times and the median bacterial count of the three tests was recorded.

Results

The number of bacteria on the different instruments is shown in Table 1. The table shows that the bacterial counts varied between 10 and 250 CFU/cm², depending on the operation site. The most contaminated instruments were used in reconstructive surgery.

Table 1 Number of bacteria on used surgical instruments

Operating room	Number of instruments	Number of bacteria (CFU/cm ²)
Abdominal surgery	12	2.10^2
Orthopedic surgery	6	$1.8.10^2$
Plastic reconstructive surgery	5	$2.5.10^2$
Cardiovascular surgery	4	60
Gynecology	3	10
Neurosurgery	3	10
Pediatric surgery	2	10
Biopsy instruments	1	No growth

Table 2 Changes in the number of bacteria over time

Time	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 25923
Time zero	1×10^2	6×10^3	1.8×10^3
2nd hour	4×10^2	6×10^2	3.2×10^3
4th hour	4×10^2	2×10^2	1×10^3
6th hour	3×10^2	4×10^2	5.4×10^3
12th hour	3×10^3	6×10^3	1.6×10^4
24th hour	2.3×10^6	1.5×10^6	4.2×10^6
36th hour	5×10^5	3.3×10^6	1.2×10^7
48th hour	1.2×10^7	2×10^6	3×10^6

It was established that the bacterial load did not change during the first 6 h but started increasing after 6 h (Table 2). The bacterial count increased logarithmically and an increase of $3 \log_{10}$ CFU/cm² was measured after 12 h. The increase lasted for 48 h. The logarithmic increase of the bacterial count over time is shown in Fig. 1.

Discussion

The bioburden level per instrument is dependent on the anatomic site where the device was used. Chu-Nancy et al. [2] found that bioburden levels varied between 0 and 4415 CFU per instrument after clinical use. Eighty eight percent of the instruments had bioburden levels lower than 1000. Chan-Myers et al. [3] showed that the bioburden level of rigid lumened medical devices after clinical use was relatively low, ranging from 10 to 10^4 CFU per device. Gordon Smith et al. [4] established that the microbial contamination on used dental handpieces varied between 42 and 250 CFU/mL. Our experiments show that the bacterial loads varied between 10 and 2.5×10^2 CFU/cm². No growth was observed on instruments used in biopsy procedures. The most contaminated instruments had been used in reconstructive surgery. This is due to the fact that the number of pseudomonas in burn wounds can be as high as 10^6 CFU/g tissue. Obviously instruments used in these interventions will be highly contaminated [8]. No growth on biopsy instruments could be observed, which can probably be explained by the short duration of the procedure.

The typical bacterial growth curve consists of four main phases. The initial stationary phase, which is also known as the lag phase, starts immediately after inoculation and lasts between 2 and 3 h. It is a period in which the cells adjust and adapt to their new environment. The

Fig. 1 Logarithmic change of bacterial load with time

duration of this phase depends on the medium, temperature, and nature of the cells. The logarithmic period of growth is characterized by the exponentially increasing numbers of bacterial organisms over time. It starts approximately after 4 h and lasts between 2 and 4 h depending on the nature and concentration of the nutrients in the medium, pH, and temperature of the incubation. The logarithmic phase is followed by the stationary phase in which the number of organisms remains stable. During this phase the rate of cells dying and of new ones developing is in balance. This condition may last for hours or days, depending on the nature of the bacteria. With the exhaustion of nutrients, overcrowding, and accumulation of toxic products in the metabolism, the culture enters the death phase. Nevertheless a small number of cells can survive for many months due to the release of nutrients by the dead cells [9].

In the present study, it is found that the lag phase lasts 6 h. The logarithmic phase starts after 6 h and lasts 18 h. The stationary phase starts approximately after 24 h and ends after 48 h (Fig. 1). As instruments are not expected to be cleaned more than 48 h after their use, it was not possible to establish when the bacteria entered the death phase.

The *F-value*—the time expressed in minutes required to reach sterility of an instrument by exposure to a certain killing agent at a certain temperature—will be longer when there are a large number of microorganisms on the instruments before disinfection and sterilization in comparison to having just a few microorganisms on the instruments. It is,

therefore, very important to reduce the bacterial load on the used instruments before the bacteria start growing [6].

In conclusion, it has been proven that the cleaning of the surgical instruments in the first 6 h after use is essential in order to ensure effective disinfection and sterilization, and thus to reach SAL.

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